
# BOOK OF ABSTRACTS

# ANTIMICROBIAL RESISTANCE IN EAST AFRICA

# 2017

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# INTRODUCTION

Sub-Saharan African countries have recorded success reducing the burden of major infectious diseases like HIV, tuberculosis (TB), and malaria. However, this success is eminently threatened by the antimicrobial resistance (AMR) developed by the causative agents of these major infectious diseases. The increasingly high rates of AMR are threatening to reverse the success to the pre-antimicrobial era, where a trivial infection could cause death. This means that if that happened, there will be higher mortality rates than recovery rates from infectious diseases. In 2014, the World Health Organization (WHO) published the first report on AMR. It revealed that this serious threat was no longer simply a prediction of the future, but rather that AMR was happening now, all over the world.

The rate of AMR is increasing, at a time when new strains of infectious agents also are emerging and deadly outbreaks are still occurring in the region. Reports show increasing rates of emerging and existing strains and species of TB, HIV, malaria, and mosquitoes that are completely resistant to the currently available antimicrobial agents. For instance, the rate of multiple drug-resistant TB (MDR-TB, and XDR-TB) is increasing. Even for drugs like pyrazinamide, new evidence shows that *Mycobacterium tuberculosis* resistance to the drug is higher than previously thought, thus raising doubts about the potential role of pyrazinamide in shortening MDR-TB treatment regimen. Reports also show the increasing rates of multidrug-resistant HIV (MDR-HIV) to antiretroviral drugs (ARVs); given the need for long term use of ARVs to suppress the virus, the increasing rate of MDR-HIV, if left unabated will cause serious challenges in preventing and treating HIV in the future. The trends are similar for malaria and mosquitoes, even to the new antimalarial and insecticidal products. Unsurprisingly, East Africa exhibits the same trends as the rest of sub-Saharan Africa.

To highlight the magnitude of antimicrobial resistance developed in the causative agents of the three major infectious diseases in the region (TB, HIV, and malaria), the East African Health Research Commission, as per its mandate, has compiled abstracts of research carried out in East Africa during the last thirty years (1985-2015). Together with evidence from current research that will be presented by experts during the 6th East African Health and Scientific Conference to be held in Bujumbura, The Republic of Burundi, in March 2017, the book of abstracts will serve as an excellent resource to support discussion during the symposium dedicated to the growing concern of AMR in East Africa. The symposium is part of the conference and aims to provide recommendations and strategies for the implementation of those recommendations. This will be one of the initiatives to address this growing health concern.

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East African Health Research Commission
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# HIV DRUG RESISTANCE

## 153 Citations

## (sorted newest to oldest)

# HIV DRUG RESISTANCE

1. **Prevalence of Transmitted Drug Resistance Mutations in HIV-1-Infected Drug-Naive Patients from Urban and Suburban Regions of Kenya.**

Onsongo S[1], Abidi SH[2], Khamadi S[3], Shah R[1], Kageha S[4], Ojwang P[1], Ali S[5], Okinda N[1].

AIDS Res Hum Retroviruses. 2016 Mar;32(3):220-5. doi: 10.1089/aid.2015.0026. Epub 2015 Sep 24.

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**ABSTRACT**

HIV was first described in Kenya in 1984-1985. Currently, Kenya has an estimated HIV-1 prevalence of 6.2%. With the introduction of antiretroviral drugs, the survival of most HIV patients has been prolonged markedly. However, this is greatly threatened by increasing rates of antiretroviral dug resistance, which may eventually lead to suboptimal treatment outcomes. The objective of this study was to characterize currently occurring antiretroviral drug resistance mutations among drug-naive patients visiting two referral hospitals in Kenya. Using polymerase chain reaction, the HIV protease gene was amplified from blood samples of 63 study participants. The sequences were used to determine HIV-1 subtype and presence/prevalence of mutations associated with resistance to protease inhibitors. Finally, the protease gene was variably measured using Shannon entropy analysis. Analysis of frequency of HIV-1 subtypes revealed subtype A to be the predominant subtype, while the analysis of drug resistance mutations revealed the presence of four minor drug resistance mutations associated weakly with resistance to protease inhibitors. Among these mutations, L33I was the most prevalent mutation. Shannon entropy analysis revealed high genomic variability, especially in region spanning nucleotides 1-55, 113-170, and 205-240. This study warrants the need for dedicated efforts to improve compliance to antiretroviral therapy and reduce transmitted resistance rates, which will greatly ensure the therapeutic efficacy of antiretroviral drugs.

PMCID: PMC4779969 [Available on 2017-03-01]
PMID: 26401720 [PubMed - indexed for MEDLINE]

2. **Maternal Neutralization-Resistant Virus Variants Do Not Predict Infant HIV Infection Risk.**

Milligan C[1], Omenda MM[2], Chohan V[2], Odem-Davis K[3], Richardson BA[4], Nduati R[5], Overbaugh J[6].

MBio. 2016 Feb 2;7(1):e02221-15. doi: 10.1128/mBio.02221-15.

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**ABSTRACT**

Mother-to-child transmission (MTCT) of HIV provides a setting for studying immune correlates of protection. Neutralizing antibodies (NAbs) are suggested to contribute to a viral bottleneck during MTCT, but their role in blocking transmission is unclear, as studies comparing the NAb sensitivities of maternal viruses have yielded disparate results. We sought to determine whether transmitting mothers differ from nontransmitting mothers in the ability to neutralize individual autologous virus variants present at transmission. Ten transmitting and 10 nontransmitting HIV-infected mothers at high risk of MTCT were included in this study. Full-length HIV envelope genes (n = 100) were cloned from peripheral blood mononuclear cells obtained near transmission from transmitting mothers and at similar time points from nontransmitting mothers. Envelope clones were tested as pseudoviruses against contemporaneous, autologous maternal plasma in neutralization assays. The association between transmission and the log2 50% inhibitory concentration (IC50) for multiple virus variants per mother was estimated by using logistic regression with clustered standard errors. t tests were used to compare proportions of neutralization-resistant viruses. Overall, transmitting mothers had a median IC50 of 317 (interquartile range [IQR], 202 to 521), and nontransmitting mothers had a median IC50 of 243 (IQR, 95 to 594). Transmission risk was not significantly associated with autologous NAb activity (odds ratio, 1.25; P = 0.3). Compared to nontransmitting mothers, transmitting mothers had similar numbers of or fewer neutralization-resistant virus variants, depending on the IC50 neutralization resistance cutoff. In conclusion, HIV-infected mothers harbor mostly neutralization-sensitive viruses, although resistant variants were detected in both transmitting and nontransmitting mothers. These results suggest that MTCT during the breastfeeding period is not driven solely by the presence of maternal neutralization escape variants.

**IMPORTANCE:**

There are limited data demonstrating whether NAbs can prevent HIV transmission and infection in humans, and for this reason, NAbs have been studied in MTCT, where maternal antibodies are present at the time of transmission. Results of these studies have varied, perhaps because of differences in methods. Importantly, studies often used cultured viruses and samples from time points outside the window of transmission, which could confound findings. Here, we considered the role of maternal NAbs against individual maternal virus variants near the time of transmission. We found no evidence that NAbs are associated with protection from infection. In fact, depending on the cutoff used to define neutralization resistance, we found evidence that nontransmitting mothers have more neutralization-resistant virus variants. These results suggest that lack of virus transmission in the early breastfeeding period is not simply due to an absence of maternal neutralization escape variants and likely includes multiple factors.

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PMCID: PMC4742716 **Free PMC Article**
PMID: 26838723 [PubMed - indexed for MEDLINE]

3. **Simplified Paper Format for Detecting HIV Drug Resistance in Clinical Specimens by Oligonucleotide Ligation.**

Panpradist N[1], Beck IA[2], Chung MH[3,4], Kiarie JN[5], Frenkel LM[4,6], Lutz BR[1].

PLoS One. 2016 Jan 11;11(1):e0145962. doi: 10.1371/journal.pone.0145962. eCollection 2016.

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**ABSTRACT**

Human immunodeficiency virus (HIV) is a chronic infection that can be managed by antiretroviral treatment (ART). However, periods of suboptimal viral suppression during lifelong ART can select for HIV drug resistant (DR) variants. Transmission of drug resistant virus can lessen or abrogate ART efficacy. Therefore, testing of individuals for drug resistance prior to initiation of treatment is recommended to ensure effective ART. Sensitive and inexpensive HIV genotyping methods are needed in low-resource settings where most HIV infections occur. The oligonucleotide ligation assay (OLA) is a sensitive point mutation assay for detection of drug resistance mutations in HIV pol. The current OLA involves four main steps from sample to analysis: (1) lysis and/or nucleic acid extraction, (2) amplification of HIV RNA or

DNA, (3) ligation of oligonucleotide probes designed to detect single nucleotide mutations that confer HIV drug resistance, and (4) analysis via oligonucleotide surface capture, denaturation, and detection (CDD). The relative complexity of these steps has limited its adoption in resource-limited laboratories. Here we describe a simplification of the 2.5-hour plate-format CDD to a 45-minute paper-format CDD that eliminates the need for a plate reader. Analysis of mutations at four HIV-1 DR codons (K103N, Y181C, M184V, and G190A) in 26 blood specimens showed a strong correlation of the ratios of mutant signal to total signal between the paper CDD and the plate CDD. The assay described makes the OLA easier to perform in low resource laboratories.

PMCID: PMC4713472 **Free PMC Article**
PMID: 26751207 [PubMed - indexed for MEDLINE]

4. **Preexposure prophylaxis-selected drug resistance decays rapidly after drug cessation.**

Weis JF,[1] Baeten JM, [2,3,4] McCoy CO, [5] Warth C, [5] Donnell D, [2,6] Thomas KK, [2] Hendrix CW, [7,8,9] Marzinke MA, [7,10] Mugo N, [2,11] Iv FA, [5] Celum C, [2,3,4] Lehman DA; [1,2] Partners PrEP Study Team.

AIDS. 2016 Jan 2;30(1):31-5. doi: 10.1097/QAD.0000000000000915.

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**ABSTRACT**

OBJECTIVE: Resistance to emtricitabine plus tenofovir disoproxil fumarate (FTC/TDF) or TDF alone used as preexposure prophylaxis (PrEP) has been detected in individuals who initiated PrEP during unrecognized acute HIV infection and, rarely, in PrEP breakthrough infections. PrEP-selected resistance could alter future treatment options, and therefore we sought to determine how long resistance persisted after PrEP cessation.

METHODS: The Partners PrEP Study was a randomized placebo-controlled trial of FTC/TDF or TDF as PrEP for HIV prevention. We previously reported that PrEP-related mutations (K65R, K70E or M184IV) were detected by 454 sequencing following seroconversion in nine individuals who acquired HIV during the Partners PrEP Study. In the current study, we used 454 sequencing to detect and quantify PrEP-

related mutations in HIV RNA-positive plasma samples prior to seroconversion, as well as in plasma from 6, 12, and 24 months after PrEP cessation from these nine individuals.

RESULTS: HIV RNA-positive, antibody-negative samples were available prior to seroconversion for four of nine individuals with resistance detected at seroconversion. In all four cases, K65R, K70E and M184IV were not detected prior to seroconversion, suggesting PrEP-related resistance was selected and not transmitted. All PrEP-selected mutations were no longer detectable by 6 months after PrEP cessation and remained undetectable at 12 and 24 months in the absence of antiretroviral therapy.

CONCLUSION: Using highly sensitive assays, PrEP-selected resistance in plasma decays below detection by 6 months following drug cessation and remains undetectable for at least 24 months. Even high levels of resistance mutations during acute infection decay rapidly in the absence of ongoing PrEP exposure.

PMCID: PMC4704103 **Free PMC Article**
PMID: 26731753 [PubMed - indexed for MEDLINE]

5. **HemaSpot, a Novel Blood Storage Device for HIV-1 Drug Resistance Testing.**

Brooks K[1], DeLong A[2], Balamane M[1], Schreier L[1], Orido M[3], Chepkenja M[3], Kemboi E[3], D'Antuono M[1], Chan PA[1], Emonyi W[4], Diero L[4], Coetzer M[1], Kantor R[5].

J Clin Microbiol. 2016 Jan;54(1):223-5. doi: 10.1128/JCM.02853-15. Epub 2015 Nov 11.

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**ABSTRACT**

HemaSpot, a novel dried-blood storage filter device, was used for HIV-1 pol resistance testing in 30 fresh United States blood samples and 54 previously frozen Kenyan blood samples. Genotyping succeeded in 79% and 58% of samples, respectively, improved with shorter storage and higher viral load, and had good (86%) resistance mutation concordance to plasma.

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PMCID: PMC4702722 **Free PMC Article**
PMID: 26560534 [PubMed - indexed for MEDLINE]

6. **HIV-1 Drug Resistance Mutations: Potential Applications for Point-of-Care Genotypic Resistance Testing.**

Rhee SY[1], Jordan MR[2], Raizes E[3], Chua A[4,5], Parkin N[6], Kantor R[7], Van Zyl GU[8,9], Mukui I[10], Hosseinipour MC[11], Frenkel LM[12], Ndembi N[13], Hamers RL[14], Rinke de Wit TF[14], Wallis CL[15], Gupta RK[16], Fokam J[17,18], Zeh C[19], Schapiro JM[20], Carmona S[21,22], Katzenstein D[1], Tang M[1], Aghokeng AF[23], De Oliveira T[24], Wensing AM[25], Gallant JE[26], Wainberg MA[27], Richman DD[28,29], Fitzgibbon JE[30], Schito M[31], Bertagnolio S[32], Yang C[3], Shafer RW[1].

PLoS One. 2015 Dec 30;10(12):e0145772. doi: 10.1371/journal.pone.0145772. eCollection 2015.

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**ABSTRACT**

The increasing prevalence of acquired and transmitted HIV-1 drug resistance is an obstacle to successful antiretroviral therapy (ART) in the low- and middle-income countries (LMICs) hardest hit by the HIV-1 pandemic. Genotypic drug resistance testing could facilitate the choice of initial ART in areas with rising transmitted drug resistance (TDR) and enable care-providers to determine which individuals with virological failure (VF) on a first- or second-line ART regimen require a change in treatment. An inexpensive near point-of-care (POC) genotypic resistance test would be useful in settings where the resources, capacity, and infrastructure to perform standard genotypic drug resistance testing are limited. Such a test would be particularly useful in conjunction with the POC HIV-1 viral load tests that are currently being introduced in LMICs. A POC genotypic resistance test is likely to involve the use of allele-specific point mutation assays for detecting drug-resistance mutations (DRMs). This study proposes that two major nucleoside reverse transcriptase inhibitor (NRTI)-associated DRMs (M184V and K65R) and four major NNRTI-associated DRMs (K103N, Y181C, G190A, and V106M) would be the most useful for POC genotypic resistance testing in LMIC settings. One or more of these six DRMs was present in 61.2% of analyzed virus sequences from ART-naïve individuals with intermediate or high-level TDR and 98.8% of analyzed virus sequences from individuals on a first-line NRTI/NNRTI-containing regimen with intermediate or high-level acquired drug resistance. The detection of one or more of these DRMs in an ART-naïve individual or in a individual with VF on a first-line NRTI/NNRTI-containing regimen may be considered an indication for a protease inhibitor (PI)-containing regimen or closer virological monitoring based on cost-effectiveness or country policy.

PMCID: PMC4696791 **Free PMC Article**
PMID: 26717411 [PubMed - indexed for MEDLINE]

7. **Virological Response and Antiretroviral Drug Resistance Emerging during Antiretroviral Therapy at Three Treatment Centers in Uganda.**

Kaleebu P[1,2,3], Kirungi W[4], Watera C[1], Asio J[1], Lyagoba F[1,2], Lutalo T[1], Kapaata AA[2], Nanyonga F[1], Parry CM[2], Magambo B[1,2], Nazziwa J[2], Nannyonjo M[2], Hughes P[2], Hladik W[5], Ruberantwari A[1], Namuwenge N[4], Musinguzi J[4], Downing R[1], Katongole-Mbidde E[1]; HIV Drug Resistance Working group.

PLoS One. 2015 Dec 23;10(12):e0145536. doi: 10.1371/journal.pone.0145536. eCollection 2015.

Collaborators: (14)
Kaleebu P, Kirungi W, Munderi P, Ssali F, Lutalo T, Etukoit B, Namayanja G, Downing R, Watera C, Byomire H, Kambugu A, Kityo C, Namuwenge N, Namagala E.

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**ABSTRACT**

BACKGROUND: With the scale-up of antiretroviral therapy (ART), monitoring programme performance is needed to maximize ART efficacy and limit HIV drug resistance (HIVDR).

METHODS: We implemented a WHO HIVDR prospective survey protocol at three treatment centers between 2012 and 2013. Data were ABSTRACTed from patient records at ART start (T1) and after 12 months (T2). Genotyping was performed in the HIV pol region at the two time points.

RESULTS: Of the 425 patients enrolled, at T2, 20 (4.7%) had died, 66 (15.5%) were lost to follow-up, 313 (73.6%) were still on first-line, 8 (1.9%) had switched to second-line, 17 (4.0%) had transferred out and 1 (0.2%) had stopped treatment. At T2, 272 out of 321 on first and second line (84.7%) suppressed below 1000 copies/ml and the HIV DR prevention rate was 70.1%, just within the WHO threshold of ≥ 70%. The proportion of participants with potential HIVDR was 20.9%, which is higher than the 18.8% based on pooled analyses from African studies. Of the 35 patients with mutations at T2, 80% had M184V/I, 65.7% Y181C, and 48.6% (54.8% excluding those not on Tenofovir) had K65R mutations. 22.9% had Thymidine Analogue Mutations (TAMs). Factors significantly associated with HIVDR prevention at T2 were: baseline viral load (VL) <100,000 copies/ml [Adjusted odds ratio (AOR) 3.13, 95% confidence interval (CI): 1.36-7.19] and facility. Independent baseline predictors for HIVDR mutations at T2 were: CD4 count < 250 cells/µl (AOR 2.80, 95% CI: 1.08-7.29) and viral load ≥ 100,000 copies/ml (AOR 2.48, 95% CI: 1.00-6.14).

CONCLUSION: Strengthening defaulter tracing, intensified follow-up for patients with low CD4 counts and/or high VL at ART initiation together with early treatment initiation above 250 CD4 cells/ul and adequate patient counselling would improve ART efficacy and HIVDR prevention. The high rate of K65R and TAMs could compromise second line regimens including NRTIs.

PMCID: PMC4689474 **Free PMC Article**
PMID: 26700639 [PubMed - indexed for MEDLINE]

8. **High Prevalence of HIV Low Abundance Drug-Resistant Variants in a Treatment-Naive Population in North Rift Kenya.**

Cheriro W[1,2], Kiptoo M[1,3], Kikuvi G[1], Mining S[4], Emonyi W[4], Songok E[1,3,5].

AIDS Res Hum Retroviruses. 2015 Dec;31(12):1274-7. doi: 10.1089/AID.2015.0039. Epub 2015 Oct 8.

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**ABSTRACT**

The advent of antiretroviral treatment (ART) has resulted in a dramatic reduction in AIDS-related morbidity and mortality. However, the emergence and spread of antiretroviral drug resistance (DR) threaten to negatively impact treatment regimens and compromise efforts to control the epidemic. It is recommended that surveillance of drug resistance occur in conjunction with scale-up efforts to ensure that appropriate first-line therapy is offered relative to the resistance that exists. However, standard resistance testing methods used in Sub-Saharan Africa rely on techniques that do not include low abundance DR variants (LADRVs) that have been documented to contribute to treatment failure. The use of next generation sequencing (NGS) has been shown to be more sensitive to LADRVS. We have carried out a preliminary investigation using NGS to determine the prevalence of LDRVS among a drug-naive population in North Rift Kenya. Antiretroviral-naive patients attending a care clinic in North Rift Kenya were requested to provide and with consent provided blood samples for DR analysis. DNA was extracted and amplified and nested PCR was conducted on the pol RT region using primers tagged with multiplex identifiers (MID). Resulting PCR amplicons were purified, quantified, and pyrosequenced using a GS FLX Titanium PicoTiterPlate (Roche). Valid pyrosequencing reads were aligned with HXB-2 and the frequency and distribution of nucleotide and amino acid changes were determined using an in-house Perl script. DR mutations were identified using the IAS-USA HIV DR mutation database. Sixty samples were successfully sequenced of which 26 were subtype A, 9 were subtype D, 2 were subtype C, and the remaining were recombinants. Forty-six (76.6%) had at least one drug resistance mutation, with 25 (41.6%) indicated as major and the remaining 21 (35%) indicated as minor. The most prevalent mutation was NRTI position K219Q/R (11/46, 24%) followed by NRTI M184V (5/46, 11%) and NNRTI K103N (4/46, 9%). Our use of NGS technology revealed a high prevalence of LADRVs among drug-naive populations in Kenya, a region with predominantly non-B subtypes. The impact of these mutations on the clinical outcome of ART can be ascertained only through long-term follow-up.
PMID: 26414430 [PubMed - indexed for MEDLINE]

9. **Pretreatment HIV drug resistance increases regimen switches in sub-Saharan Africa.**

Boender TS[1], Hoenderboom BM[1], Sigaloff KC[2], Hamers RL[2], Wellington M[3], Shamu T[3], Siwale M[4], Labib Maksimos EE[5], Nankya I[6], Kityo CM[6], Adeyemo TA[7], Akanmu AS[7], Mandaliya K[8], Botes ME[9], Ondoa P[1], Rinke de Wit TF[1].

Clin Infect Dis. 2015 Dec 1;61(11):1749-58. doi: 10.1093/cid/civ656. Epub 2015 Aug 3.

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**ABSTRACT**

BACKGROUND: After the scale-up of antiretroviral therapy (ART) for human immunodeficiency virus (HIV) infection in Africa, increasing numbers of patients have pretreatment drug resistance.

METHODS: In a large multicountry cohort of patients starting standard first-line ART in six African countries, pol genotyping was retrospectively performed if viral load (VL) ≥1000 cps/mL. Pretreatment drug resistance was defined as a decreased susceptibility to ≥1 prescribed drug. We assessed the effect of pretreatment drug resistance on all-cause mortality, new AIDS events and switch to second-line ART due to presumed treatment failure, using Cox models.

RESULTS: Among 2579 participants for whom a pretreatment genotype was available, 5.5% had pretreatment drug resistance. Pretreatment drug resistance was associated with an increased risk of regimen switch (adjusted hazard ratio [aHR] 3.80; 95% confidence interval [CI], 1.49-9.68; P =.005) but was not associated with mortality (aHR 0.75, 95% CI,.24-2.35; P =.617) or new AIDS events (aHR 1.06, 95% CI,.68-1.64; P =.807). During three years of follow up, 106 (4.1%) participants switched to second-line, of whom 18 (17.0%) switched with VL < 1000 cps/mL, 7 (6.6%) with VL ≥ 1000 cps/mL and no drug resistance mutations (DRMs), 46 (43.4%) with VL ≥ 1000 cps/mL and ≥1 DRMs; no HIV RNA data was available for 32 (30.2%) participants.

CONCLUSIONS: Given rising pretreatment HIV drug resistance levels in sub-Saharan Africa, these findings underscore the need for expanded access to second-line ART. VL monitoring can improve the accuracy of failure detection and efficiency of switching practices.

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**Free Article**
PMID: 26240203 [PubMed - indexed for MEDLINE]

10. **Epidemiology of HBV infection in a cohort of Ugandan HIV-infected patients and rate and pattern of lamivudine-resistant HBV infection in patients receiving antiretroviral therapy.**

Calisti G[1], Muhindo R[2], Boum Y 2nd[3], Wilson LA[2], Foster GM[4], Geretti AM[4], Bhagani S[5].

Trans R Soc Trop Med Hyg. 2015 Nov;109(11):723-9. doi: 10.1093/trstmh/trv077. Epub 2015 Sep 18.

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**ABSTRACT**

BACKGROUND: Many HIV-infected patients in sub-Saharan Africa are not routinely screened for hepatitis B virus (HBV) infection and are on antiretroviral therapy (ART) regimens containing only lamivudine as anti-HBV active drug.

METHODS: In 2009-2011, we screened for hepatitis B surface antigen (HBsAg) in 2820 HIV-infected adults patients at the Mbarara Hospital Uganda and investigated risk factors for HBV infection. Using samples of dried plasma or blood spots, we tested for HBV viral load and HBV drug resistance mutations in all HBsAg-positive patients on ART for ≥ 12 months.

RESULTS: In this study, 109 patients tested HBsAg positive (3.9%; 109/2820). HBsAg-positive patients were more likely to have had >4 lifetime sexual partners ($p<0.01$). Of the 55 HBsAg-positive patients on ART for ≥ 12 months, 53 were only on lamivudine as anti-HBV active drug and two were on tenofovir and lamivudine. HBV-DNA was detected in 30 patients (54.5%; 30/55), all on lamivudine-monotherapy. Of the 23 patients in whom HBV-DNA sequencing was successful, 17 had lamivudine-resistant HBV strains harbouring rtM204V/I mutations accompanied by secondary/compensatory mutations.

CONCLUSIONS: Our study suggests that sexual transmission may represent a major mode of spread of HBV in southwest Uganda and confirms the importance of screening for HBV and of using ART regimens containing tenofovir in HIV/HBV co-infected patients.

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PMID: 26386408 [PubMed - indexed for MEDLINE]

11. **Comparison of 454 Ultra-Deep Sequencing and Allele-Specific Real-Time PCR with Regard to the Detection of Emerging Drug-Resistant Minor HIV-1 Variants after Antiretroviral Prophylaxis for Vertical Transmission.**

Hauser A[1], Kuecherer C[1], Kunz A[2], Dabrowski PW[3], Radonić A[3], Nitsche A[3], Theuring S[2], Bannert N[1], Sewangi J[4], Mbezi P[5], Dugange F[6], Harms G[2], Meixenberger K[1].

PLoS One. 2015 Oct 15;10(10):e0140809. doi: 10.1371/journal.pone.0140809. eCollection 2015.

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**ABSTRACT**

BACKGROUND: Pregnant HIV-infected women were screened for the development of HIV-1 drug resistance after implementation of a triple-antiretroviral transmission prophylaxis as recommended by the WHO in 2006. The study offered the opportunity to compare amplicon-based 454 ultra-deep sequencing (UDS) and allele-specific real-time PCR (ASPCR) for the detection of drug-resistant minor variants in the HIV-1 reverse transcriptase (RT).

METHODS: Plasma samples from 34 Tanzanian women were previously analysed by ASPCR for key resistance mutations in the viral RT selected by AZT, 3TC, and NVP (K70R, K103N, Y181C, M184V, T215Y/F). In this study, the RT region of the same samples was investigated by amplicon-based UDS for resistance mutations using the 454 GS FLX System.

RESULTS: Drug-resistant HIV-variants were identified in 69% (20/29) of women by UDS and in 45% (13/29) by ASPCR. The absolute number of resistance mutations identified by UDS was twice that identified by ASPCR (45 vs 24). By UDS 14 of 24 ASPCR-detected resistance mutations were identified at the same position. The overall concordance between UDS and ASPCR was 61.0% (25/41). The proportions of variants quantified by UDS were approximately 2-3 times lower than by ASPCR. Amplicon generation from samples with viral loads below 20,000 copies/ml failed more frequently by UDS compared to ASPCR (limit of detection = 650 copies/ml), resulting in missing or insufficient sequence coverage.

CONCLUSIONS: Both methods can provide useful information about drug-resistant minor HIV-1 variants. ASPCR has a higher sensitivity than UDS, but is restricted to single resistance mutations. In contrast, UDS is limited by its requirement for high viral loads to achieve sufficient sequence coverage, but the sequence information reveals the complete resistance patterns within the genomic region

analysed. Improvements to the UDS limit of detection are in progress, and UDS could then facilitate monitoring of drug-resistant minor variants in the HIV-1 quasispecies.

PMCID: PMC4607442 **Free PMC Article**
PMID: 26469189 [PubMed - indexed for MEDLINE]

12. **Routine use of Xpert® MTB/RIF in areas with different prevalences of HIV and drug-resistant tuberculosis.**

Page AL[1], Ardizzoni E[2], Lassovsky M[3], Kirubi B[4], Bichkova D[5], Pedrotta A[6], Lastrucci C[7], de la Tour R[8], Bonnet M[1], Varaine F[7].

Int J Tuberc Lung Dis. 2015 Sep;19(9):1078-83, i-iii. doi: 10.5588/ijtld.14.0951.

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**ABSTRACT**

SETTING: Despite the widespread introduction of Xpert(®) MTB/RIF in developing countries, reports of its use and value in routine conditions remain limited.

OBJECTIVE: To describe Xpert results in relation to microscopy, treatment initiation, cost and workload under routine conditions at four sites in Cambodia, Georgia, Kenya and Swaziland.

DESIGN: Laboratory and clinical information on presumed TB patients were obtained from routine registers over a period of at least 6 months between March and November 2012.

RESULTS: Among the 6086 presumed TB patients included in the analysis, Xpert testing increased the number of biologically confirmed cases by 15% to 67% compared to microscopy. Up to 12% of the initial Xpert results were inconclusive. Between 56% and 83% of patients were started on treatment based on microscopy and/or Xpert results, with median delays of 1-16 days. Rifampicin resistance was detected in 3-19% of Xpert-positive patients.

CONCLUSION: Despite the additional numbers of cases detected by Xpert compared to microscopy, large proportions of patients are still started on treatment empirically in routine practice. Patient and specimen flow should be optimised to reduce delays in treatment initiation. Simple, non-sputum-based point-of-care tests with high sensitivity are needed to improve TB diagnosis and management.
PMID: 26260829 [PubMed - indexed for MEDLINE]

13. **Disseminated tuberculosis in an HIV-infected child: rifampicin resistance detected by GeneXpert in a lymph node aspirate but not in cerebrospinal fluid.**

Gamell A[1], Ntamatungiro AJ[2], Battegay M[3], Letang E[1].

BMJ Case Rep. 2015 Aug 3;2015. pii: bcr2014207997. doi: 10.1136/bcr-2014-207997.

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**ABSTRACT**

A 9-year-old HIV-infected child previously treated with inadequate doses of antitubercular drugs based on weight was admitted 5 months after initial tuberculosis (TB) diagnosis with acute hemiplegia and inguinal lymphadenopathies in a rural hospital in Tanzania. He was diagnosed with TB meningitis and lymphadenitis using Xpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) assay. Rifampicin resistance was detected in the lymph node aspirate but not in the cerebrospinal fluid. His TB therapy was optimised based on available medications and antiretroviral treatment was initiated 6 weeks later. Despite these efforts, the clinical evolution was poor and the child died 12 weeks after admission.

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PMID: 26240094 [PubMed - indexed for MEDLINE]

14. **Nevirapine Resistance in Previously Nevirapine-Unexposed HIV-1-Infected Kenyan Infants Initiating Early Antiretroviral Therapy.**

Chohan BH[1,2,3], Tapia K[2], Benki-Nugent S[2], Khasimwa B[4], Ngayo M[3], Maleche-Obimbo E[4], Wamalwa D[4], Overbaugh J[5], John-Stewart G[2,6,7,8].

AIDS Res Hum Retroviruses. 2015 Aug;31(8):783-91. doi: 10.1089/AID.2014.0370. Epub 2015 Apr 22.

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**ABSTRACT**

Nevirapine (NVP) resistance occurs frequently in infants following NVP use in prevention of mother-to-child transmission (PMTCT) regimens. However, among previously NVP-unexposed infants treated with NVP-antiretroviral therapy (ART), the development and impact of NVP resistance have not been well characterized. In a prospective clinical trial providing early ART to HIV-infected infants <5 months of age in Kenya (OPH03 study), we followed NVP-unexposed infants who initiated NVP-ART for 12 months. Viral loads were assessed and resistance determined using a population-based genotypic resistance assay. Of 99 infants screened, 33 had no prior NVP exposure, 22 of whom were initiated on NVP-ART. Among 19 infants with follow-up, seven (37%) infants developed resistance: one at 3 months and six at 6 months after ART initiation. The cumulative probability of NVP resistance was 5.9% at 3 months and 43.5% at 6 months. Baseline HIV RNA levels (p=0.7) and other characteristics were not associated with developing resistance. Post-ART, higher virus levels at visits preceding the detection of resistance were significantly associated with increased detection of resistance (p=0.004). Virus levels after 6 and 12 months of ART were significantly higher in infants with resistance than those without (p=0.007, p=0.030, respectively). Among infants without previous NVP exposure, development of NVP resistance was frequent and was associated with virologic failure during the first year of ART. Earlier development of NVP resistance in infants than in adults initiating NVP-ART may be due to longer viremia following ART or inadequate NVP levels resulting from NVP lead-in dosing. The development of NVP resistance may, in part, explain the superiority of protease inhibitor-based ART in infants.

PMCID: PMC4533021 **Free PMC Article**
PMID: 25819584 [PubMed - indexed for MEDLINE]

15. **Doubt, defiance, and identity: Understanding resistance to male circumcision for HIV prevention in Malawi.**

Parkhurst JO[1], Chilongozi D[2], Hutchinson E[3].

Soc Sci Med. 2015 Jun;135:15-22. doi: 10.1016/j.socscimed.2015.04.020. Epub 2015 Apr 23.

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**ABSTRACT**

Global policy recommendations to scale up of male circumcision (MC) for HIV prevention tend to frame the procedure as a simple and efficacious public health intervention. However, there has been variable uptake of MC in countries with significant HIV epidemics. Kenya, for example, has embraced MC and has been dubbed a 'leader' by the global health community, while Malawi has been branded a 'laggard' in its slow adoption of a national programme, with a strong political discourse of resistance forming around MC. Regardless of any epidemiological or technical evidence, the uptake of international recommendations will be shaped by how a policy, and the specific artefacts that constitute that policy,

intersect with local concerns. MC holds particular significance within many ethnic and religious groups, serving as an important rite of passage, but also designating otherness or enabling the identification of the social and political self. Understanding how the artefact of MC intersects with local social, economic, and political contexts, is therefore essential to understand the acceptance or resistance of global policy recommendations. In this paper we present an in-depth analysis of Malawi's political resistance to MC, finding that ethnic and religious divisions dominating recent political movements aligned well with differing circumcision practices. Political resistance was further found to manifest through two key narratives: a 'narrative of defiance' around the need to resist donor manipulation, and a 'narrative of doubt' which seized on a piece of epidemiological evidence to refute global claims of efficacy. Further, we found that discussions over MC served as an additional arena through which ethnic identities and claims to power could themselves be negotiated, and therefore used to support claims of political legitimacy.
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**Free Article**

PMID: 25939072 [PubMed - indexed for MEDLINE]

16. **Insulin resistance change and antiretroviral therapy exposure in HIV-infected and uninfected Rwandan women: a longitudinal analysis.**

Mutimura E[1], Hoover DR[2], Shi Q[3], Dusingize JC[1], Sinayobye JD[1], Cohen M[4], Anastos K[5].

PLoS One. 2015 Apr 16;10(4):e0123936. doi: 10.1371/journal.pone.0123936. eCollection 2015.

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**ABSTRACT**

BACKGROUND: We longitudinally assessed predictors of insulin resistance (IR) change among HIV-uninfected and HIV-infected (ART-initiators and ART-non-initiators) Rwandan women.

METHODOLOGY: HIV-infected (HIV+) and uninfected (HIV-) women provided demographic and clinical measures: age, body mass index (BMI) in Kg/(height in meters)2, Fat-Mass (FMI) and Fat-Free-Mass (FFMI) index, fasting serum glucose and insulin. Homeostasis Model Assessment (HOMA) was calculated to estimate IR change over time in log10 transformed HOMA measured at study enrollment or prior to ART initiation in 3 groups: HIV- (n = 194), HIV+ ART-non-initiators (n=95) and HIV+ ART-initiators (n=371). ANCOVA linear regression models of change in log10-HOMA were fit with all models included the first log10 HOMA as a predictor.

RESULTS: Mean±SD log10-HOMA was -0.18±0.39 at the 1st and -0.21±0.41 at the 2nd measure, with mean change of 0.03±0.44. In the final model (all women) BMI at 1st HOMA measure (0.014; 95% CI=0.006-0.021 per kg/m2; p<0.001) and change in BMI from 1st to 2nd measure (0.024; 95% CI=0.013-0.035 per kg/m2; p<0.001) predicted HOMA change. When restricted to subjects with FMI measures, FMI at 1st HOMA measure (0.020; 95% CI=0.010-0.030 per kg/m2; p<0.001) and change in FMI from 1st to 2nd measure (0.032; 95% CI=0.020-0.043 per kg/m2; p<0.0001) predicted change in HOMA. While ART use did not predict change in log10-HOMA, untreated HIV+ women had a significant decline in IR over time. Use or duration of AZT, d4T and EFV was not associated with HOMA change in HIV+ women.

CONCLUSIONS: Baseline BMI and change in BMI, and in particular fat mass and change in fat mass predicted insulin resistance change over ~3 years in HIV-infected and uninfected Rwandan women. Exposure to specific ART (d4T, AZT, EFV) did not predict insulin resistance change in ART-treated HIV-infected Rwandan women.

PMCID: PMC4400132 **Free PMC Article**
PMID: 25880634 [PubMed - indexed for MEDLINE]

17. **Risk factors and mortality associated with resistance to first-line antiretroviral therapy: multicentric cross-sectional and longitudinal analyses.**

Pinoges L,[1] Schramm B,[1] Poulet E,[1] Balkan S,[2] Szumilin E,[2] Ferreyra C,[3] Pujades-Rodríguez M.[1,4]

J Acquir Immune Defic Syndr. 2015 Apr 15;68(5):527-35. doi: 10.1097/QAI.0000000000000513.

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**ABSTRACT**

BACKGROUND: Understanding the factors associated with HIV drug resistance development and subsequent mortality is important to improve clinical patient management.

METHODS: Analysis of individual electronic health records from 4 HIV programs in Malawi, Kenya, Uganda, and Cambodia, linked to data from 5 cross-sectional virological studies conducted among patients receiving first-line antiretroviral therapy (ART) for ≥6 months. Adjusted logistic and Cox-regression models were used to identify risk factors for drug resistance and subsequent mortality.

RESULTS: A total of 2257 patients (62% women) were included. At ART initiation, median CD4 cell count was 100 cells per microliter (interquartile range, 40-165). A median of 25.1 months after therapy start, 18% of patients had ≥400 and 12.4% ≥1000 HIV RNA copies per milliliter. Of 180 patients with drug resistance data, 83.9% had major resistance(s) to nucleoside or nonnucleoside reverse transcriptase inhibitors, and 74.4% dual resistance. Resistance to nevirapine, lamivudine, and efavirenz was common, and 6% had etravirine cross-resistance. Risk factors for resistance were young age (<35

years), low CD4 cell count (<200 cells/μL), and poor treatment adherence. During 4978 person-years of follow-up after virological testing (median = 31.8 months), 57 deaths occurred [rate = 1.14/100 person-years; 95% confidence interval (CI): 0.88 to 1.48]. Mortality was higher in patients with resistance (hazard ratio = 2.08; 95% CI: 1.07 to 4.07 vs. <400 copies/mL), and older age (hazard ratio = 2.41; 95% CI: 1.24 to 4.71 for ≥43 vs. ≤34 years), and lower in those receiving ART for >30 months.

CONCLUSIONS: Our findings underline the importance of optimal treatment adherence and adequate virological response monitoring and emphasize the need for resistance surveillance initiatives even in HIV programs achieving high virological suppression rates.
PMID: 25585301 [PubMed - indexed for MEDLINE]

18. **Risk of drug resistance among persons acquiring HIV within a randomized clinical trial of single- or dual-agent preexposure prophylaxis.**

Lehman DA[1], Baeten JM[2], McCoy CO[3], Weis JF[4], Peterson D[4], Mbara G[1], Donnell D[5], Thomas KK[6], Hendrix CW[7], Marzinke MA[7], Frenkel L[8], Ndase P[6], Mugo NR[9], Celum C[2], Overbaugh J[10], Matsen FA[3]; Partners PrEP Study Team.

J Infect Dis. 2015 Apr 15;211(8):1211-8. doi: 10.1093/infdis/jiu677. Epub 2015 Jan 13.

Collaborators: (28)
Celum C, Baeten JM, Donnell D, Coombs RW, Frenkel L, Hendrix CW, Marzinke MA, Lingappa J, McElrath MJ, Fife K, Were E, Tumwesigye E, Ndase P, Katabira E, Katabira E, Ronald A, Bukusi E, Cohen C, Wangisi J, Campbell J, Tappero J, Kiarie J, Farquhar C, John-Stewart G, Mugo NR, Campbell J, Tappero J, Wangisi J.

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**Comment in:**

- [Pre-exposure prophylaxis and selection of HIV resistant strains: Attention should be paid to the pre-exposure prophylaxis initiated during the seronegative initial period of HIV primary infection ]. [Med Mal Infect. 2015]
- Weighing the risk of drug resistance with the benefits of HIV preexposure prophylaxis. [J Infect Dis. 2015]

**ABSTRACT**

BACKGROUND: Preexposure prophylaxis (PrEP) with emtricitabine plus tenofovir disoproxil fumarate (FTC/TDF) or TDF alone reduces the risk of human immunodeficiency virus (HIV) acquisition. Understanding the risk of antiretroviral resistance selected by PrEP during breakthrough infections is important because of the risk of treatment failure during subsequent antiretroviral use.

METHODS: Within the largest randomized trial of FTC/TDF versus TDF as PrEP, plasma samples were tested for HIV with resistance mutations associated with FTC (K65R and M184IV) and TDF (K65R and K70E), using 454 sequencing.

RESULTS: Of 121 HIV seroconverters, 25 received FTC/TDF, 38 received TDF, and 58 received placebo. Plasma drug levels in 26 individuals indicated PrEP use during or after HIV acquisition, of which 5 had virus with resistance mutations associated with their PrEP regimen. Among those with PrEP drug detected during infection, resistance was more frequent in the FTC/TDF arm (4 of 7 [57%]), compared with the TDF arm (1 of 19 [5.3%]; P =.01), owing to the FTC-associated mutation M184IV. Of these cases, 3 had unrecognized acute infection at PrEP randomization, and 2 were HIV negative at enrollment.

CONCLUSIONS: These results suggest that resistance selected by PrEP is rare but can occur both with PrEP initiation during acute seronegative HIV infection and in PrEP breakthrough infections and that FTC is associated with a greater frequency of resistance mutations than TDF.

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PMCID: PMC4402339 **Free PMC Article**
PMID: 25587020 [PubMed - indexed for MEDLINE]

19. **Virologic and immunologic failure, drug resistance and mortality during the first 24 months postpartum among HIV-infected women initiated on antiretroviral therapy for life in the Mitra plus Study, Dar es Salaam, Tanzania.**

Ngarina M[1,2,3], Kilewo C[4], Karlsson K[5], Aboud S[6], Karlsson A[7], Marrone G[8], Leyna G[9], Ekström AM[10,11], Biberfeld G[12,13].

BMC Infect Dis. 2015 Apr 8;15:175. doi: 10.1186/s12879-015-0914-z.

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**ABSTRACT**

BACKGROUND: In the Mitra plus study of prevention of mother-to-child transmission of HIV-1, which included 501 women in Dar es Salaam, Tanzania, triple antiretroviral therapy (ART) was given from late pregnancy throughout breastfeeding up to 6 months postnatally. Here we report findings in a sub-cohort of women with ≤200 CD4cells/μL at enrolment who were continued on ART for life and followed up during 24 months after delivery to determine virologic and immunologic responses, drug resistance and mortality.

METHODS: Blood samples for viral load and CD4 counts testing were collected at enrolment and at 3, 6, 12 and 24 months postpartum. HIV drug resistance testing was performed at 12 months. Data analysis included descriptive statistics and multivariate analysis using Generalized Estimated Equations of 73 women with at least two postpartum assessments. The mortality analysis included 84 women who had delivered.

RESULTS: The proportion of women with a viral load≥400 copies/mL was 97% (71/73) at enrolment, 16% (11/67), 22% (15/69), 61% (36/59) and 86% (48/56) at 3, 6, 12 and 24 months postpartum, respectively. The proportion of women with immunologic failure was 12% (8/69), 25% (15/60) and 41% (24/58) at 6, 12 and 24 months, respectively. At 12 months, drug resistance was demonstrated in 34% (20/59), including 12 with dual-class resistance. Self-report on drug adherence was 95% (64/68), 85% (56/66), 74% (39/53) and 65% (30/46) at 3, 6, 12 and 24 months, respectively. The mortality rate was 5.9% (95% CI 2.5-13.7%) at 24 months. The probability of virologic and immunologic failure was

significantly higher among women who reported non-perfect adherence to ART at month 24 postpartum.

CONCLUSIONS: Following an initial decline of viral load, virologic failure was common at 12 and 24 months postpartum among women initiated on ART for life during pregnancy because of low CD4 cell counts. A high proportion of viremic mothers also had resistance mutations. However, at 24 months follow-up, the mortality rate was still fairly low. Continuous adherence counseling and affordable means of monitoring of the virologic response are crucial for successful implementation of the WHO Option B+ guidelines to start all HIV-infected pregnant women on ART for life.

PMCID: PMC4392730 **Free PMC Article**
PMID: 25886277 [PubMed - indexed for MEDLINE]

20. **Frequent detection of antiretroviral drug resistance in HIV-1-infected orphaned children followed at a donor-funded rural pediatric clinic in Dodoma, Tanzania.**

Meini G[1], Balestrieri M, Cianchino S, Tacconi D, Rossi de Gasperis M, Concato C, Vicenti I, Rosi A, Saladini F, Callea F, Zazzi M.

AIDS Res Hum Retroviruses. 2015 Apr;31(4):448-51. doi: 10.1089/AID.2014.0251. Epub 2014 Dec 31.

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**ABSTRACT**

A total of 81 HIV-1 protease (PR) and reverse transcriptase (RT) sequences were obtained from 46 drug-naive and 35 pretreated individual HIV-1-infected orphaned children followed at a donor-funded rural pediatric clinic in Dodoma, Tanzania. PR and RT sequencing was performed by home-brew technology on 70 plasma samples and 11 dried blood spot specimens. Nucleoside RT inhibitor (NRTI) resistance mutations were detected in 2.2% of drug-naive and 82.9% of pretreated children. Nonnucleoside RT inhibitor (NNRTI) resistance mutations were detected in 69.6% of drug-naive and 91.4% of pretreated children. Resistance to protease inhibitors was rare (8.6% in pretreated children). Based on few complete treatment records, only around 20% of the treated children had undetectable plasma HIV-1 RNA. The rate of NRTI and NNRTI resistance in this donor-funded rural pediatric clinic was high and appeared to limit virological response to treatment.
PMID: 25492218 [PubMed - indexed for MEDLINE]

21. **Characteristics of pyogenic odontogenic infection in patients attending Mulago Hospital, Uganda: a cross-sectional study.**

Kityamuwesi R[1], Muwaz L[2], Kasangaki A[3], Kajumbula H[4], Rwenyonyi CM[5].

BMC Microbiol. 2015 Feb 25;15:46. doi: 10.1186/s12866-015-0382-z.

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**ABSTRACT**

BACKGROUND: Predisposing factors of pyogenic odontogenic infection include dental caries, pericoronitis, periodontitis, trauma to the dentition and the supporting structures or complications of dental procedures. The infections are usually polymicrobial involving normal endogenous flora. We characterised pyogenic odontogenic infection in patients attending Mulago Hospital, Uganda.

RESULTS: Of the 130 patients, 62 (47.7%) were female. The most frequently involved fascial spaces were: the buccal, 52 (25.4%); submasseteric, 46 (22.4%) and the submandibular space, 36 (17.5%). Dental caries was the most prevalent predisposing factor, particularly of the lower third molar teeth. Viridans Streptococci Group and Staphylococcus aureus were the most frequent bacterial isolates: 23.5% and 19.4%, respectively. All Viridans Streptococci isolates were resistant to penicillin G, sulfamethoxazole/trimethoprim (cotrimoxazole), ampicillin and tetracycline, but susceptible to vancomycin. All Staphylococcus aureus strains were resistant to cotrimoxazole and ampicillin while retaining susceptibility to vancomycin, cefotaxime, linezolid, moxifloxacin and amoxicillin/clavulanate. Thirty five (26.9%) patients were HIV infected and the HIV status did not significantly influence the pattern of odontogenic infection.

CONCLUSIONS: Dental caries was the most prevalent predisposing factor for pyogenic odontogenic infection. High prevalence of bacterial resistance to ampicillin and cotrimoxazole suggests the need for regular antibiotic susceptibility tests of isolates and rational use of antibiotics in the management of these infections. Prevention requires strengthening of oral health in the community.

PMCID: PMC4344792 **Free PMC Article**
PMID: 25881243 [PubMed - indexed for MEDLINE]

22. **Update on HIV-1 acquired and transmitted drug resistance in Africa.**

Ssemwanga D[1], Lihana RW[2], Ugoji C[3], Abimiku A[4], Nkengasong J[5], Dakum P[3], Ndembi N[3].

AIDS Rev. 2015 Jan-Mar;17(1):3-20.

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**ABSTRACT**

The last ten years have witnessed a significant scale-up and access to antiretroviral therapy in Africa, which has improved patient quality of life and survival. One major challenge associated with increased access to antiretroviral therapy is the development of antiretroviral resistance due to inconsistent drug supply and/or poor patient adherence. We review the current state of both acquired and transmitted drug resistance in Africa over the past ten years (2001-2011) to identify drug resistance associated with the different drug regimens used on the continent and to help guide affordable strategies for drug resistance surveillance. A total of 161 references (153 articles, six reports and two conference ABSTRACTs) were reviewed. Antiretroviral resistance data was available for 40 of 53 African countries. A total of 5,541 adult patients from 99 studies in Africa were included in this analysis. The pooled prevalence of drug resistance mutations in Africa was 10.6%, and Central Africa had the highest prevalence of 54.9%. The highest prevalence of nucleoside reverse transcriptase inhibitor mutations was in the west (55.3%) and central (54.8%) areas; nonnucleoside reverse transcriptase inhibitor mutations were highest in East Africa (57.0%) and protease inhibitors mutations highest in Southern Africa (16.3%). The major nucleoside reverse transcriptase inhibitor mutation in all four African regions was M184V. Major nonnucleoside reverse transcriptase inhibitor as well as protease inhibitor mutations varied by region. The prevalence of drug resistance has remained low in several African countries although the emergence of drug resistance mutations varied across countries. Continued surveillance of antiretroviral therapy resistance remains crucial in gauging the effectiveness of country antiretroviral therapy programs and strategizing on effective and affordable strategies for successful treatment.
PMID: 25427100 [PubMed - indexed for MEDLINE]

23. **The Mycobacterium tuberculosis Uganda II family and resistance to first-line anti-tuberculosis drugs in Uganda.**

Ezati N[1], Lukoye D[2], Wampande EM[3,4], Musisi K[5], Kasule GW[6], Cobelens FG[7], Kateete DP[8], Joloba ML[9,10].

BMC Infect Dis. 2014 Dec 19;14:703. doi: 10.1186/s12879-014-0703-0.

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**ABSTRACT**

BACKGROUND: The global increase in the burden of multidrug-resistant tuberculosis (MDR-TB) underscores an urgent need for data on factors involved in generation and spread of TB drug resistance. We performed molecular analyses on a representative sample of Mycobacterium tuberculosis (MTB) isolates. Basing on findings of the molecular epidemiological study in Kampala, we hypothesized that the predominant MTB strain lineage in Uganda is negatively associated with anti-TB drug resistance and we set out to test this hypothesis.

METHODS: We extracted DNA from mycobacterial isolates collected from smear-positive TB patients in the national TB drug resistance survey and carried out IS6110-PCR. To identify MTB lineages/sub lineages RT-PCR SNP was performed using specific primers and hybridization probes and the 'melting curve' analysis was done to distinguish the Uganda II family from other MTB families. The primary outcome was the distribution of the Uganda II family and its associations with anti-TB drug resistance and HIV infection.

RESULTS: Out of the 1537 patients enrolled, MTB isolates for 1001 patients were available for SNP analysis for identification of Uganda II family, of which 973 (97%) had conclusive RT-PCR results. Of these 422 (43.4%) were of the Uganda II family, mostly distributed in the south west zone (55.0%; OR = 4.6 for comparison with other zones; 95% CI 2.83-7.57; $p < 0.001$) but occurred in each of the other seven geographic zones at varying levels. Compared to the Uganda II family, other genotypes as a group were more likely to be resistant to any anti-TB drug (OR(adj) =2.9; 95% CI 1.63-5.06; $p = 0.001$) or MDR (OR(adj) 4.9; 95% CI, 1.15-20.60; $p = 0.032$), even after adjusting for geographic zone, patient category, sex, residence and HIV status. It was commonest in the 25-34 year age group 159/330 (48.2%). No association was observed between Uganda II family and HIV infection.

CONCLUSION: The Uganda II family is a major cause of morbidity due to TB in all NTLP zones in Uganda. It is less likely to be resistant to anti-TB drugs than other MTB strain lineages.

PMCID: PMC4367914 **Free PMC Article**
PMID: 25523472 [PubMed - indexed for MEDLINE]

24. **HIV type 1 drug resistance patterns among patients failing first and second line antiretroviral therapy in Nairobi, Kenya.**

Koigi P, Ngayo MO, Khamadi S, Ngugi C, Nyamache AK[1].

BMC Res Notes. 2014 Dec 9;7:890. doi: 10.1186/1756-0500-7-890.

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**ABSTRACT**

BACKGROUND: The ever-expanding rollout of antiretroviral therapy in poor resource settings without routine virological monitoring has been accompanied with development of drug resistance that has resulted in limited treatment success.

METHODS: A cross-sectional study with one time viral load was conducted during the period between 2012 and 2013 to determine treatment failure and drug resistance mutations among adults receiving first-line (44) (3TC_d4T/AZT_NVP/EFV) and second-line (20) (3TC/AZT/LPV/r) in Nairobi, Kenya. HIV-1 pol-RT genotyping for drug resistance was performed using an in-house protocol.

RESULTS: A total of 64 patients were recruited (mean age 36.9 yrs.) during the period between 2012 and 2013 of the 44 adult patients failing first-line 24 (40.9%) had drug resistance mutations. Eight (8) patients had NRTI resistance mutations with NAMS M184V (54.2%) and K65R (8.4%) mutations being the highest followed by TAMs T215Y and K70R (12.5%). In addition, among patients failing second-line (20), six patients (30%) had NNRTI resistance; two patients on K103N and G190A mutations while V106A, Y184V, A98G, Y181C mutations per patient were also detected. However, for NRTI two patients had TAM T215Y. M184V mutation occurred in one patient.

CONCLUSIONS: The study findings showed that HIV-1 drug resistance was significantly high in the study population. The detected accumulated resistance strains show that emergence of HIV drug resistance will continue to be a big challenge and should be given more attention as the scale up of treatment in the country continues.

PMCID: PMC4295353 **Free PMC Article**
PMID: 25487529 [PubMed - indexed for MEDLINE]

25. **Increasing the use of second-line therapy is a cost-effective approach to prevent the spread of drug-resistant HIV: a mathematical modelling study.**

Nichols BE[1], Sigaloff KC[2], Kityo C[3], Hamers RL[2], Baltussen R[4], Bertagnolio S[5], Jordan MR[6], Hallett TB[7], Boucher CA[8], de Wit TF[9], van de Vijver DA[8].

J Int AIDS Soc. 2014 Dec 5;17:19164. doi: 10.7448/IAS.17.1.19164. eCollection 2014.

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**ABSTRACT**

INTRODUCTION: Earlier antiretroviral therapy (ART) initiation reduces HIV-1 incidence. This benefit may be offset by increased transmitted drug resistance (TDR), which could limit future HIV treatment options. We analyze the epidemiological impact and cost-effectiveness of strategies to reduce TDR.

METHODS: We develop a deterministic mathematical model representing Kampala, Uganda, to predict the prevalence of TDR over a 10-year period. We then compare the impact on TDR and cost-effectiveness of: (1) introduction of pre-therapy genotyping; (2) doubling use of second-line treatment to 80% (50-90%) of patients with confirmed virological failure on first-line ART; and (3) increasing viral load monitoring from yearly to twice yearly. An intervention can be considered cost-effective if it costs less than three times the gross domestic product per capita per quality adjusted life year (QALY) gained, or less than $3420 in Uganda.

RESULTS: The prevalence of TDR is predicted to rise from 6.7% (interquartile range [IQR] 6.2-7.2%) in 2014, to 6.8% (IQR 6.1-7.6%), 10.0% (IQR 8.9-11.5%) and 11.1% (IQR 9.7-13.0%) in 2024 if treatment is initiated at a CD4 <350, <500, or immediately, respectively. The absolute number of TDR cases is predicted to decrease 4.4-8.1% when treating earlier compared to treating at CD4 <350 due to the preventative effects of earlier treatment. Most cases of TDR can be averted by increasing second-line treatment (additional 7.1-10.2% reduction), followed by increased viral load monitoring (<2.7%) and pre-therapy genotyping (<1.0%). Only increasing second-line treatment is cost-effective, ranging from $1612 to $2234 (IQR $450-dominated) per QALY gained.

CONCLUSIONS: While earlier treatment initiation will result in a predicted increase in the proportion of patients infected with drug-resistant HIV, the absolute numbers of patients infected with drug-resistant HIV is predicted to decrease. Increasing use of second-line treatment to all patients with confirmed

failure on first-line therapy is a cost-effective approach to reduce TDR. Improving access to second-line ART is therefore a major priority.

PMCID: PMC4260459 **Free PMC Article**
PMID: 25491351 [PubMed - indexed for MEDLINE]

26. **HIV-1 drug resistance mutations among infants born to HIV-positive mothers in Busia, Kenya.**

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AIDS Res Hum Retroviruses. 2014 Dec;30(12):1236-8. doi: 10.1089/AID.2014.0158.

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**ABSTRACT**

To determine HIV-1 subtypes and transmitted HIV-1 drug-resistant mutations among HIV-1-positive children born to HIV-positive mothers in Busia County, blood samples were collected from 53 children aged between 6 weeks and 5 years in 2011. Their mothers were HIV-1 positive and on antiretroviral therapy at the time the children were born. The samples were analyzed for HIV-1 drug resistance and subtypes through sequencing of portions of the HIV-1 pol gene. The generated sequences were analyzed for subtype diversity using the REGA and BLAST subtyping tools. HIV-1 drug resistance was determined using the Stanford University HIV database. Of the 53 samples that were successfully amplified and sequenced, 69.8% (37/53) were determined to be HIV-1 subtype A, 22.6% (12/53) were subtype D, 5.6% (3/53) were subtype C, and 1.8% (1/53) were subtype A1C. The prevalence of HIV-1 drug resistance mutations of any kind was 22.6% (12/53).
PMID: 25171915 [PubMed - indexed for MEDLINE]

27. **HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in western Kenya.**

Kantor R[1], DeLong A[2], Balamane M[3], Schreier L[4], Lloyd RM Jr[5], Injera W[6], Kamle L[6], Mambo F[6], Muyonga S[6], Katzenstein D[7], Hogan J[2], Buziba N[8], Diero L[6].

J Int AIDS Soc. 2014 Nov 18;17:19262. doi: 10.7448/IAS.17.1.19262. eCollection 2014.

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**ABSTRACT**

INTRODUCTION: Antiretroviral resistance leads to treatment failure and resistance transmission. Resistance data in western Kenya are limited. Collection of non-plasma analytes may provide additional resistance information.

METHODS: We assessed HIV diversity using the REGA tool, transmitted resistance by the WHO mutation list and acquired resistance upon first-line failure by the IAS-USA mutation list, at the Academic Model Providing Access to Healthcare (AMPATH), a major treatment programme in western Kenya. Plasma and four non-plasma analytes, dried blood-spots (DBS), dried plasma-spots (DPS), ViveST(TM)-plasma (STP) and ViveST-blood (STB), were compared to identify diversity and evaluate sequence concordance.

RESULTS: Among 122 patients, 62 were treatment-naïve and 60 treatment-experienced; 61% were female, median age 35 years, median CD4 182 cells/μL, median viral-load 4.6 log10 copies/mL. One hundred and ninety-six sequences were available for 107/122 (88%) patients, 58/62 (94%) treatment-naïve and 49/60 (82%) treated; 100/122 (82%) plasma, 37/78 (47%) attempted DBS, 16/45 (36%) attempted DPS, 14/44 (32%) attempted STP from fresh plasma and 23/34 (68%) from frozen plasma, and 5/42 (12%) attempted STB. Plasma and DBS genotyping success increased at higher VL and shorter shipment-to-genotyping time. Main subtypes were A (62%), D (15%) and C (6%). Transmitted resistance was found in 1.8% of plasma sequences, and 7% combining analytes. Plasma resistance mutations were identified in 91% of treated patients, 76% NRTI, 91% NNRTI; 76% dual-class; 60% with intermediate-high predicted resistance to future treatment options; with novel mutation co-occurrence patterns. Nearly 88% of plasma mutations were identified in DBS, 89% in DPS and 94% in STP. Of 23 discordant mutations, 92% in plasma and 60% in non-plasma analytes were mixtures. Mean whole-sequence discordance from frozen plasma reference was 1.1% for plasma-DBS, 1.2% plasma-DPS, 2.0% plasma-STP and 2.3% plasma-STB. Of 23 plasma-STP discordances, one mutation was identified in plasma and 22 in STP ($p<0.05$). Discordance was inversely significantly related to VL for DBS.

CONCLUSIONS: In a large treatment programme in western Kenya, we report high HIV-1 subtype diversity; low plasma transmitted resistance, increasing when multiple analytes were combined; and high-acquired resistance with unique mutation patterns. Resistance surveillance may be augmented by using non-plasma analytes for lower-cost genotyping in resource-limited settings.

PMCID: PMC4238965 **Free PMC Article**
PMID: 25413893 [PubMed - indexed for MEDLINE]

28. **Detection and management of drug-resistant tuberculosis in HIV-infected patients in lower-income countries.**

Ballif M, Nhandu V, Wood R, Dusingize JC, Carter EJ, Cortes CP, McGowan CC, Diero L, Graber C, Renner L, Hawerlander D, Kiertiburanakul S, Du QT, Sterling TR, Egger M, Fenner L; International epidemiological Databases to Evaluate AIDS (IeDEA).

Int J Tuberc Lung Dis. 2014 Nov;18(11):1327-36. doi: 10.5588/ijtld.14.0106.

Collaborators: (383)
Ajayi S, Anastos K, Ballif M, Bashi J, Bishai W, Boulle A, Braitstein P, Carriquiry G, Carter JE, Cegielski P, Chimbetete C, Conrad J, Cortes CP, Davies MA, Diero L, Duda S, Durier N, Dusingize JC, Egger M, Eboua TF, Fenner L, Gasser A, Geng E, Hardwicke L, Hoffmann C, Huebner R, Kancheya N, Kiertiburanakul S, Kim P, Lameck D, Leroy V, Lewden C, Lindegren ML, Mandalakas A, Maskew M, McKaig R, Mofenson L, Mpoudi- Etame M, Okwara B, Phiri S, Prasitsuebsai W, Petit A, Prozesky H, Reid SE, Renner L, Reubenson G, Sohn A, Sterling TR, Vo Q, Walker D, Wehbe F, Wejse C, Wester W, Williams C, Wood R, Wools-Kaloustian K, Yao Z, Yunihastuti E, Zhang FJ, Zhao HX, Han N, Merati TP, Wirawan DN, Yuliana F, Ditangco R, Uy E, Bantique R, Phanuphak P, Ruxrungtham K, Avihingsanon A, Mengthaisong T, Kiertiburanakul S, Sungkanuparph S, Sanmeema N, Chaiwarith R, Sirisanthana T, Kotarathititum W, Pham TT, Cuong DD, Ha HL, Nguyen VK, Bui VH, Cao TT, Sohn AH, Durier N, Petersen B, Cooper DA, Law MG, Jiamsakul A, Boettiger DC, Wati DK, Atmikasari LP, Malino IY, Nallusamy R, Chan KC, Lumbiganon P, Kosalaraksa P, Tharnprisan P, Udomphanit T, Chokephaibulkit K, Lapphra K, Phongsamart W, Wittawatmongkol O, Dung KT, Lam NV, An PN, Loan NT, Truong HK, Du TQ, Chau NH, Do CV, Ha MT, Sohn AH, Durier N, Nipathakosol P, Cooper DA, Law MG, Kariminia A, Dusingize JC, Mutimura E, Gitembagara A, Anastos K, Tatwangire J, Izabelle I, Niyongabo T, Twizere C, Baramperanye E, Edmonds A, Yotebieng M, Azinyue I, Ayangma L, Yiannoutsos C, Ayaya S, Bukusi EA, Lewis-Kulzer J, Somi G, Lyamuya R, Ngonyani K, Ssali J, Ssemuwemba H, Mwebesa BB, Kambugu A, Hermans S, Nalugoda F, Jjingo K, Chimbetete C, Dickinsonn D, Eley B, Fritz C, Garone D, Giddy J, Hoffmann C, MacPhail P, Moultrie H, Ndirangu J, Pestilli S, Phiri S, Prozesky H, Rabie H, Stringer J, Technau K, Wood R, Egger M, Graber C, Kaeser F, Keiser O, Boulle A, Cornell M, Davies MA, Maxwell N, Zannou DM, Ahouada C, Akakpo J, Ahomadegbé C, Bashi J, Gougounon-Houéto A, Azon-Kouanou A, Houngbé F, Sehonou J, Koumakäı S, Alihonou F, d'Almeida M, Hodonou I, Hounhoui G, Sagbo G, Tossa-Bagnan L, Adjide H, Drabo J, Bognounou R, Dienderé A, Traore E, Zoungrana L, Zerbo B, Sawadogo AB, Zoungrana J, Héma A, Soré I, Bado G, Tapsoba A, Yé D, Kouéta F, Ouedraogo S, Ouédraogo R, Hiembo W, Gansonré M, Messou E, Gnokoro JC, Koné M, Kouakou GM, Bosse CA, Brou K, Assi AI, Chenal H, Hawerlander D, Soppi F, Minga A, Yoboue JM, Eholié SP, Amego MD, Andavi V, Diallo Z, Ello F, Tanon AK, Koule SO, Anzan KC, Guehi C, Aka EA, Issouf KL, Kouakou JC, N'Gbeche MS, Pety T, Avit- Edi D, Kouakou K, Moh M, Yao VA, Folquet MA, Dainguy ME, Kouakou C, Méa- Assande VT, Oka-Berete G, Zobo N, Acquah P, Kokora MB, Eboua TF, Timité -Konan M, Diecket Ahoussou L, Assouan JK, Sami MF, Kouadio S, Renner L, Goka B, Welbeck J, Sackey A, Owiafe SN, Wejse C, Da Silva ZJ, Paulo J, Rodrigues A, da Silva D, Medina C, Oliviera-Souto I, Østergaard L, Laursen A, Sodemann M, Aaby P, Fomsgaard A, Erikstrup C, Eugen-Olsen J, Mäýga MY, Diakité FF, Kalle A, Katile D, Traore HA, Minta D, Cissé T, Dembeél M, Doumbia M, Fomba M, Kaya AS, Traoré AM, Traoré H, Toure A, Dicko F, Sylla M, Berthé A, Traoré HC, Köýta A, Koné N, N'Diaye C, Coulibaly ST, Traoré M, Traoré N, Charurat M, Ajayi S, Alim G, Dapiap S, Igbinoba F, Benson O, Adebamowo C, James J, Osakede P, Olasode J, Seydi M, Sow PS, Diop B, Manga NM, Tine JM, Bassabi CC, Sy HS, Ba A, Diagne A, Dior H, Faye M, Gueye RD, Mbaye AD, Albert CH, Patassi A, Kotosso A, Kariyare BG, Gbadamassi G, Komi A, Mensah-Zukong KE, Pakpame P, Lawson-Evi AK, Atakouma Y, Takassi E, Djeha A, Ephoévi-gah A, Djibril Sel-H, Dabis F, Bissagnene E, Arrive E, Coffie P, Ekouevi D, Jaquet A, Leroy V, Lewden C, Sasco AJ, Amani D, Azani JC, Balestre E, Bessekon S, Bohossou F, Gilbert C,

Karcher S, Gonsan JM, Carrou JL, Lenaud S, Nchot C, Malateste K, Yao AR, Siloue B, Clouet G, Dosso M, Doring A, Kouakou A, Rabourdin E, Rivenc J, Anglaret X, Ba B, Essanin JB, Ciaranello A, Datte S, Desmonde S, Elvis JS, Gottlieb GS, GHoro A, Kangah SN, Malvy D, Meless D, Mounkaila- Harouna A, Ndondoki C, Shiboski C, Tchounga B, Thiébaut R, Wandeler G, McGowan C, Cahn P, Gotuzzo E, Grinsztejn B, Pape J, Padgett D, Madero JS.

**ABSTRACT**

SETTING: Drug resistance threatens tuberculosis (TB) control, particularly among human immunodeficiency virus (HIV) infected persons.

OBJECTIVE: To describe practices in the prevention and management of drug-resistant TB under antiretroviral therapy (ART) programs in lower-income countries.

DESIGN: We used online questionnaires to collect program-level data on 47 ART programs in Southern Africa (n = 14), East Africa (n = 8), West Africa (n = 7), Central Africa (n = 5), Latin America (n = 7) and the Asia-Pacific (n = 6 programs) in 2012. Patient-level data were collected on 1002 adult TB patients seen at 40 of the participating ART programs.

RESULTS: Phenotypic drug susceptibility testing (DST) was available in 36 (77%) ART programs, but was only used for 22% of all TB patients. Molecular DST was available in 33 (70%) programs and was used in 23% of all TB patients. Twenty ART programs (43%) provided directly observed therapy (DOT) during the entire course of treatment, 16 (34%) during the intensive phase only, and 11 (23%) did not follow DOT. Fourteen (30%) ART programs reported no access to second-line anti-tuberculosis regimens; 18 (38%) reported TB drug shortages.

CONCLUSIONS: Capacity to diagnose and treat drug-resistant TB was limited across ART programs in lower-income countries. DOT was not always implemented and drug supplies were regularly interrupted, which may contribute to the global emergence of drug resistance.

PMCID: PMC4323497 **Free PMC Article**
PMID: 25299866 [PubMed - indexed for MEDLINE]

29. **Oligonucleotide ligation assay detects HIV drug resistance associated with virologic failure among antiretroviral-naive adults in Kenya.**

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J Acquir Immune Defic Syndr. 2014 Nov 1;67(3):246-53. doi: 10.1097/QAI.0000000000000312.

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**ABSTRACT**

BACKGROUND: Transmitted drug resistance (TDR) is increasing in some areas of Africa. Detection of TDR may predict virologic failure of first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy (ART). We evaluated the utility of a relatively inexpensive oligonucleotide ligation assay (OLA) to detect clinically relevant TDR at the time of ART initiation.

METHODS: Pre-ART plasmas from ART-naive Kenyans initiating an NNRTI-based fixed-dose combination ART in a randomized adherence trial conducted in 2006 were retrospectively analyzed by OLA for mutations conferring resistance to NNRTI (K103N, Y181C, and G190A) and lamivudine (M184V). Post-ART plasmas were analyzed for virologic failure (≥1000 copies/mL) at 6-month intervals over 18-month follow-up. Pre-ART plasmas of those with virologic failure were evaluated for drug resistance by consensus and 454-pyrosequencing.

RESULTS: Among 386 participants, TDR was detected by OLA in 3.89% (95% confidence interval: 2.19 to 6.33) and was associated with a 10-fold higher rate of virologic failure (hazard ratio: 10.39; 95% confidence interval: 3.23 to 32.41; P < 0.001) compared with those without TDR. OLA detected 24 TDR mutations (K103N: n = 13; Y181C: n = 5; G190A: n = 3; M184V: n = 3) in 15 subjects (NNRTI: n = 15; 3TC: n = 3). Among 51 participants who developed virologic failure, consensus sequencing did not detect additional TDR mutations conferring high-level resistance, and pyrosequencing only detected additional mutations at frequencies <2%. Mutant frequencies <2% at ART initiation were significantly less likely to be found at the time of virologic failure compared with frequencies ≥2% (22% vs. 63%; P < 0.001).

CONCLUSIONS: Detection of TDR by a point mutation assay may prevent the use of suboptimal ART.

PMCID: PMC4197120 **Free PMC Article**
PMID: 25140907 [PubMed - indexed for MEDLINE]

30. **Prevalence and virologic consequences of transmitted HIV-1 drug resistance in Uganda.**

Lee GQ[1], Bangsberg DR, Muzoora C, Boum Y, Oyugi JH, Emenyonu N, Bennett J, Hunt PW, Knapp D, Brumme CJ, Harrigan PR, Martin JN.

AIDS Res Hum Retroviruses. 2014 Sep;30(9):896-906. doi: 10.1089/AID.2014.0043. Epub 2014 Jul 29.

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**ABSTRACT**

Few reports have examined the impact of HIV-1 transmitted drug resistance (TDR) in resource-limited settings where there are fewer regimen choices and limited pretherapy/posttherapy resistance testing. In this study, we examined TDR prevalence in Kampala and Mbarara, Uganda and assessed its virologic consequences after antiretroviral therapy initiation. We sequenced the HIV-1 protease/reverse transcriptase from n=81 and n=491 treatment-naive participants of the Uganda AIDS Rural Treatment Outcomes (UARTO) pilot study in Kampala (AMU 2002-2004) and main cohort in Mbarara (MBA 2005-2010). TDR-associated mutations were defined by the WHO 2009 surveillance mutation list. Posttreatment viral load data were available for both populations. Overall TDR prevalence was 7% (Kampala) and 3% (Mbarara) with no significant time trend. There was a slight but statistically nonsignificant trend indicating that the presence of TDR was associated with a worse treatment outcome. Virologic suppression (≤400 copies/ml within 6 months posttherapy initiation) was achieved in 87% and 96% of participants with wildtype viruses versus 67% and 83% of participants with TDR (AMU, MBA p=0.2 and 0.1); time to suppression (log-rank p=0.3 and p=0.05). Overall, 85% and 96% of study participants achieved suppression regardless of TDR status. Surprisingly, among the TDR cases, approximately half still achieved suppression; the presence of pretherapy K103N while on nevirapine and fewer active drugs in the first regimen were most often observed with failures. The majority of patients benefited from the local HIV care system even without resistance monitoring. Overall, TDR prevalence was relatively low and its presence did not always imply treatment failure.

PMCID: PMC4151058 **Free PMC Article**
PMID: 24960249 [PubMed - indexed for MEDLINE]

31. **Drug susceptibility and resistance mutations after first-line failure in resource limited settings.**

Wallis CL[1], Aga E[2], Ribaudo H[2], Saravanan S[3], Norton M[4], Stevens W[5], Kumarasamy N[3], Bartlett J[6], Katzenstein D[7]; A5230 team.

Clin Infect Dis. 2014 Sep 1;59(5):706-15. doi: 10.1093/cid/ciu314. Epub 2014 May 1.

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**ABSTRACT**

BACKGROUND: The development of drug resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) has been associated with baseline human immunodeficiency virus (HIV)-1 RNA level (VL), CD4 cell counts (CD4), subtype, and treatment

failure duration. This study describes drug resistance and levels of susceptibility after first-line virologic failure in individuals from Thailand, South Africa, India, Malawi, Tanzania.

METHODS: CD4 and VL were captured at AIDs Clinical Trial Group (ACTG) A5230 study entry, a study of lopinavir/ritonavir (LPV/r) monotherapy after first-line virologic failure on an NNRTI regimen. HIV drug-resistance mutation associations with subtype, site, study entry VL, and CD4 were evaluated using Fisher exact and Kruskall-Wallis tests.

RESULTS: Of the 207 individuals who were screened for A5230, sequence data were available for 148 individuals. Subtypes observed: subtype C (n = 97, 66%) AE (n = 27, 18%), A1 (n = 12, 8%), and D (n = 10, 7%). Of the 148 individuals, 93% (n = 138) and 96% (n = 142) had at least 1 reverse transcriptase (RT) mutation associated with NRTI and NNRTI resistance, respectively. The number of NRTI mutations was significantly associated with a higher study screening VL and lower study screening CD4 (P <.001). Differences in drug-resistance patterns in both NRTI and NNRTI were observed by site.

CONCLUSIONS: The degree of NNRTI and NRTI resistance after first-line virologic failure was associated with higher VL at study entry. Thirty-two percent of individuals remained fully susceptible to etravirine and rilpivirine, protease inhibitor resistance was rare. Some level of susceptibility to NRTI remained; however, VL monitoring and earlier virologic failure detection may result in lower NRTI resistance.

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PMCID: PMC4148601 **Free PMC Article**
PMID: 24795328 [PubMed - indexed for MEDLINE]

32. **Short communication: east meets west: a description of HIV-1 drug resistance mutation patterns of patients failing first line therapy in PEPFAR clinics from Uganda and Nigeria.**

Crawford KW[1], Wakabi S, Kibuuka H, Magala F, Keshinro B, Okoye I, Akintunde E, Hamm TE.

AIDS Res Hum Retroviruses. 2014 Aug;30(8):796-9. doi: 10.1089/AID.2013.0294. Epub 2014 Jun 6.

[1]Global Health Programs, U.S. Military HIV Research Program (MHRP)/Walter Reed Army Institute of Research (WRAIR), Bethesda, Maryland.

**ABSTRACT**

HIV-1 viral load (VL) monitoring is recommended but seldom performed in resource-constrained countries. An evaluation of patients receiving first-line antiretroviral therapy in a multicountry PEPFAR program (RV288) was performed to determine the rates and predictors of virologic suppression. Resistance data from treatment failures are available from Uganda and Nigeria. Each country enrolled 325 subjects into this cross-sectional study. Subjects on first-line therapy were randomly selected for HIV RNA testing (viral load). Regimens included efavirenz or nevirapine with zidovudine/lamivudine or tenofovir/lamivudine. VL was determined from plasma using the Roche COBAS TaqMan HIV-1 Test, High Pure System v1.0 (47 copies/ml). Genotypic resistance testing was performed on samples with VL>1,000 copies/ml. From Uganda, 85% of subjects were undetectable while 7% (23/325) had VL>1,000 copies/ml. The HIV-1 subtype distribution was as follows: A=47.6%, C=14.3%, and D=38.1%. No

resistance mutations were found in 14% of subjects. All subjects with resistance had the M184V mutation. Of subjects failing a zidovudine regimen less than 1 year, 88% (7/8) had no thymidine analogue mutations (TAMs), compared to 50% (4/8) failing greater than 1 year. Four subjects (25%) had more than two mutations from the TAM-1 pathway (41L, 210W, 215Y). In Nigeria, 82% were undetectable while 14% (45/325) had VL>1,000 copies/ml. HIV-1 subtype distribution was as follows: 62.8%=CRF02_AG, 34%=pure G, and 2.8%=A. Of the 35 genotyped subjects, 14% (5/35) had no resistance mutations. Of the remainder, 10% (3/30) had no nucleoside analogue mutations while 33% (10/30) had only M184V along with nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations. Forty percent (10/25) of subjects on zidovudine failed without TAMs. Another 25% (5/25) of subjects failing on zidovudine had more than two TAM-1 mutations. Individuals failing first-line antiretroviral therapy (ART) may retain sensitivity to one or more nucleoside analogues from the regimen. Knowledge of drug resistance patterns allow for selection of drugs that can be recycled in future regimens. Accumulation of resistance mutations may compromise future treatment options.
PMID: 24798614 [PubMed - indexed for MEDLINE]

33. **Field study of dried blood spot specimens for HIV-1 drug resistance genotyping.**

Parry CM[1], Parkin N[2], Diallo K[3], Mwebaza S[4], Batamwita R[4], DeVos J[3], Bbosa N[5], Lyagoba F[6], Magambo B[6], Jordan MR[7], Downing R[5], Zhang G[3], Kaleebu P[6], Yang C[8], Bertagnolio S[9].

J Clin Microbiol. 2014 Aug;52(8):2868-75. doi: 10.1128/JCM.00544-14. Epub 2014 May 28.

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**ABSTRACT**

Dried blood spots (DBS) are an alternative specimen type for HIV drug resistance genotyping in resource-limited settings. Data relating to the impact of DBS storage and shipment conditions on genotyping efficiency under field conditions are limited. We compared the genotyping efficiencies and resistance profiles of DBS stored and shipped at different temperatures to those of plasma specimens collected in parallel from patients receiving antiretroviral therapy in Uganda. Plasma and four DBS cards from anti-coagulated venous blood and a fifth card from finger-prick blood were prepared from 103 HIV patients with a median viral load (VL) of 57,062 copies/ml (range, 1,081 to 2,964,191). DBS were stored at ambient temperature for 2 or 4 weeks or frozen at -80 °C and shipped from Uganda to the United States at ambient temperature or frozen on dry ice for genotyping using a broadly sensitive in-house method. Plasma (97.1%) and DBS (98.1%) stored and shipped frozen had similar genotyping

efficiencies. DBS stored frozen (97.1%) or at ambient temperature for 2 weeks (93.2%) and shipped at ambient temperature also had similar genotyping efficiencies. Genotyping efficiency was reduced for DBS stored at ambient temperature for 4 weeks (89.3%, P = 0.03) or prepared from finger-prick blood and stored at ambient temperature for 2 weeks (77.7%, P < 0.001) compared to DBS prepared from venous blood and handled similarly. Resistance profiles were similar between plasma and DBS specimens. This report delineates the optimal DBS collection, storage, and shipping conditions and opens a new avenue for cost-saving ambient-temperature DBS specimen shipments for HIV drug resistance (HIVDR) surveillances in resource-limited settings.

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PMCID: PMC4136191 **Free PMC Article**
PMID: 24871219 [PubMed - indexed for MEDLINE]

34. **Clinical and virological response to antiretroviral drugs among HIV patients on first-line treatment in Dar-es-Salaam, Tanzania.**

Mosha F[1], Ledwaba J, Ndugulile F, Ng'ang'a Z, Nsubuga P, Morris L, Kasubi M, Swai A, Vercauteren J, Vandamme AM.

J Infect Dev Ctries. 2014 Jul 14;8(7):845-52. doi: 10.3855/jidc.3879.

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**ABSTRACT**

INTRODUCTION: In Tanzania, the follow-up on antiretroviral therapy (ART) response is based on clinical outcomes. We investigated virological response and ARV resistance mutations in relation to clinical response in ARV-treated patients.

METHODOLOGY: A cross-sectional study of a cohort of 150 patients taking first-line ART in Dar-es-Salaam was conducted. Data were collected using standardized questionnaires and patients' blood samples. HIV viral load testing and genotyping was performed on all viremic samples. Statistical analyses compared clinical responders and non-responders.

RESULTS: The median time on ART was 20 months; 71 (47%) patients were ART clinical responders. Clinical non-responders were more likely to have started ART with advanced disease with significantly lower median percentage weight gain (6% versus 20%) with respect to pre-treatment levels. Sixty-one (86%) and 64 (81%) of clinical responders and non-responders, respectively, had undetectable viral loads. Genotyping was successful in 24 (96%) virologically failing patients, among whom 83% had resistance mutations; 67% had dual nucleoside reverse transcriptase inhibitor (NRTI)/non-NRTI (NNRTI) resistance mutations. Seventeen (71%) and 19 (79%) patients had NRTI and NNRTI resistance mutations, respectively, which were related to the ART in use, with no difference between clinical responders and non-responders. The most prevalent subtypes were A and C, found in 9 (38%) and 7 (29%) patients, respectively.

CONCLUSIONS: The observed virological response was high and did not correlate with clinical response. The prevalence of ARV resistance mutations was high in viraemic patients and was related to the ARV prescribed. We recommend use of viral load monitoring during ART in Tanzania.

**Free Article**

PMID: 25022294 [PubMed - indexed for MEDLINE]

35. **HIV-1 drug mutations in children from northern Tanzania.**

Shao ER[1], Kifaro EG[2], Chilumba IB[2], Nyombi BM[3], Moyo S[4], Gaseitsiwe S[4], Musonda R[4], Johannessen A[5], Kibiki G[3], Essex M[4].

J Antimicrob Chemother. 2014 Jul;69(7):1928-32. doi: 10.1093/jac/dku087. Epub 2014 Apr 11.

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**ABSTRACT**

OBJECTIVES: In resource-limited settings, it is a challenge to get quality clinical specimens due to poor infrastructure for their collection, transportation, processing and storage. Using dried blood spots (DBS) might be an alternative to plasma for HIV-1 drug resistance testing in this setting. The objectives of this study were to determine mutations associated with antiretroviral resistance among children <18 months old born to HIV-1-infected mothers enrolled in prevention of mother-to-child transmission services in northern Tanzania.

PATIENTS AND METHODS: Kilimanjaro Christian Medical Center (KCMC) Clinical Laboratory is the zonal centre for early infant diagnosis using DBS in northern Tanzania. DBS were collected from January 2011 to December 2012. Mothers were kept on triple therapy and single-dose nevirapine before pregnancy and during labour, respectively. Infants were given single-dose nevirapine and most of them were breastfed. Genotypic resistance was determined in those with a viral load of >400 copies/mL.

RESULTS: Genotypic resistance mutations were detected in 13 of 46 children (28%). HIV-1 genotypes were A1 (n = 27), C (n = 10), A/D (n = 4), D (n = 3) and CRF10_CD (n = 2). The median age was 12 weeks (IQR 6-28). The mean log10 viral load was 3.87 copies/mL (SD 0.995). All major mutations were detected in the reverse transcriptase gene and none in the protease gene region. The most frequent mutations were Y181C (n = 8) and K103N (n = 4), conferring resistance to non-nucleoside reverse transcriptase inhibitors.

CONCLUSIONS: One-third of infants newly diagnosed with HIV in northern Tanzania harboured major drug resistance mutations to currently used antiretroviral regimens. These mutations were detected from DBS collected from the field and stored at room temperature. Surveillance of drug resistance among this population in resource-limited settings is warranted.

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PMID: 24729604 [PubMed - indexed for MEDLINE]

36. **Phenotypic and genotypic analyses to guide selection of reverse transcriptase inhibitors in second-line HIV therapy following extended virological failure in Uganda.**

Goodall RL, Dunn DT, Pattery T, van Cauwenberge A, Nkurunziza P, Awio P, Ndembi N, Munderi P, Kityo C, Gilks CF, Kaleebu P, Pillay D; DART Virology Group and Trial Teams.

J Antimicrob Chemother. 2014 Jul;69(7):1938-44. doi: 10.1093/jac/dku052. Epub 2014 Mar 14.

Collaborators: (134)
Kaleebu P, Pillay D, Awio P, Chirara M, Dunn D, Gibb DM, Gilks C, Goodall R, Kapaata A, Katuramur M, Lyagoba F, Magala R, Magambo B, Mataruka K, McCormick A, Mugarura L, Musunga T, Nabankkema M, Nkalubo J, Nkurunziza P, Parry C, Robertson V, Spyer M, Yirrell D, Grosskurth H, Munderi P, Kabuye G, Nsibambi D, Kasirye R, Zalwango E, Nakazibwe M, Kikaire B, Nassuna G, Massa R, Fadhiru K, Namyalo M, Zalwango A, Generous L, Khauka P, Rutikarayo N, Nakahima W, Mugisha A, Todd J, Levin J, Muyingo S, Ruberantwari A, Kaleebu P, Yirrell D, Ndembi N, Lyagoba F, Hughes P, Aber M, Medina Lara A, Foster S, Amurwon J, Nyanzi Wakholi B, Mugyenyi P, Kityo C, Ssali F, Tumukunde D, Otim T, Kabanda J, Musana H, Akao J, Kyomugisha H, Byamukama A, Sabiiti J, Komugyena J, Wavamunno P, Mukiibi S, Drasiku A, Byaruhanga R, Labeja O, Katundu P, Tugume S, Awio P, Namazzi A, Bakeinyaga GT, Katabira H, Abaine D, Tukamushaba J, Anywar W, Ojiambo W, Angweng E, Murungi S, Haguma W, Atwiine S, Kigozi J, Latif A, Hakim J, Robertson V, Reid A, Chidziva E, Bulaya-Tembo R, Musoro G, Taziwa F, Chimbetete C, Chakonza L, Mawora A, Muvirimi C, Tinago G, Svovanapasis P, Simango M, Chirema O, Machingura J, Mutsai S, Phiri M, Bafana T, Chirara M, Muchabaiwa L, Muzambi M, Katabira E, Ronald A, Kambungu A, Lutwama F, Nanfuka A, Walusimbi J, Nabankema E, Nalumenya R, Namuli T, Kulume R, Namata I, Nyachwo L, Florence A, Kusiima A, Lubwama E, Nairuba R, Oketta F, Buluma E, Waita R, Ojiambo H, Sadik F, Wanyama J, Nabongo P.

**ABSTRACT**

OBJECTIVES: We investigated phenotypic and genotypic resistance after 2 years of first-line therapy with two HIV treatment regimens in the absence of virological monitoring.

METHODS: NORA [Nevirapine OR Abacavir study, a sub-study of the Development of AntiRetroviral Therapy in Africa (DART) trial] randomized 600 symptomatic HIV-infected Ugandan adults (CD4 cell count <200 cells/mm(3)) to receive zidovudine/lamivudine plus abacavir (cABC arm) or nevirapine (cNVP arm). All virological tests were performed retrospectively, including resistance tests on week 96

plasma samples with HIV RNA levels ≥1000 copies/mL. Phenotypic resistance was expressed as fold-change in IC(50) (FC) relative to wild-type virus.

RESULTS: HIV-1 RNA viral load ≥1000 copies/mL at week 96 was seen in 58/204 (28.4%) cABC participants and 21/159 (13.2%) cNVP participants. Resistance results were available in 35 cABC and 17 cNVP participants; 31 (89%) cABC and 16 (94%) cNVP isolates had a week 96 FC below the biological cut-off for tenofovir (2.2). In the cNVP arm, 16/17 participants had resistance mutations synonymous with high-level resistance to nevirapine and efavirenz; FC values for etravirine were above the biological cut-off in 9 (53%) isolates. In multivariate regression models, K65R, Y115F and the presence of thymidine analogue-associated mutations were associated with increased susceptibility to etravirine in the cABC arm.

CONCLUSIONS: Our data support the use of tenofovir following failure of a first-line zidovudine-containing regimen and shed further light on non-nucleoside reverse transcriptase inhibitor hypersusceptibility.

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PMCID: PMC4054985 **Free PMC Article**
PMID: 24633208 [PubMed - indexed for MEDLINE]

37. **Validation of an oligonucleotide ligation assay for quantification of human immunodeficiency virus type 1 drug-resistant mutants by use of massively parallel sequencing.**

Beck IA[1], Deng W[2], Payant R[1], Hall R[2], Bumgarner RE[2], Mullins JI[3], Frenkel LM[4].

J Clin Microbiol. 2014 Jul;52(7):2320-7. doi: 10.1128/JCM.00306-14. Epub 2014 Apr 16.

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**ABSTRACT**

Global HIV treatment programs need sensitive and affordable tests to monitor HIV drug resistance. We compared mutant detection by the oligonucleotide ligation assay (OLA), an economical and simple test, to massively parallel sequencing. Nonnucleoside reverse transcriptase inhibitor (K103N, V106M, Y181C, and G190A) and lamivudine (M184V) resistance mutations were quantified in blood-derived plasma RNA and cell DNA specimens by OLA and 454 pyrosequencing. A median of 1,000 HIV DNA or RNA

templates (range, 163 to 1,874 templates) from blood specimens collected in Mozambique (n = 60) and Kenya (n = 51) were analyzed at 4 codons in each sample (n = 441 codons assessed). Mutations were detected at 75 (17%) codons by OLA sensitive to 2.0%, at 71 codons (16%; P = 0.78) by pyrosequencing using a cutoff value of ≥ 2.0%, and at 125 codons (28%; P < 0.0001) by pyrosequencing sensitive to 0.1%. Discrepancies between the assays included 15 codons with mutant concentrations of ∼2%, one at 8.8% by pyrosequencing and not detected by OLA, and one at 69% by OLA and not detected by pyrosequencing. The latter two cases were associated with genetic polymorphisms in the regions critical for ligation of the OLA probes and pyrosequencing primers, respectively. Overall, mutant concentrations quantified by the two methods correlated well across the codons tested (R(2) > 0.8). Repeat pyrosequencing of 13 specimens showed reproducible detection of 5/24 mutations at <2% and 6/6 at ≥ 2%. In conclusion, the OLA and pyrosequencing performed similarly in the quantification of nonnucleoside reverse transcriptase inhibitor and lamivudine mutations present at >2% of the viral population in clinical specimens. While pyrosequencing was more sensitive, detection of mutants below 2% was not reproducible.

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PMCID: PMC4097683 **Free PMC Article**
PMID: 24740080 [PubMed - indexed for MEDLINE]

38. **Monitoring prevention or emergence of HIV drug resistance: results of a population-based foundational survey of early warning indicators in mainland Tanzania.**

Juma JM[1], Tiberio JK, Abuya MI, Kilama BK, Somi GR, Sambu V, Banda R, Jullu BS, Ramadhani AA.

BMC Infect Dis. 2014 Apr 11;14:196. doi: 10.1186/1471-2334-14-196.

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**ABSTRACT**

BACKGROUND: In Tanzania, routine individual-level testing for HIV drug resistance (HIVDR) using laboratory genotyping and phenotyping is not feasible due to resource constraints. To monitor the prevention or emergence of HIVDR at a population level, WHO developed generic strategies to be adapted by countries, which include a set of early warning indicators (EWIs).

METHODS: To establish a baseline of EWIs, we conducted a retrospective longitudinal survey of 35 purposively sampled care and treatment clinics in 17 regions of mainland Tanzania. We extracted data relevant for four EWIs (ART prescribing practices, patients lost to follow-up 12 months after ART initiation, retention on first-line ART at 12 months, and ART clinic appointment keeping in the first 12 months) from the patient monitoring system on patients who initiated ART at each respective facility in 2010. We uploaded patient information into WHO HIVResNet excel-based tool to compute national and facility averages of the EWIs and tested for associations between various programmatic factors and EWI performance using Fisher's Exact Test.

RESULTS: All sampled facilities met the WHO EWI target (100%) for ART prescribing practices. However, the national averages for patients lost to follow-up 12 months after ART initiation, retention on first-

line ART at 12 months, and ART clinic appointment keeping in the first 12 months fell short, at 26%, 54% and 38%, respectively, compared to the WHO targets ≤ 20%, ≥ 70%, and ≥ 80%. Clinics with fewer patients lost to follow-up 12 months after ART initiation and more patients retained on first-line-ART at 12 months were more likely to have their patients spend the longest time in the facility (including wait-time and time with providers), (p = 0.011 and 0.007, respectively).

CONCLUSION: Tanzania performed very well in EWI 1a, ART prescribing practices. However, its performance in other three EWIs was far below the WHO targets. This study provides a baseline for future monitoring of EWIs in Tanzania and highlights areas for improvement in the management of ART patients in order not only to prevent emergence of HIVDR due to programmatic factors, but also to improve the quality of life for ART patients.

PMCID: PMC3999848 **Free PMC Article**
PMID: 24725750 [PubMed - indexed for MEDLINE]

39. **Acting locally: innate mucosal immunity in resistance to HIV-1 infection in Kenyan commercial sex workers.**

Yao XD[1], Omange RW[2], Henrick BM[1], Lester RT[3], Kimani J[4], Ball TB[2], Plummer FA[2], Rosenthal KL[1].

Mucosal Immunol. 2014 Mar;7(2):268-79. doi: 10.1038/mi.2013.44. Epub 2013 Jun 26.

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**ABSTRACT**

Cohort studies of female commercial sex workers (CSWs) in Kenya were among the first to identify highly HIV-1-exposed seronegative (HESN) individuals. As natural resistance is usually mediated by innate immune mechanisms, we focused on determining whether expression and function of innate signaling pathways were altered locally in the genital mucosa of HESN CSWs. Our results demonstrated that selected pattern-recognition receptors (PRRs) were significantly reduced in expression in cervical mononuclear cells (CMCs) from HESN compared with the new HIV-negative (HIV-N) and HIV-positive (HIV-P) groups. Although baseline levels of secreted cytokines were reduced in CMCs of HESN, they were highly stimulated following exposure to ssRNA40 in vitro. Importantly, cervical epithelial cells from HESN also expressed reduced levels of PRRs, but Toll-like receptor 3 (TLR3) and TLR7 as well as nuclear factor-κB and activator protein 1 were highly expressed and activated. Lastly, inflammatory cytokines interleukin (IL)-1β, IL-8, and RANTES (regulated and normal T cell expressed and secreted) were detected at lower levels in cervicovaginal lavage of HESN compared with the HIV-N and HIV-P groups. Overall, our study reveals a local microenvironment of HIV resistance in the genital mucosa

consisting of a finely controlled balance of basal immune quiescence with a focused and potent innate anti-viral response critical to resistance to sexual transmission of HIV-1.

PMID: 23801306 [PubMed - indexed for MEDLINE]

40. **Occurrence of etravirine/rilpivirine-specific resistance mutations selected by efavirenz and nevirapine in Kenyan patients with non-B HIV-1 subtypes failing antiretroviral therapy.**

Crawford KW,[1,2] Njeru D,[3] Maswai J,[4] Omondi M,[4] Apollo D,[3] Kimetto J,[4] Gitonga L,[3] Munyao J,[4] Langat R,[4] Aoko A,[4] Tarus J,[4] Khamadi S,[5] Hamm TE.[1,2]

AIDS. 2014 Jan 28;28(3):442-5. doi: 10.1097/QAD.0000000000000140.

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[4]Kenya Medical Research Institute (KEMRI)/Walter Reed Program, South Rift Valley (SRV), US Army Medical Research Unit-Kenya (USAMRU-K), Kerich, Kenya
[5]Southern Highlands Walter Reed Program, Mbeya, Tanzania.

**ABSTRACT**

Resistance to efavirenz and nevirapine has not been associated with mutations at position 138 of reverse transcriptase. In an evaluation of virologic suppression rates in PEPFAR (President's Emergency Plan For AIDS Relief) clinics in Kenya among patients on first-line therapy (RV288), 63% (617/975) of randomly selected patients on antiretroviral therapy were suppressed (HIV RNA<400 copies/ml). Among those with non-nucleoside reverse transcriptase inhibitor resistance (n = 101), 14 (13.8%) had substitutions at 138 (A, G, K or Q), mutations selected only by etravirine and rilpivirine in subtype B viruses. All 14 patients received efavirenz or nevirapine, not etravirine or rilpivirine, and were predominantly subtype A1. This may be the first report of efavirenz and nevirapine selecting these mutations in these subtypes.

PMID: 24670527 [PubMed - indexed for MEDLINE]

41. **Averted HIV infections due to expanded antiretroviral treatment eligibility offsets risk of transmitted drug resistance: a modeling study.**

Nichols BE,[1] Sigaloff KC,[2,3] Kityo C,[4] Mandaliya K,[5] Hamers RL,[2,3] Bertagnolio S,[6] Jordan MR,[7] Boucher CA,[1] Rinke de Wit TF,[2,3] van de Vijver DA.[1]

AIDS. 2014 Jan 2;28(1):73-83. doi: 10.1097/01.aids.0000433239.01611.52.

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**ABSTRACT**

BACKGROUND: Earlier antiretroviral therapy initiation can reduce the incidence of HIV-1. This benefit can be offset by increased transmitted drug resistance (TDR). We compared the preventive benefits of reducing incident infections with the potential TDR increase in East Africa.

METHODS: A mathematical model was constructed to represent Kampala, Uganda, and Mombasa, Kenya. We predicted the effect of initiating treatment at different immunological thresholds (<350, <500 CD4 cells/μl) on infections averted and mutation-specific TDR prevalence over 10 years compared to initiating treatment at CD4 cell count below 200 cells/μl.

RESULTS: When initiating treatment at CD4 cell count below 350 cells/μl, we predict 18 [interquartile range (IQR) 11-31] and 46 (IQR 30-83) infections averted for each additional case of TDR in Kampala and Mombasa, respectively, and 22 (IQR 17-35) and 32 (IQR 21-57) infections averted when initiating at below 500. TDR is predicted to increase most strongly when initiating treatment at CD4 cell count below 500 cells/μl, from 8.3% (IQR 7.7-9.0%) and 12.3% (IQR 11.7-13.1%) in 2012 to 19.0% (IQR 16.5-21.8%) and 19.2% (IQR 17.1-21.5%) in 10 years in Kampala and Mombasa, respectively. The TDR epidemic at all immunological thresholds was comprised mainly of resistance to non-nucleoside reverse transcriptase inhibitors. When 80-100% of individuals with virological failure are timely switched to second-line therapy, TDR is predicted to decline irrespective of treatment initiation threshold.

CONCLUSION: Averted HIV infections due to the expansion of antiretroviral treatment eligibility offset the risk of transmitted drug resistance, as defined by more infections averted than TDR gained. The effectiveness of first-line non-nucleoside reverse transcriptase inhibitor-based therapy can be preserved by improving switching practices to second-line therapy.
PMID: 23921620 [PubMed - indexed for MEDLINE]

42. **Our bodies are our own: resistance to ABC-based HIV-prevention programmes in northern Tanzanian conservation organisations.**

Reid-Hresko J[1].

Cult Health Sex. 2014;16(7):765-79. doi: 10.1080/13691058.2014.911959. Epub 2014 May 12.

[1]Division of Social Science, Quest University Canada, British Columbia, Canada.

**ABSTRACT**

ABC-based HIV-prevention programmes have been widely employed in northern Tanzanian wildlife conservation settings in an attempt to (re)shape the sexual behaviours of conservation actors. Utilising findings from 66 semi-structured interviews conducted in 2009-2010, this paper examines ABC prevention as a form of Foucauldian governmentality--circulating technologies of power that mobilise disciplinary technologies and attempt to transform such efforts into technologies of the self--and explores how individuals understand and respond to attempts to govern their behaviour. ABC regimes attempt to rework subjectivity, positioning HIV-related behaviours within a risk-based neoliberal rationality. However, efforts to use ABC as a technology to govern populations and individual bodies are largely incommensurate with existing Tanzanian sociocultural formations, including economic and gendered inequalities, and local understandings of sexuality. The language research participants used to talk about ABC and the justifications they offered for non-compliance illuminate this discrepancy. Data reveal that the recipients of ABC campaigns are active producers of understandings that work for them in their lives, but may not produce the behavioural shifts envisioned by programme goals. These findings corroborate previous research, which questions the continued plausibility of ABC as a stand-alone HIV- prevention framework.
PMID: 24816078 [PubMed - indexed for MEDLINE]

43. **Long-term effectiveness of combination antiretroviral therapy and prevalence of HIV drug resistance in HIV-1-infected children and adolescents in Rwanda.**

Mutwa PR[1,2,3], Boer KR[2,3,4], Rusine J[2,3,5], Muganga N[1], Tuyishimire D[2,3,6], Schuurman R[7], Reiss P[2,3], Lange JM[2,3], Geelen SP[2,3,8].

Pediatr Infect Dis J. 2014 Jan;33(1):63-9. doi: 10.1097/INF.0b013e31829e6b9f.

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Philippe R. Mutwa MD, mutwaph@gmail.com

**ABSTRACT**

OBJECTIVE: To determine the long-term outcomes of treatment and prevalence of genotypic drug resistance in children and adolescents on combination antiretroviral therapy.

METHODS: A cross-sectional study (September 2009 to October 2010) in which clinical, immunologic and virologic outcomes were assessed at a single-study visit and through patient records in a cohort of HIV-infected children and adolescents. Risk factors for clinical and immunologic responses and virologic outcome were evaluated using logistic regression, and the accuracy of clinical and immunologic criteria in identifying virologic failure was assessed.

RESULTS: Four hundred twenty-four patients were enrolled with a median age of 10.8 years (range: 1.7-18.8) and a median duration on combination antiretroviral therapy of 3.4 years (range: 1.0-8.1). Thirty-three percent were stunted and 17% underweight. Eighty-four percent (95% confidence interval: 79-87) of children >5 years had CD4 ≥350 cells/mm and in 74% (95% confidence interval: 62-84) of younger children CD4% was ≥25. CD4 values and age at combination antiretroviral therapy initiation were independently associated with CD4 outcomes; 124 (29%) had HIV-1 RNA ≥1000 copies/mL, with no significant predictors. Sensitivity for weight-for-age and height-for-age and CD4 cells (<350/mm) remained under 50% (15-42%); CD4 cells showed the best specificity, ranging from 91% to 97%. Of 52 samples tested, ≥1 mutations were observed in 91% (nucleoside reverse transcriptase inhibitors) and 95% (non-nucleoside reverse transcriptase inhibitors); 1 to 2 thymidine analogue-associated mutations were detected in 16 (31%) and ≥3 thymidine analogue-associated mutations in 7 (13%).

CONCLUSION: Nearly 1 in 3 children showed virologic failure, and >10% of the subgroup of children with treatment failure in whom genotyping was performed demonstrated multiple HIV drug resistance mutations. Neither clinical condition nor CD4 cells were good indicators for treatment failure.
PMID: 24352189 [PubMed - indexed for MEDLINE]

44. **HIV-1 drug resistance-associated mutations among HIV-1 infected drug-naïve antenatal clinic attendees in rural Kenya.**

Kiptoo M[1], Brooks J, Lihana RW, Sandstrom P, Ng'ang'a Z, Kinyua J, Lagat N, Okoth F, Songok EM.

BMC Infect Dis. 2013 Nov 4;13:517. doi: 10.1186/1471-2334-13-517.

[1]Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya. Mkiptoo@kemri.org.

**ABSTRACT**

BACKGROUND: Access to antiretroviral therapy (ART) has increased dramatically in Sub-Saharan Africa. In Kenya, 560,000 people had access to ART by the end of 2011. This scaling up of ART has raised challenges to the Kenyan health system due to emergence of drug resistant viruses among those on treatment and possible onward transmission. To counter this, and come up with an effective treatment strategy, it has become vital to determine baseline mutations associated with drug resistance among the circulating strains of HIV-1 in Kenya.

METHODS: The prevalence of mutations associated with drug resistance in HIV-1 protease (PR) and reverse transcriptase (RT) regions from 188 HIV-1 infected treatment-naïve pregnant women was investigated in Kapsabet, Nandi Hills and Kitale district hospitals of Kenya. Blood samples were collected between April 2005 and June 2006. The HIV-1 pol gene was amplified using primers for HIV-1 PR and RT and sequenced using the BigDye chemistry. The mutations were analyzed based on the IAS algorithm as well as the Stanford University HIV Drug Resistance Database.

RESULTS: Based on the PR and RT sequences, HIV-1 subtypes A1 (n=117, 62.2%), A2 (n=2, 1.1%), D (n=27, 14.4%), C (n=13, 6.9%), G (n=3, 1.6%), and possible recombinants (n=26, 13.8%) were detected. Mutations associated with nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside RTI (NNRTI)-resistance were detected in 1.6% (3 of 188) and 1.1% (2 of 188), respectively. Mutations associated with PI resistance were detected in 0.5% (1 of 188) of the study population.

CONCLUSION: The prevalence of drug resistance among drug-naïve pregnant women in rural North Rift, Kenya in 2006 was 3.2%. Major drug resistance mutations associated with PIs, NRTIs and NNRTIs do exist among treatment-naïve pregnant women in North Rift, Kenya. There is a need for consistent follow-up of drug-naïve individuals in this region to determine the impact of mutations on treatment outcomes.

PMCID: PMC4228423 **Free PMC Article**
PMID: 24180455 [PubMed - indexed for MEDLINE]

45. **Effect of 7 days of phenytoin on the pharmacokinetics of and the development of resistance to single-dose nevirapine for perinatal HIV prevention: a randomized pilot trial.**

Fillekes Q[1], Muro EP, Chunda C, Aitken S, Kisanga ER, Kankasa C, Thomason MJ, Gibb DM, Walker AS, Burger DM.

J Antimicrob Chemother. 2013 Nov;68(11):2609-15. doi: 10.1093/jac/dkt246. Epub 2013 Jul 17.

[1]Department of Pharmacy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

**ABSTRACT**

OBJECTIVES: To confirm whether 7 days of phenytoin, an enzyme inducer, would decrease the elimination half-life of single-dose nevirapine and to investigate its effect on the development of nevirapine resistance in pregnant, HIV-infected women.

METHODS: In a pharmacokinetic pilot trial (NCT01187719), HIV-infected, antiretroviral (ARV)-naive pregnant women ≥18 years old from Zambia and Tanzania and with CD4 cell counts >350 cells/mm(3) were randomized 1 : 1 to a control (zidovudine pre-delivery, single-dose nevirapine/zidovudine/lamivudine at delivery and zidovudine/lamivudine for 7 days post-delivery) or an intervention (control plus 184 mg of phenytoin once daily for 7 days post-delivery) group. Primary endpoints were the pharmacokinetics of and resistance to nevirapine.

RESULTS: Thirty-five and 37 women were allocated to the control and intervention groups, with median (IQR) ages of 27 (23-31) and 27 (23-33) years, respectively. Twenty-three and 23 women had detectable nevirapine levels at delivery and subsequent samples in the control and the intervention

groups, respectively. Geometric mean (GM) (95% CI) plasma levels of nevirapine at delivery were 1.02 (0.58-1.78) mg/L and 1.14 (0.70-1.86) mg/L in the control and intervention groups, respectively (P = 0.76). One week after delivery, 0/23 (0%) and 15/22 (68%) control and intervention mothers, respectively, had undetectable levels of nevirapine (<0.05 mg/L; P<0.001). One week later, the figures were 10/21 (48%) and 18/19 (95%) mothers, respectively (P = 0.002). The GM (95% CI) half-life of nevirapine was 63.2 (52.8-75.7) versus 25.5 (21.6-30.1) h in the control group versus the intervention group (P < 0.001). New nevirapine mutations were found in 0/20 (0%) intervention-group mothers versus 1/21 (5%) control-group mothers. Overall, there was no difference in adverse events reported between the control and intervention arms (P > 0.28).

CONCLUSIONS: Adding 7 days of an enzyme inducer to single-dose nevirapine to prevent mother-to-child transmission of HIV significantly reduced subtherapeutic nevirapine levels by shortening the half-life of nevirapine. As prolonged subtherapeutic nevirapine dosage leads to the emergence of resistance, single-dose nevirapine could be used with phenytoin as an alternative if other ARVs were unavailable.

**Free Article**

PMID: 23864647 [PubMed - indexed for MEDLINE]

46. **Antiretroviral treatment interruptions induced by the Kenyan postelection crisis are associated with virological failure.**

Mann M[1], Diero L[2,3], Kemboi E[2,3], Mambo F[2,3], Rono M[2,3], Injera W[2,3], Delong A[4], Schreier L[5], Wools-Kaloustian K[6], Sidle J[7], Buziba N[2,3], Kantor R[5].

J Acquir Immune Defic Syndr. 2013 Oct 1;64(2):220-4. doi: 10.1097/QAI.0b013e31829ec485.

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[7]Department of Medicine, Division of General Internal Medicine, Indiana University, Indianapolis, IN.

**Erratum in:**

- J Acquir Immune Defic Syndr. 2013 Nov 1;64(3):323. Kaloustian, Kara W [corrected to Wools-Kaloustian, Kara].

**ABSTRACT**

BACKGROUND: Antiretroviral treatment interruptions (TIs) cause suboptimal clinical outcomes. Data on TIs during social disruption are limited.

METHODS: We determined effects of unplanned TIs after the 2007-2008 Kenyan postelection violence on virological failure, comparing viral load (VL) outcomes in HIV-infected adults with and without conflict-induced TI.

RESULTS: Two hundred and one patients were enrolled, median 2.2 years after conflict and 4.3 years on treatment. Eighty-eight patients experienced conflict-related TIs and 113 received continuous treatment. After adjusting for preconflict CD4, patients with TIs were more likely to have detectable VL, VL >5,000 and VL >10,000.

CONCLUSIONS: Unplanned conflict-related TIs are associated with increased likelihood of virological failure.

PMCID: PMC3920989 **Free PMC Article**
PMID: 24047971 [PubMed - indexed for MEDLINE]

47. **HIV-1 drug resistance in recently HIV-infected pregnant mother's naïve to antiretroviral therapy in Dodoma urban, Tanzania.**

Vairo F[1], Nicastri E, Liuzzi G, Chaula Z, Nguhuni B, Bevilacqua N, Forbici F, Amendola A, Fabeni L, De Nardo P, Perno CF, Cannas A, Sakhoo C, Capobianchi MR, Ippolito G; AMANI Study Group.

BMC Infect Dis. 2013 Sep 21;13:439. doi: 10.1186/1471-2334-13-439.

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**ABSTRACT**

BACKGROUND: HIV resistance affects virological response to therapy and efficacy of prophylaxis in mother-to-child-transmission. The study aims to assess the prevalence of HIV primary resistance in pregnant women naïve to antiretrovirals.

METHODS: Cross sectional baseline analysis of a cohort of HIV + pregnant women (HPW) enrolled in the study entitled Antiretroviral Management of Antenatal and Natal HIV Infection (AMANI, peace in Kiswahili language). The AMANI study began in May 2010 in Dodoma, Tanzania. In this observational cohort, antiretroviral treatment was provided to all women from the 28th week of gestation until the end of the breastfeeding period. Baseline CD4 cell count, viral load and HIV drug-resistance genotype were collected.

RESULTS: Drug-resistance analysis was performed on 97 naïve infected-mothers. The prevalence of all primary drug resistance and primary non-nucleoside reverse-transcriptase inhibitors resistance was 11.9% and 7.5%, respectively. K103S was found in two women with no M184V detection. HIV-1

subtype A was the most commonly identified, with a high prevalence of subtype A1, followed by C, D, C/D recombinant, A/C recombinant and A/D recombinant. HIV drug- resistance mutations were detected in A1 and C subtypes.

CONCLUSION: Our study reports an 11.9% prevalence rate of primary drug resistance in naïve HIV-infected pregnant women from a remote area of Tanzania. Considering that the non-nucleoside reverse-transcriptase inhibitors are part of the first-line antiretroviral regimen in Tanzania and all of Africa, resistance surveys should be prioritized in settings where antiretroviral therapy programs are scaled up.

PMCID: PMC3849050 **Free PMC Article**
PMID: 24053581 [PubMed - indexed for MEDLINE]

48. **Prevalence of drug resistance mutations and HIV type 1 subtypes in an HIV type 1-infected cohort in rural Tanzania.**

Masimba P[1], Kituma E, Klimkait T, Horvath E, Stoeckle M, Hatz C, Mossdorf E, Mwaigomole E, Khamis S, Jullu B, Abdulla S, Tanner M, Felger I.

AIDS Res Hum Retroviruses. 2013 Sep;29(9):1229-36. doi: 10.1089/AID.2011.0367.

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland.

**ABSTRACT**

The development of resistance mutations in drug-targeted HIV-1 genes compromises the success of antiretroviral therapy (ART) programs. Genotyping of these mutations enables adjusted therapeutic decisions both at the individual and population level. We investigated over time the prevalence of HIV-1 primary drug resistance mutations in treatment-naive patients and described the HIV-1 subtype distribution in a cohort in rural Tanzania at the beginning of the ART rollout in 2005-2007 and later in 2009. Viral RNA was analyzed in 387 baseline plasma samples from treatment-naive patients over a period of 5 years. The reverse transcriptase (RT) and protease genes were reversely transcribed, polymerase chain reaction (PCR) amplified, and directly sequenced to identify HIV-1 subtypes and single nucleotide polymorphisms associated with drug resistance (DR-SNPs). The prevalence of major DR-SNPs in 2005-2007 in the RT gene was determined: K103N (5.0%), Y181C (2.5%), M184V (2.5%), and G190A (1.7%), and M41L, K65KR, K70KR, and L74LV (0.8%). In samples from 2009 only K103N (3.3%), M184V, and T215FY (0.8%) were detected. Initial frequencies of subtypes C, A, D, and recombinants were 43%, 32%, 18%, and 7%, respectively. Later similar frequencies were found except for the recombinants, which were found twice as often (15%), highlighting the subtype diversity and a relatively stable subtype frequency in the area. DR-SNPs were found at initiation of the cohort despite very low previous ART use in the area. Statistically, frequencies of major mutations did not change significantly over the studied 5-year interval. These mutations could reflect primary resistances and may indicate a possible risk for treatment failure.

PMCID: PMC3749719 **Free PMC Article**
PMID: 23806135 [PubMed - indexed for MEDLINE]

49. **Low primary and secondary HIV drug-resistance after 12 months of antiretroviral therapy in human immune-deficiency virus type 1 (HIV-1)-infected individuals from Kigali, Rwanda.**

Rusine J[1], Asiimwe-Kateera B, van de Wijgert J, Boer KR, Mukantwali E, Karita E, Gasengayire A, Jurriaans S, de Jong M, Ondoa P.

PLoS One. 2013 Aug 12;8(8):e64345. doi: 10.1371/journal.pone.0064345. eCollection 2013.

[1]Amsterdam Institute for Global Health and Development (AIGHD), Department of Global Health, Academic Medical Center, Amsterdam, The Netherlands.

**ABSTRACT**

Treatment outcomes of HIV patients receiving antiretroviral therapy (ART) in Rwanda are scarcely documented. HIV viral load (VL) and HIV drug-resistance (HIVDR) outcomes at month 12 were determined in a prospective cohort study of antiretroviral-naïve HIV patients initiating first-line therapy in Kigali. Treatment response was monitored clinically and by regular CD4 counts and targeted HIV viral load (VL) to confirm drug failure. VL measurements and HIVDR genotyping were performed retrospectively on baseline and month 12 samples. One hundred and fifty-eight participants who completed their month 12 follow-up visit had VL data available at month 12. Most of them (88%) were virologically suppressed (VL≤1000 copies/mL) but 18 had virological failure (11%), which is in the range of WHO-suggested targets for HIVDR prevention. If only CD4 criteria had been used to classify treatment response, 26% of the participants would have been misclassified as treatment failure. Pre-therapy HIVDR was documented in 4 of 109 participants (3.6%) with an HIVDR genotyping results at baseline. Eight of 12 participants (66.7%) with virological failure and HIVDR genotyping results at month 12 were found to harbor mutation(s), mostly NNRTI resistance mutations, whereas 4 patients had no HIVDR mutations. Almost half (44%) of the participants initiated ART at CD4 count ≤200 cell/µl and severe CD4 depletion at baseline (<50 cells/µl) was associated with virological treatment failure ($p = 0.008$). Although the findings may not be generalizable to all HIV patients in Rwanda, our data suggest that first-line ART regimen changes are currently not warranted. However, the accumulation of acquired HIVDR mutations in some participants underscores the need to reinforce HIVDR prevention strategies, such as increasing the availability and appropriate use of VL testing to monitor ART response, ensuring high quality adherence counseling, and promoting earlier identification of HIV patients and enrollment into HIV care and treatment programs.

PMCID: PMC3741294 **Free PMC Article**
PMID: 23950859 [PubMed - indexed for MEDLINE]

50. **Multidrug- and isoniazid-resistant tuberculosis in three high HIV burden African regions.**

Sanchez-Padilla E[1], Ardizzoni E, Sauvageot D, Ahoua L, Martin A, Varaine F, Adatu-Engwau F, Akeche G, Salaniponi F, Bonnet M.

Int J Tuberc Lung Dis. 2013 Aug;17(8):1036-42. doi: 10.5588/ijtld.12.0842.

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**ABSTRACT**

SETTING: Despite major progress in the surveillance of drug-resistant tuberculosis (TB), data are lacking for many low-resource countries. World Health Organization estimates of multidrug-resistant TB (MDR-TB) rates in Africa are low, and based on very limited data from the African continent.

OBJECTIVE: To measure MDR-TB prevalence in sub-Saharan African regions with a high prevalence of human immunodeficiency virus (HIV).

METHOD: We conducted three anti-tuberculosis drug resistance surveys in sub-Saharan African regions with high HIV-TB coinfection prevalence: Homa Bay (Kenya), Chiradzulu (Malawi) and West Nile region (Uganda).

RESULTS: The prevalence of MDR-TB in new patients was found to be low in the three regions: 1.4% (95%CI 0.2-2.6) in Homa Bay, 2.0% (95%CI 0.4-3.6) in Chiradzulu and 0.6% (95%CI 0.0-1.5) in the West Nile region. We found no significant association between MDR-TB and HIV infection. Nonetheless, ≥ 10% of the new cases surveyed were resistant to isoniazid (INH).

CONCLUSION: The relatively high rate of resistance to INH highlights the need for rapid detection of INH resistance in addition to rifampicin (RMP) resistance, to allow rapid modification of treatment to avoid the acquisition of RMP resistance. Drug resistance should be monitored periodically.
PMID: 23827027 [PubMed - indexed for MEDLINE]

51. **Treatment failure and drug resistance is more frequent in HIV-1 subtype D versus subtype A-infected Ugandans over a 10-year study period.**

Kyeyune F, Nankya I, Metha S, Akao J, Ndashimye E, Tebit DM, Rodriguez B, Kityo C, Salata RA, Mugyenyi P, Arts E; JCRC Drug Resistance Working Group.

AIDS. 2013 Jul 31;27(12):1899-909.

Collaborators: (6)
Bagenda L, Nanyonjo H, Immonen T, Ssali F, Semanda M, Bukuru A.

**ABSTRACT**

OBJECTIVES: To determine the impact of HIV-1 subtype on treatment outcomes and the emergence of drug resistance in the resource limited setting of Kampala, Uganda.

DESIGN: The Joint Clinical Research Centre (JCRC) in Kampala, Uganda has provided over 2000 drug-resistant genotypes (DRGs) over the past 10 years as standard of care for patients failing therapy and 1403 from treatment-naive and experienced patients over the past 10 years have been analyzed for this study.

METHOD: Viral loads, CD4 cell count, treatment histories and other relevant clinical data was compared with the infecting HIV-1 subtype and DRGs of Ugandan patients failing treatment.

RESULTS: Patients failing HAART with DRGs (n = 937) were more frequently infected with subtype D than expected on the basis of the subtype distribution in the treatment-naive population (n = 655) in Kampala (P < 0.001). Higher proportions of treatment failures among subtype D-infected patients were driven by resistance to nucleoside reverse transcriptase inhibitors (NRTI) (P < 0.0002) more than to non-NRTIs (P > 0.04) or protease inhibitors.

CONCLUSION: Higher rates of treatment failure among subtype D as compared with subtype A-infected Ugandans was analogous to the faster disease progression in subtype D-infected patients. The mechanism(s) by which drug resistance may emerge faster in subtype D HIV-1 may relate to higher replicative fitness and increased propensity for a CXCR4 tropism.

PMCID: PMC4494684 **Free PMC Article**
PMID: 23727942 [PubMed - indexed for MEDLINE]

52. **Short communication: high rates of thymidine analogue mutations and dual-class resistance among HIV-infected Ugandan children failing first-line antiretroviral therapy.**

Sigaloff KC[1], Kayiwa J, Musiime V, Calis JC, Kaudha E, Mukuye A, Matama C, Nankya I, Nakatudde L, Dekker JT, Hamers RL, Mugyenyi P, Rinke De Wit TF, Kityo C.

AIDS Res Hum Retroviruses. 2013 Jun;29(6):925-30. doi: 10.1089/AID.2012.0218. Epub 2013 Apr 17.

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**ABSTRACT**

HIV-infected children are at high risk of acquiring drug-resistant viruses, which is of particular concern in settings where antiretroviral drug options are limited. We aimed to assess resistance patterns and predict viral drug susceptibility among children with first-line antiretroviral therapy (ART) failure in Uganda. A cross-sectional analysis of children switching ART regimens due to first-line failure was performed at three clinical sites in Uganda. HIV-RNA determination and genotypic resistance testing on all specimens with HIV-RNA >1,000 copies/ml were performed. Major drug resistance mutations were scored using the 2011 International Antiviral Society-USA list. The Stanford algorithm was used to predict drug susceptibility. At the time of switch, 44 genotypic resistance tests were available for 50 children. All children harbored virus with nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance [95% confidence interval (CI) 92-100%] and NRTI resistance was present in 98% (95% CI 88-100%). Forty-six percent (95% CI 30-61%) of children harbored ≥2 thymidine analog mutations. M184V was identified as the only NRTI mutation in 27% (95% CI 15-43%). HIV susceptibility to NRTIs, with the exception of tenofovir, was reduced in ≥60% of children. Ugandan children experiencing first-line ART failure in our study harbored high rates of dual-class and accumulated HIV drug resistance. Methods to prevent treatment failure, including adequate pediatric formulations and alternative second-line treatment options, are urgently needed.
PMID: 23517497 [PubMed - indexed for MEDLINE]

53. **Short communication: HIV type 1 transmitted drug resistance and evidence of transmission clusters among recently infected antiretroviral-naive individuals from Ugandan fishing communities of Lake Victoria.**

Nazziwa J[1], Njai HF, Ndembi N, Birungi J, Lyagoba F, Gershim A, Nakiyingi-Miiro J, Nielsen L, Mpendo J, Nanvubya A, Debont J, Grosskurth H, Kamali A, Seeley J, Kaleebu P; Chivtum Study Team.

AIDS Res Hum Retroviruses. 2013 May;29(5):788-95. doi: 10.1089/AID.2012.0123.

Collaborators: (22)
Sembatya J, Ssebunya B, Kwigenga V, Nassuna R, Kivumbi NA, Kamulegeya I, Basajja V, Makaire F, Nabaleera H, Sebayinda M, Aling E, Wambuuzi M, Ssekitoleko M, Bucyana A, Nuwamanya S, Nyende D, Wampande L, Agaba C, Kalina B, Nabaggala G, Sebyala Z, Muhumuza R.

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**ABSTRACT**

Human immunodeficiency virus type 1 (HIV-1) prevalence and incidence in the fishing communities on Lake Victoria in Uganda are high. This population may play a role in driving the HIV epidemic in Uganda including the spread of transmitted drug resistance (TDR). We report data on TDR in this population among antiretroviral (ARV)-naive, recently infected individuals about 5 years after ARV scaling-up in Uganda. We identified phylogenetic transmission clusters and combined these with volunteer life histories in order to understand the sexual networks within this population. From a prospective cohort of 1,000 HIV-negative individuals recruited from five communities, 51 seroconverters were identified over a period of 2 years. From these, whole blood was collected and population sequencing of the HIV-1 pol gene (protease/reverse transcriptase) was performed from plasma. Drug resistance mutations (DRMs) were scored using the 2009 WHO list for surveillance of TDR. TDR prevalence categories were estimated using the WHO recommended truncated sampling technique for the surveillance of TDR for use in resource-limited settings (RLS). Of the samples 92% (47/51) were successfully genotyped. HIV-1 subtype frequencies were 15/47 (32%) A1, 20/47 (43%) D, 1/47 (2%) C, 1/47 (2%) G, and 10/47 (21%) unique recombinant forms. Nonnucleoside reverse transcriptase inhibitor (NNRTI) drug resistance mutation K103N was identified in two individuals and V106A in one (6%) suggesting that the level of TDR was moderate in this population. No nucleoside/tide reverse transcriptase inhibitor (NRTI) or protease inhibitor (PI) DRMs were detected. In this study, we identified five transmission clusters supported by high bootstrap values and low genetic distances. Of these, one pair included the two individuals with K103N. Two of the genotypic clusters corresponded with reported sexual partnerships as detected through prior in-depth interviews. The level of TDR to NNRTIs in these ARV-naive individuals was moderate by WHO threshold survey categorization. The transmission clusters suggest a high degree of sexual partner mixing between members of these communities.

PMCID: PMC3636596 **Free PMC Article**
PMID: 23173702 [PubMed - indexed for MEDLINE]

54. **Impact of maternal and infant antiretroviral drug regimens on drug resistance in HIV-infected breastfeeding infants.**

Fogel JM[1], Mwatha A, Richardson P, Brown ER, Chipato T, Alexandre M, Moodley D, Elbireer A, Mirochnick M, George K, Mofenson LM, Zwerski S, Coovadia HM, Eshleman SH.

Pediatr Infect Dis J. 2013 Apr;32(4):e164-9. doi: 10.1097/INF.0b013e31827f44ee.

[1]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

**ABSTRACT**

BACKGROUND: The HIV Prevention Trials Network (HPTN) 046 trial evaluated the efficacy of extended infant nevirapine (NVP) administration for prevention of HIV transmission through breastfeeding. Infants received daily NVP up to 6 weeks of age. HIV-uninfected infants (the intent-to-treat group) received daily NVP or placebo up to 6 months of age. We analyzed emergence of NVP resistance in infants who acquired HIV infection despite prophylaxis.

METHODS: HIV genotyping was performed using the ViroSeq HIV Genotyping System. Medians and proportions were used to summarize data. Two-sided Fisher exact tests were used to evaluate associations between categorical variables.

RESULTS: NVP resistance was detected in 12 (92.3%) of 13 infants who were HIV-infected by 6 weeks and in 7 (28%) of 25 infants who were HIV-uninfected at 6 weeks and HIV-infected at 6 months of age (6/8 = 75% in the NVP arm, 1/17 = 5.9% in the placebo arm, P = 0.001). Among those 25 infants, 4 had mothers who initiated an antiretroviral treatment regimen by 6 months postpartum. In all 4 cases, the treatment regimen included a non-nucleoside reverse transcriptase inhibitor (NVP or efavirenz). NVP resistance was detected in all 4 of those infants by 6 months of age (4/4 = 100%). In contrast, only 3 (14.2%) of the remaining 21 HIV-infected infants whose mothers did not initiate antiretroviral treatment developed NVP resistance (P = 0.003).

CONCLUSIONS: Extended NVP prophylaxis significantly increased the risk of NVP resistance in infants who acquired HIV infection after 6 weeks of age. Treatment of maternal HIV infection was also associated with emergence of NVP resistance in HIV-infected, breastfed infants.

PMCID: PMC3826537 **Free PMC Article**
PMID: 23249916 [PubMed - indexed for MEDLINE]

55. **Antiretroviral drug resistance profiles and response to second-line therapy among HIV type 1-infected Ugandan children.**

Musiime V[1], Kaudha E, Kayiwa J, Mirembe G, Odera M, Kizito H, Nankya I, Ssali F, Kityo C, Colebunders R, Mugyenyi P.

AIDS Res Hum Retroviruses. 2013 Mar;29(3):449-55. doi: 10.1089/aid.2012.0283. Epub 2013 Jan 11.

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**ABSTRACT**

We sought to determine the pattern of resistance-associated mutations (RAMs) among HIV-1-infected children failing first-line antiretroviral therapy (ART) and ascertain their response to second-line regimens in 48 weeks of follow-up. The design involved a cohort study within an HIV care program. We studied records of 142 children on ART with virological failure to first-line ART and switched to second-line ART with prior genotypic resistance testing. The pattern of RAMs was determined in frequency runs and the factors associated with accumulation of≥3 thymidine analogue mutations (TAMs) and K103N were determined using multivariate logistic models. Changes in weight, height, CD4, and viral load at weeks 24 and 48 after switch to second-line therapy were determined using descriptive statistics. The children were mean age 10.9±4.6 years and 55.6% were male. The commonest nucleoside reverse transcriptase inhibitor (NRTI) RAM was M184V in 129/142 (90.8%) children. TAMs,≥3 TAMs, 69 insertion complex, K65R/N, and Q151M were observed in 43.0%, 10.6%, 18.3%, 2.8%, and 2.1% of the children, respectively. The commonest nonnucleoside reverse transcriptase inhibitor (NNRTI) RAM was K103N in 72/142 (50.7%) children. The starting ART regimen was associated with accumulation of both≥3 TAMs (p=0.046) and K103N (p<0.0001), while a history of poor adherence was associated with K103N accumulation (p=0.0388). After 24 weeks and 48 weeks of follow-up on lopinavir-ritonavir based second-line ART, 86/108 (79.6%) and 84.5% (87/103) of the children had viral loads<400 copies/ml, respectively. The mean CD4 absolute count increased by 173 cells/µl and 267cells/µl at weeks 24 and 48, respectively. Increments were also observed in mean weight (1.6 kg and 4.3 kg) and height (1.8 cm and 5.8 cm) at weeks 24 and 48, respectively. Multiple RAMs were observed among HIV-1-infected children with virological failure on first-line ART with M184V and K103N most frequent. The children responded favorably to boosted PI-based second-line ART.
PMID: 23308370 [PubMed - indexed for MEDLINE]

56. **Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy.**

Wamalwa DC[1], Lehman DA, Benki-Nugent S, Gasper MA, Gichohi R, Maleche-Obimbo E, Farquhar C, John-Stewart GC, Overbaugh J.

J Acquir Immune Defic Syndr. 2013 Mar 1;62(3):267-74. doi: 10.1097/QAI.0b013e31827b4ac8.

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**ABSTRACT**

BACKGROUND: HIV-infected children may require the use of combination antiretroviral treatment (cART) into adulthood. However, regimens are limited to first line and second line in many African settings. Therefore, understanding the long-term rate of virologic failure and drug resistance during prolonged antiretroviral treatment is important for establishing treatment strategies in African pediatric cohorts.

METHODS: Children aged 18 months to 12 years initiated first-line cART and were followed every 1-3 months, for up to 5.5 years. Treatment was switched to second-line cART based on clinical and immunologic criteria according to national guidelines. Virologic failure was determined retrospectively as defined by ≥2 viral loads >5000 copies per milliliter. Drug resistance was assessed during viral failure by population-based sequencing.

RESULTS: Among 100 children on first-line cART followed for a median of 49 months, 34% children experienced virologic failure. Twenty-three (68%) of the 34 children with viral failure had detectable resistance mutations, of whom 14 (61%) had multiclass resistance. Fourteen (14%) children were switched to second-line regimens and followed for a median of 28 months. Retrospective analysis revealed that virologic failure had occurred at a median of 12 months before switching to second line. During prolonged first-line treatment in the presence of viral failure, additional resistance mutations accumulated; however, only 1 (7%) of 14 children had persistent viremia during second-line treatment.

DISCUSSION: Virologic suppression was maintained on first-line cART in two-thirds of HIV-infected children for up to 5 years. Switch to second line based on clinical/immunologic criteria occurred ~1 year after viral failure, but the delay did not consistently compromise second-line treatment.

PMCID: PMC3593972 **Free PMC Article**
PMID: 23196827 [PubMed - indexed for MEDLINE]

57. **Associations of human leukocyte antigen-G with resistance and susceptibility to HIV-1 infection in the Pumwani sex worker cohort.**

Turk WJ[1], Kimani J, Bielawny T, Wachihi C, Ball TB, Plummer FA, Luo M.

AIDS. 2013 Jan 2;27(1):7-15. doi: 10.1097/QAD.0b013e32835ab1f2.

[1]Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Manitoba, Canada.

**ABSTRACT**

OBJECTIVE: To determine the association between human leukocyte antigens (HLA)-G genotypes and resistance or susceptibility to HIV-1.

DESIGN: A group of sex workers in Pumwani, Kenya can be epidemiologically defined as resistant to HIV-1 infection despite frequent exposure and provide an example of natural protective immunity. HLA class I and II molecules have been shown to be associated with resistance/susceptibility to infection in

this cohort. HLA-G is a nonclassical class I allele that is primarily involved in mucosal and inflammatory response, which is of interest in HIV-1 resistance.

METHODS: In this study, we used a sequence-based typing method to genotype HLA-G for 667 women enrolled in this cohort and examined the influence of HLA-G genotypes on resistance or susceptibility to HIV-1 infection.

RESULTS: The G*01 : 01:01 genotype was significantly enriched in the HIV-1-resistant women [P = 0.002, Odds ratio: 2.11, 95% confidence interval (CI): 0.259-0.976], whereas the G*01 : 04:04 genotype was significantly associated with susceptibility to HIV-1 infection (P = 0.039, OR:0.502, 95% CI:0.259-0.976). Kaplan-Meier survival analysis correlated with these results. G*01 : 01:01 genotype was associated with significantly lower rate of seroconversion (P = 0.001). Whereas, G*01 : 04:04 genotype was significantly associated with an increased rate of seroconversion (P = 0.013). The associations of these HLA-G alleles are independent of other HLA class I and II alleles identified in this population.

CONCLUSION: Our study showed that specific HLA-G alleles are associated with resistance or susceptibility to HIV-1 acquisition in this high-risk population. Further studies are needed to understand its functional significance in HIV-1 transmission.
PMID: 23032415 [PubMed - indexed for MEDLINE]

58. **Low prevalence of transmitted HIV type 1 drug resistance among antiretroviral-naive adults in a rural HIV clinic in Kenya.**

Hassan AS[1], Mwaringa SM, Obonyo CA, Nabwera HM, Sanders EJ, Rinke de Wit TF, Cane PA, Berkley JA.

AIDS Res Hum Retroviruses. 2013 Jan;29(1):129-35. doi: 10.1089/AID.2012.0167. Epub 2012 Sep 11.

[1]KEMRI/Wellcome Trust Research Programme, Kilifi, Kenya. ahassan@kemri-wellcome.org

**ABSTRACT**

Low levels of HIV-1 transmitted drug resistance (TDR) have previously been reported from many parts of sub-Saharan Africa (sSA). However, recent data, mostly from urban settings, suggest an increase in the prevalence of HIV-1 TDR. Our objective was to determine the prevalence of TDR mutations among HIV-1-infected, antiretroviral (ARV)-naive adults enrolling for care in a rural HIV clinic in Kenya. Two cross-sectional studies were carried out between July 2008 and June 2010. Plasma samples from ARV-naive adults (>15 years old) at the time of registering for care after HIV diagnosis and before starting ARVs were used. A portion of the pol subgenomic region of the virus containing the protease and part of the reverse transcriptase genes was amplified and sequenced. TDR mutations were identified and interpreted using the Stanford HIV drug resistance database and the WHO list for surveillance of drug resistance strains. Overall, samples from 182 ARV-naive adults [mean age (95% CI): 34.9 (33.3-36.4) years] were successfully amplified and sequenced. Two TDR mutations to nucleoside reverse transcriptase inhibitors [n=1 (T215D)] and protease inhibitors [n=1 (M46L)] were identified, giving an overall TDR prevalence of 1.1% (95% CI: 0.1-3.9). Despite reports of an increase in the prevalence of HIV-1 TDR in some urban settings in sSA, we report a prevalence of HIV-1 TDR of less than 5% at a rural

HIV clinic in coastal Kenya. Continued broader surveillance is needed to monitor the extent of TDR in sSA.

PMCID: PMC3537300 **Free PMC Article**
PMID: 22900472 [PubMed - indexed for MEDLINE]

59. **Diversity of HIV type 1 and drug resistance mutations among injecting drug users in Kenya.**

Osman S[1], Lihana RW, Kibaya RM, Ishizaki A, Bi X, Okoth FA, Ichimura H, Lwembe RM.

AIDS Res Hum Retroviruses. 2013 Jan;29(1):187-90. doi: 10.1089/AID.2012.0182. Epub 2012 Sep 18.

[1]Kenya Medical Research Institute, Nairobi, Kenya.

**ABSTRACT**

Drug use in Kenya dates back to the precolonial period but research among drug users in relation to human immunodeficiency virus (HIV)-associated risk and intervention strategies has been low. To evaluate HIV-1 diversity and drug resistance among injecting drug users (IDUs), a cross-sectional study involving 58 patients was carried out in Mombasa between February and March 2010. HIV-1 RNA was extracted from plasma and polymerase chain reaction using specific primers for HIV-1 reverse transcriptase was done. Population sequencing was done and subtypes were determined phylogenetically. The prevalent HIV-1 subtypes were A1 (52/58), D (5/58), and C (2/58). The prevalence of drug resistance was 13.8% (8/58) with detection of nucleoside reverse transcriptase inhibitor (NRTI) mutations, T215F (n=5), K219Q (n=3), M184V (n=1), and nonnucleoside RTI mutation, K103N (n=1). Antiretroviral therapy (ART) and its monitoring among infected Kenyan IDUs is feasible. Policymakers and service providers in HIV prevention initiatives should improve service delivery so as to measure ART coverage among IDUs to prevent further transmission of drug-resistant variants.
PMID: 22856626 [PubMed - indexed for MEDLINE]

60. **Prevalence, clinical and virologic outcomes of hepatitis B virus co-infection in HIV-1 positive Kenyan women on antiretroviral therapy.**

Day SL[1], Odem-Davis K, Mandaliya KN, Jerome KR, Cook L, Masese LN, Scott J, Kim HN, Graham SM, McClelland RS.

PLoS One. 2013;8(3):e59346. doi: 10.1371/journal.pone.0059346. Epub 2013 Mar 18.

[1]Department of Medicine, University of Washington, Seattle, Washington, United States of America.

**ABSTRACT**

BACKGROUND: Sub-Saharan Africa carries a high burden of co-infection with HIV-1 and hepatitis B virus (HBV). In this region, individuals with HIV-1/HBV co-infection on antiretroviral therapy (ART) frequently receive lamivudine as the only agent active against HBV, raising concerns for development of HBV resistance to lamivudine. We aimed to determine the prevalence, clinical, and virologic outcomes of

chronic HBV infection, including HBV resistance to lamivudine, in a cohort of HIV-1 seropositive Kenyan women on long-term ART.

METHODS: In this prospective cohort study, HIV-1 seropositive women initiated three-drug ART regimens that included lamivudine as the single drug active against HBV. Archived samples were tested for HBsAg, with further testing to determine HBeAg seroprevalence, HBV DNA suppression, and lamivudine resistance. We estimated the prevalence of chronic HBV and examined associations between HBV co-infection and clinical and virologic outcomes with chi-square tests, logistic regression, Kaplan-Meier and Cox regression.

RESULTS: In a cohort of 159 women followed for a median of 3.4 years (interquartile range 1.4-4.5), 11 (6.9%; 95% CI 3.1-10.7) had chronic HBV infection. Of these, 9 (82%) achieved undetectable plasma HBV DNA levels. One woman developed lamivudine resistance, for an incidence of 3 per 100 person-years. The HBV co-infected women were at greater risk for abnormal ALT elevations compared to HIV-1 mono-infected women (HR 2.37; 95% CI 1.1-5.3). There were no differences between HBV-infected and uninfected women in mortality, CD4 count, or HIV-1 RNA suppression.

CONCLUSION: The prevalence of chronic HBV in this cohort was similar to recent studies from other African populations. Given our long-term follow-up, lamivudine resistance was lower than expected for HIV-1/HBV co-infected patients. Improved screening for HBV and extended follow-up of HIV-1/HBV co-infected individuals are needed to better understand the impact of different ART regimens on clinical outcomes in this population.

PMCID: PMC3601052 **Free PMC Article**
PMID: 23527168 [PubMed - indexed for MEDLINE]

61. **Low drug resistance levels among drug-naive individuals with recent HIV type 1 infection in a rural clinical cohort in southwestern Uganda.**

Ssemwanga D[1], Kapaata A, Lyagoba F, Magambo B, Nanyonjo M, Mayanja BN, Parry CM, Kaleebu P.

AIDS Res Hum Retroviruses. 2012 Dec;28(12):1784-7. doi: 10.1089/AID.2012.0090. Epub 2012 Aug 3.

[1]MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda.

**ABSTRACT**

To investigate the prevalence of transmitted drug resistance (TDR) among individuals with recent HIV-1 infection between February 2004 and January 2010 in a rural clinical cohort, samples from 72 participants were analyzed. Results from the 72 participants showed no protease inhibitor and nucleoside reverse transcriptase inhibitor-associated mutations. One participant (1.4%, 95% CI: 0.04-7.5%) had two nonnucleoside reverse transcriptase inhibitor mutations (G190E and P225H). HIV-1 subtype frequencies were A 22 (30.6%), D 38 (52.8%), and C 1 (1.4%); 11 (15.3%) were A/D unique recombinant forms. Seven years after the scale up of antiretroviral therapy (ART) in a rural clinical cohort in Uganda, the prevalence of TDR among recently HIV-1-infected individuals was low at 1.4%. Since our findings from an HIV study cohort may not be generalizable to the general population,

routine TDR surveys in specific populations may be necessary to inform policy on the magnitude and prevention strategies of TDR.

PMID: 22616647 [PubMed - indexed for MEDLINE]

62. **Antiretroviral drug susceptibility among HIV-infected adults failing antiretroviral therapy in Rakai, Uganda.**

Reynolds SJ[1], Laeyendecker O, Nakigozi G, Gallant JE, Huang W, Hudelson SE, Quinn TC, Newell K, Serwadda D, Gray RH, Wawer MJ, Eshleman SH.

AIDS Res Hum Retroviruses. 2012 Dec;28(12):1739-44. doi: 10.1089/AID.2011.0352. Epub 2012 Apr 26.

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**ABSTRACT**

We analyzed antiretroviral drug susceptibility in HIV-infected adults failing first- and second-line antiretroviral treatment (ART) in Rakai, Uganda. Samples obtained from participants at baseline (pretreatment) and at the time of failure on first-line ART and second-line ART were analyzed using genotypic and phenotypic assays for antiretroviral drug resistance. Test results were obtained from 73 samples from 38 individuals (31 baseline samples, 36 first-line failure samples, and six second-line failure samples). Four (13%) of the 31 baseline samples had mutations associated with resistance to nucleoside or nonnucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively). Among the 36 first-line failure samples, 31 (86%) had NNRTI resistance mutations and 29 (81%) had lamivudine resistance mutations; only eight (22%) had other NRTI resistance mutations. None of the six individuals failing a second-line protease inhibitor (PI)-based regimen had PI resistance mutations. Six (16%) of the participants had discordant genotypic and phenotypic test results. Genotypic resistance to drugs included in first-line ART regimens was detected prior to treatment and among participants failing first-line ART. PI resistance was not detected in individuals failing second-line ART. Surveillance for transmitted and acquired drug resistance remains a priority for scale-up of ART.

PMCID: PMC3505045 **Free PMC Article**
PMID: 22443282 [PubMed - indexed for MEDLINE]

63. **Effect of injectable contraceptive use on response to antiretroviral therapy among women in Rakai, Uganda.**

Polis CB[1], Nakigozi G, Ssempijja V, Makumbi FE, Boaz I, Reynolds SJ, Ndyanabo A, Lutalo T, Wawer MJ, Gray RH.

Contraception. 2012 Dec;86(6):725-30. doi: 10.1016/j.contraception.2012.05.001. Epub 2012 Jun 18.

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**ABSTRACT**

BACKGROUND: There is limited evidence on the effect of injectable contraception on response to antiretroviral therapy (ART).

DESIGN: Using modified Poisson regression, we assessed data from 418 female Ugandan ART initiators to examine the effect of injectable contraceptive use on a composite virologic failure outcome (defined as failure to achieve virologic suppression, switch to second line therapy, or death within 12 months of ART initiation) and also assessed ART adherence.

RESULTS: About 12% of women reported using injectable contraceptives at ART initiation, and their composite virologic failure rates 12 months later were similar to women not using injectable contraceptives at ART initiation (11% vs. 12%, p=0.99). Multivariable Poisson regression suggested no significant differences in virologic failure by injectable contraceptive use at baseline (prevalence risk ratio: 0.85, p=0.71), but power was limited. Adherence to ART increased with time since ART initiation, and did not appear to differ between injectable contraceptive users and non-users.

CONCLUSIONS: Consistent with current World Health Organization guidelines, our results suggest no deleterious effect of injectable contraceptive use on response to ART, but power was limited, injectable contraceptive use patterns over time were inconsistent and additional evidence is needed.

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PMCID: PMC3449005 **Free PMC Article**
PMID: 22717186 [PubMed - indexed for MEDLINE]

64. **A genetic polymorphism of FREM1 is associated with resistance against HIV infection in the Pumwani sex worker cohort.**

Luo M[1], Sainsbury J, Tuff J, Lacap PA, Yuan XY, Hirbod T, Kimani J, Wachihi C, Ramdahin S, Bielawny T, Embree J, Broliden K, Ball TB, Plummer FA.

J Virol. 2012 Nov;86(21):11899-905. doi: 10.1128/JVI.01499-12. Epub 2012 Aug 22.

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**ABSTRACT**

A subgroup of women enrolled in the Pumwani sex worker cohort remain seronegative and PCR negative for human immunodeficiency virus type 1 despite repeated exposure through high-risk sex work. Studies have shown that polymorphisms of genes involved in antigen presentation and viral restriction factors are associated with resistance to HIV infection. To discover other possible genetic factors underlying this HIV-resistant phenotype, we conducted an exploratory nonbiased, low-resolution, genome-wide single-nucleotide polymorphism (SNP) analysis comparing 60 HIV-resistant women to 48 HIV-infected controls. The SNP minor allele rs1552896, in an intron of FREM1, was significantly associated with the resistant phenotype (P = 1.68 × 10(-5); adjusted P = 2.37 × 10(-4); odds

ratio [OR], 9.51; 95% confidence interval [CI], 2.82 to 32.05). We expanded the sample size by genotyping rs1552896 in the Pumwani cohort and comparing 114 HIV-resistant women to 609 HIV-infected controls and confirmed the association (P = 1.7 × 10(-4); OR, 2.67; 95% CI, 1.47 to 4.84). To validate the association in a second cohort, we genotyped 783 women enrolled in a mother-child health study and observed the minor allele of rs1552896 enriched in HIV-uninfected women (n = 488) compared to HIV-infected enrollees (n = 295) (P = 0.036; OR, 1.69; 95% CI, 0.98 to 2.93). Quantitative reverse transcription-PCR showed that FREM1 mRNA was highly expressed in tissues relevant for HIV-1 infection, and immunohistochemical analysis revealed that FREM1 protein is expressed in the ectocervical mucosa of HIV-resistant women. The significant association of rs1552896 with an HIV-resistant phenotype, together with the expression profile of FREM1 in tissues relevant to HIV infection, suggests that FREM1 is a potentially novel candidate gene for resistance to HIV infection.

PMCID: PMC3486297 **Free PMC Article**
PMID: 22915813 [PubMed - indexed for MEDLINE]

65. **Anti-tuberculosis drug resistance pattern among pulmonary tuberculosis patients with or without HIV infection in Mwanza, Tanzania.**

Range N[1], Friis H, Mfaume S, Magnussen P, Chanualucha J, Kilale A, Mugomela A, Andersen AB.

Tanzan J Health Res. 2012 Oct;14(4):243-9.

[1]National Institute for Medical Research, Muhimbili Medical Research Centre, Dar es Salaam, Tanzania. hrange08@gmail.com

**ABSTRACT**

Anti-tuberculosis drug resistance is a major problem in tuberculosis (TB) control, particularly multi-drug resistance TB (MDR-TB). The objective of this study was to determine the prevalence of primary and acquired anti-TB drug resistance among newly diagnosed pulmonary TB (PTB) and relapse cases. Sputa were collected from newly diagnosed and relapse PTB patients. Drug susceptibility tests (DST) were performed on sputum culture positive isolates of Mycobacterium tuberculosis using resistance ratio method on four first-line anti-TB drugs: rifampicin, isoniazid, ethambutol and streptomycin. Demographic and anthropometric information was collected and HIV status was determined. Of the 523 culture positive isolates, DST results were available for 503 (96%), 455 were new and 48 were relapse cases. Resistance to at least one of the four drugs was observed in 7.8% (39/503) of the isolates, 7.3% (33/455) were new and 12.5% (6/48) were from relapse cases. Mono resistance to isoniazid was higher in both among new 45.5% (15/33) and relapse 50.0% (3/6) cases. Resistance to rifampicin and streptomycin alone was equal 4/33 (12.1%) and only among new cases. Resistance to ethambutol alone was only one among new cases. Overall MDR-TB prevalence was 2.4% (12/503), nine were new and three were relapse cases. MDR-TB was 17.9% (7/39) for rifampicin and isoniazid. Prevalence of HIV was 43.3% and was similar among new and relapse cases and not risk factor for drug resistance. Majority of PTB patients (52%) had BMI below 18 kg/m2. Those with BMI greater than 18 kg/m2 were more likely to develop drug resistance than those with BMI below 18 kg/m2 (P=0.004). With the resurgence of TB and the high prevalence of HIV among TB patients, prevalence of drug resistance is still low both among new and relapses cases. Despite the current low drug resistance, there is a need for continuous monitoring of the resistance.

PMID: 26591721 [PubMed - indexed for MEDLINE]

66. **Short communication: High prevalence of transmitted antiretroviral drug resistance among newly HIV type 1 diagnosed adults in Mombasa, Kenya.**

Sigaloff KC[1], Mandaliya K, Hamers RL, Otieno F, Jao IM, Lyagoba F, Magambo B, Kapaata A, Ndembi N, Rinke de Wit TF.

AIDS Res Hum Retroviruses. 2012 Sep;28(9):1033-7. doi: 10.1089/AID.2011.0348. Epub 2012 Feb 2.

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**ABSTRACT**

ABSTRACT In view of the recent antiretroviral therapy (ART) scale-up in Kenya, surveillance of transmitted HIV drug resistance (TDR) is important. A cross-sectional survey was conducted among newly HIV-1 diagnosed, antiretroviral-naive adults in Mombasa, Kenya. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list. HIV-1 subtypes were determined using REGA and SCUEAL subtyping tools. Genotypic test results were obtained for 68 of 81 participants, and SDRMs were identified in 9 samples. Resistance to nonnucleoside reverse transcriptase inhibitors (K103N) occurred in five participants, yielding a TDR prevalence of 7.4% (95% confidence interval 2.4-16.3%). Frequencies of HIV-1 subtypes were A (70.6%), C (5.9%), D (2.9%), and unique recombinant forms (20.6%). The TDR prevalence found in this survey is higher than previously reported in different regions in Kenya. These findings justify increased vigilance with respect to TDR surveillance in African regions where ART programs are scaled-up in order to inform treatment guidelines.
PMID: 22149307 [PubMed - indexed for MEDLINE]

67. **Integration of HIV testing in tuberculosis drug resistance surveillance in Kazakhstan and Kenya.**

Klinkenberg E[1], van den Hof S, Tursynbayeva A, Kipruto H, Wahogo J, Pak S, Kutwa A, L'Herminez R.

Int J Tuberc Lung Dis. 2012 May;16(5):615-7. doi: 10.5588/ijtld.11.0262. Epub 2012 Mar 8.

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**ABSTRACT**

In Kenya and Kazakhstan, integration of human immunodeficiency virus (HIV) testing results into the routine surveillance of multidrug-resistant tuberculosis (MDR-TB) proved feasible and useful. The integration process improved overall data quality and data validation capacity, and integrated data are a useful addition to routine cohort and treatment outcome data. Besides their importance for individual patient care, they provide trends on the association of MDR-TB and HIV in the routine programme setting. They also form a useful epidemiological basis for more specific studies, such as on nosocomial outbreaks. Whether the system itself is sensitive enough to monitor possible outbreaks needs further investigation.
PMID: 22409816 [PubMed - indexed for MEDLINE]

68. **Intrapartum single-dose carbamazepine reduces nevirapine levels faster and may decrease resistance after a single dose of nevirapine for perinatal HIV prevention.**

Muro EP[1], Fillekes Q, Kisanga ER, L'homme R, Aitken SC, Mariki G, Van der Ven AJ, Dolmans W, Schuurman R, Walker AS, Gibb DM, Burger DM.

J Acquir Immune Defic Syndr. 2012 Mar 1;59(3):266-73. doi: 10.1097/QAI.0b013e31824234d8.

[1]Department of Pharmacology, Kilimanjaro Christian Medical College, Moshi, Tanzania.

**ABSTRACT**

BACKGROUND: World Health Organization guidelines recommend zidovudine + lamivudine for 7 days from labor onset in HIV-infected women receiving single-dose nevirapine (sdNVP) to cover prolonged subtherapeutic nevirapine concentrations. Although effective, this is complicated and does not eliminate resistance; alternative strategies could add benefit.

METHODS: Antiretroviral-naive HIV-infected pregnant women aged 18-40 years, with CD4 >200 cells per cubic millimeter, able to regularly attend the antenatal clinics in Moshi, Tanzania, were enrolled 1:1 by alternate allocation to receive 200 mg sdNVP alone or in combination with open-label 400-mg single-dose carbamazepine (sdNVP/CBZ) at delivery (ClinicalTrials.gov NCT00294892). The coprimary outcomes were nevirapine plasma concentrations 1 week and nevirapine resistance mutations 6 weeks postpartum. Analyses were based on those still eligible at delivery.

RESULTS: Ninety-seven women were assigned to sdNVP and 95 to sdNVP/CBZ during pregnancy, of whom 75 sdNVP and 83 sdNVP/CBZ were still eligible at delivery at study sites. The median (interquartile range) nevirapine plasma concentration was 1.55 (0.88-1.84) mg/L in sdNVP (n = 61) and 1.40 (0.93-1.97) mg/L in sdNVP/CBZ (n = 72) at delivery (P = 0.91), but 1 week later was significantly lower in sdNVP/CBZ [n = 63; 0.09 (0.05-0.20) mg/L] than in sdNVP [n = 52; 0.20 (0.09-0.31) mg/L; rank-sum: P = 0.004] (geometric mean ratio: 0.64, 95% confidence interval: 0.43 to 0.96; P = 0.03). Six weeks postpartum, nevirapine mutations were observed in 11 of 52 (21%) in sdNVP and 6 of 55 (11%) in sdNVP/CBZ (odds ratio = 0.46, 95% confidence interval: 0.16 to 1.34; P = 0.15).

CONCLUSIONS: Addition of single-dose carbamazepine to sdNVP at labor onset in HIV-infected, pregnant women did not affect nevirapine plasma concentration at delivery, but significantly reduced it 1 week postpartum, with a trend toward fewer nevirapine resistance mutations.

PMID: 22134145 [PubMed - indexed for MEDLINE]

69. **Altered dendritic cell-natural killer interaction in Kenyan sex workers resistant to HIV-1 infection.**

Ghadially H[1], Keynan Y, Kimani J, Kimani M, Ball TB, Plummer FA, Mandelboim O, Meyers AF.

AIDS. 2012 Feb 20;26(4):429-36. doi: 10.1097/QAD.0b013e32834f98ea.

[1]The Lautenberg Center for General and Tumor Immunology, The Hebrew University Hadassah Medical School, Jerusalem, Israel.

**ABSTRACT**

BACKGROUND: Natural killer (NK) cells are members of the innate immune system that play an important role in the defense against viral infection. They are also involved in the regulation of adaptive immune responses through cytokine secretion and the interaction with antigen-presenting cells. However, their role in HIV infection is only partially understood.

OBJECTIVE: Here we studied the phenotype and function of NK cells of highly HIV-exposed but seronegative (HESN) uninfected commercial sex workers from Kenya who can be epidemiologically defined as relatively resistant to HIV infection.

DESIGN: The purpose of this study was to gain insight into the role of NK cells in mediating resistance to HIV-1. This information can be used to better understand protection from infection which can be used for informing future design of effective prophylactics and therapeutics for HIV.

METHODS: Whole blood samples were collected from study participants and isolated NK cells and dendritic cells were used in assays for phenotyping and cell function.

RESULTS: Activated NK cells from resistant women killed autologous immature dendritic cells more efficiently and also secreted more interferon (IFN)-γ than those of uninfected, susceptible women. Interestingly, NK cells from HIV-resistant women were significantly more effective in inducing secretion of IL-12 in immature dendritic cells.

CONCLUSIONS: These data suggest that an altered NK cell-dendritic cell interaction plays an important role in the protection from infection with HIV-1.
PMID: 22156969 [PubMed - indexed for MEDLINE]

70. **Low prevalence of Pneumocystis pneumonia (PCP) but high prevalence of pneumocystis dihydropteroate synthase (dhps) gene mutations in HIV-infected persons in Uganda.**

Taylor SM[1], Meshnick SR, Worodria W, Andama A, Cattamanchi A, Davis JL, Yoo SD, Byanyima P, Kaswabuli S, Goodman CD, Huang L; International HIV-associated Opportunistic Pneumonias Study.

PLoS One. 2012;7(11):e49991. doi: 10.1371/journal.pone.0049991. Epub 2012 Nov 16.

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**ABSTRACT**

Pneumocystis jirovecii pneumonia (PCP) is an important opportunistic infection in patients infected with HIV, but its burden is incompletely characterized in those areas of sub-Saharan Africa where HIV is prevalent. We explored the prevalence of both PCP in HIV-infected adults admitted with pneumonia to a tertiary-care hospital in Uganda and of putative P. jirovecii drug resistance by mutations in fungal dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr). In 129 consecutive patients with sputum smears negative for mycobacteria, 5 (3.9%) were diagnosed with PCP by microscopic examination of Giemsa-stained bronchoalveolar lavage fluid. Concordance was 100% between Giemsa stain and PCR (dhps and dhfr). PCP was more prevalent in patients newly-diagnosed with HIV (11.4%) than in patients with known HIV (1.1%; p = 0.007). Mortality at 2 months after discharge was 29% overall: 28% among PCP-negative patients, and 60% (3 of 5) among PCP-positive patients. In these 5 fungal isolates and an additional 8 from consecutive cases of PCP, all strains harbored mutant dhps haplotypes; all 13 isolates harbored the P57S mutation in dhps, and 3 (23%) also harbored the T55A mutation. No non-synonymous dhfr mutations were detected. PCP is an important cause of pneumonia in patients newly-diagnosed with HIV in Uganda, is associated with high mortality, and putative molecular evidence of drug resistance is prevalent. Given the reliability of field diagnosis in our cohort, future studies in sub-Saharan Africa can investigate the clinical impact of these genotypes.

PMCID: PMC3500344 **Free PMC Article**
PMID: 23166805 [PubMed - indexed for MEDLINE]

71. **Emergence of minor drug-resistant HIV-1 variants after triple antiretroviral prophylaxis for prevention of vertical HIV-1 transmission.**

Hauser A[1], Sewangi J, Mbezi P, Dugange F, Lau I, Ziske J, Theuring S, Kuecherer C, Harms G, Kunz A.

PLoS One. 2012;7(2):e32055. doi: 10.1371/journal.pone.0032055. Epub 2012 Feb 23.

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**ABSTRACT**

BACKGROUND: WHO-guidelines for prevention of mother-to-child transmission of HIV-1 in resource-limited settings recommend complex maternal antiretroviral prophylaxis comprising antenatal zidovudine (AZT), nevirapine single-dose (NVP-SD) at labor onset and AZT/lamivudine (3TC) during labor and one week postpartum. Data on resistance development selected by this regimen is not available. We therefore analyzed the emergence of minor drug-resistant HIV-1 variants in Tanzanian women following complex prophylaxis.

METHOD: 1395 pregnant women were tested for HIV-1 at Kyela District Hospital, Tanzania. 87/202 HIV-positive women started complex prophylaxis. Blood samples were collected before start of prophylaxis, at birth and 1-2, 4-6 and 12-16 weeks postpartum. Allele-specific real-time PCR assays specific for HIV-1 subtypes A, C and D were developed and applied on samples of mothers and their vertically infected infants to quantify key resistance mutations of AZT (K70R/T215Y/T215F), NVP (K103N/Y181C) and 3TC (M184V) at detection limits of <1%.

RESULTS: 50/87 HIV-infected women having started complex prophylaxis were eligible for the study. All women took AZT with a median duration of 53 days (IQR 39-64); all women ingested NVP-SD, 86% took 3TC. HIV-1 resistance mutations were detected in 20/50 (40%) women, of which 70% displayed minority species. Variants with AZT-resistance mutations were found in 11/50 (22%), NVP-resistant variants in 9/50 (18%) and 3TC-resistant variants in 4/50 women (8%). Three women harbored resistant HIV-1 against more than one drug. 49/50 infants, including the seven vertically HIV-infected were breastfed, 3/7 infants exhibited drug-resistant virus.

CONCLUSION: Complex prophylaxis resulted in lower levels of NVP-selected resistance as compared to NVP-SD, but AZT-resistant HIV-1 emerged in a substantial proportion of women. Starting AZT in pregnancy week 14 instead of 28 as recommended by the current WHO-guidelines may further increase the frequency of AZT-resistance mutations. Given its impact on HIV-transmission rate and drug-resistance development, HAART for all HIV-positive pregnant women should be considered.

PMCID: PMC3285650 **Free PMC Article**
PMID: 22384138 [PubMed - indexed for MEDLINE]

72. **Microarray analysis of HIV resistant female sex workers reveal a gene expression signature pattern reminiscent of a lowered immune activation state.**

Songok EM[1], Luo M, Liang B, Mclaren P, Kaefer N, Apidi W, Boucher G, Kimani J, Wachihi C, Sekaly R, Fowke K, Ball BT, Plummer FA.

PLoS One. 2012;7(1):e30048. doi: 10.1371/journal.pone.0030048. Epub 2012 Jan 26.

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**ABSTRACT**

To identify novel biomarkers for HIV-1 resistance, including pathways that may be critical in anti-HIV-1 vaccine design, we carried out a gene expression analysis on blood samples obtained from HIV-1 highly exposed seronegatives (HESN) from a commercial sex worker cohort in Nairobi and compared their profiles to HIV-1 negative controls. Whole blood samples were collected from 43 HIV-1 resistant sex workers and a similar number of controls. Total RNA was extracted and hybridized to the Affymetrix HUG 133 Plus 2.0 micro arrays (Affymetrix, Santa Clara CA). Output data was analysed through ArrayAssist software (Agilent, San Jose CA). More than 2,274 probe sets were differentially expressed in the HESN as compared to the control group (fold change ≥1.3; p value ≤0.0001, FDR <0.05). Unsupervised hierarchical clustering of the differentially expressed genes readily distinguished HESNs from controls. Pathway analysis through the KEGG signaling database revealed a majority of the impacted pathways (13 of 15, 87%) had genes that were significantly down regulated. The most down expressed pathways were glycolysis/gluconeogenesis, pentose phosphate, phosphatidyl inositol, natural killer cell cytotoxicity and T-cell receptor signaling. Ribosomal protein synthesis and tight junction genes were up regulated. We infer that the hallmark of HIV-1 resistance is down regulation of genes in key signaling pathways that HIV-1 depends on for infection.

PMCID: PMC3266890 **Free PMC Article**
PMID: 22291902 [PubMed - indexed for MEDLINE]

73. **HBV lamivudine resistance among hepatitis B and HIV coinfected patients starting lamivudine, stavudine and nevirapine in Kenya.**

Kim HN[1], Scott J, Cent A, Cook L, Morrow RA, Richardson B, Tapia K, Jerome KR, Lule G, John-Stewart G, Chung MH.

J Viral Hepat. 2011 Oct;18(10):e447-52. doi: 10.1111/j.1365-2893.2011.01466.x. Epub 2011 May 13.

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**ABSTRACT**

Widespread use of lamivudine in antiretroviral therapy may lead to hepatitis B virus resistance in HIV-HBV coinfected patients from endemic settings where tenofovir is not readily available. We evaluated 389 Kenyan HIV-infected adults before and for 18 months after starting highly active antiretroviral therapy with stavudine, lamivudine and nevirapine. Twenty-seven (6.9%) were HBsAg positive and anti-HBs negative, 24 were HBeAg negative, and 18 had HBV DNA levels ≤ 10,000 IU/mL. Sustained HBV suppression to <100 IU/mL occurred in 89% of 19 evaluable patients. Resistance occurred in only two subjects, both with high baseline HBV DNA levels. Lamivudine resistance can emerge in the setting of incomplete HBV suppression but was infrequently observed among HIV-HBV coinfected patients with low baseline HBV DNA levels.

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PMCID: PMC3177102 **Free PMC Article**
PMID: 21914062 [PubMed - indexed for MEDLINE]

74. **HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study.**

Hamers RL[1], Wallis CL, Kityo C, Siwale M, Mandaliya K, Conradie F, Botes ME, Wellington M, Osibogun A, Sigaloff KC, Nankya I, Schuurman R, Wit FW, Stevens WS, van Vugt M, de Wit TF; PharmAccess African Studies to Evaluate Resistance (PASER).
Collaborators: (39)
Mandaliya K, Abdallah S, Jao I, Dolan M, Schuurman R, Wensing AM, Hamers RL, Sigaloff KC, Straatsma E, Wit FW, van Vugt M, Lange JM, Rinke de Wit TF, Osibogun A, Akanmu S, Botes ME, Conradie F, Ive P, Sanne I, Wallis CL, Letsoalo E, Stevens WS, Hardman M, Kityo C, Namayanja G, Nakatudde L, Nankya I, Kiconco M, Abwola M, Mugyenyi P, Ndembi N, Lyagoba F, Kaleebu P, Siwale M, Njovu C, Labib M, Menke J, Wellington M, Luthy R.

Lancet Infect Dis. 2011 Oct;11(10):750-9. doi: 10.1016/S1473-3099(11)70149-9. Epub 2011 Jul 27.

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**Comment in:**

- HIV-1 drug resistance in antiretroviral-naive patients in sub-Saharan Africa. [Lancet Infect Dis. 2012]
- Time to get serious about HIV antiretroviral resistance. [Lancet Infect Dis. 2011]

**ABSTRACT**

BACKGROUND: There are few data on the epidemiology of primary HIV-1 drug resistance after the roll-out of antiretroviral treatment (ART) in sub-Saharan Africa. We aimed to assess the prevalence of primary resistance in six African countries after ART roll-out and if wider use of ART in sub-Saharan Africa is associated with rising prevalence of drug resistance.

METHODS: We did a cross-sectional study in antiretroviral-naive adults infected with HIV-1 who had not started first-line ART, recruited between 2007 and 2009 from 11 regions in Kenya, Nigeria, South Africa, Uganda, Zambia, and Zimbabwe. We did population-based sequencing of the pol gene on plasma specimens with greater than 1000 copies per mL of HIV RNA. We identified drug-resistance mutations with the WHO list for transmitted resistance. The prevalence of sequences containing at least one drug-resistance mutation was calculated accounting for the sampling weights of the sites. We assessed the risk factors of resistance with multilevel logistic regression with random coefficients.

FINDINGS: 2436 (94.1%) of 2590 participants had a pretreatment genotypic resistance result. 1486 participants (57.4%) were women, 1575 (60.8%) had WHO clinical stage 3 or 4 disease, and the median CD4 count was 133 cells per µL (IQR 62-204). Overall sample-weighted drug-resistance prevalence was 5.6% (139 of 2436; 95% CI 4.6-6.7), ranging from 1.1% (two of 176; 0.0-2.7) in Pretoria, South Africa, to 12.3% (22 of 179; 7.5-17.1) in Kampala, Uganda. The pooled prevalence for all three Ugandan sites was 11.6% (66 of 570; 8.9-14.2), compared with 3.5% (73 of 1866; 2.5-4.5) for all other sites. Drug class-specific resistance prevalence was 2.5% (54 of 2436; 1.8-3.2) for nucleoside reverse-transcriptase inhibitors (NRTIs), 3.3% (83 of 2436; 2.5-4.2) for non-NRTIs (NNRTIs), 1.3% (31 of 2436; 0.8-1.8) for protease inhibitors, and 1.2% (25 of 2436; 0.7-1.7) for dual-class resistance to NRTIs and NNRTIs. The most common drug-resistance mutations were K103N (43 [1.8%] of 2436), thymidine analogue mutations (33 [1.6%] of 2436), M184V (25 [1.2%] of 2436), and Y181C/I (19 [0.7%] of 2436). The odds ratio for drug resistance associated with each additional year since the start of the ART roll-out in a region was 1.38 (95% CI 1.13-1.68; p=0.001).

INTERPRETATION: The higher prevalence of primary drug resistance in Uganda than in other African countries is probably related to the earlier start of ART roll-out in Uganda. Resistance surveillance and prevention should be prioritised in settings where ART programmes are scaled up.

FUNDING: Ministry of Foreign Affairs of the Netherlands.

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75. **Immunovirological response to combined antiretroviral therapy and drug resistance patterns in children: 1- and 2-year outcomes in rural Uganda.**

Ahoua L[1], Guenther G, Rouzioux C, Pinoges L, Anguzu P, Taburet AM, Balkan S, Olson DM, Olaro C, Pujades-Rodríguez M.

BMC Pediatr. 2011 Jul 26;11:67. doi: 10.1186/1471-2431-11-67.

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**ABSTRACT**

BACKGROUND: Children living with HIV continue to be in urgent need of combined antiretroviral therapy (ART). Strategies to scale up and improve pediatric HIV care in resource-poor regions, especially in sub-Saharan Africa, require further research from these settings. We describe treatment outcomes in children treated in rural Uganda after 1 and 2 years of ART start.

METHODS: Cross-sectional assessment of all children treated with ART for 12 (M12) and 24 (M24) months was performed. CD4 counts, HIV RNA levels, antiretroviral resistance patterns, and non-nucleoside reverse transcriptase inhibitor (NNRTI) plasma concentrations were determined. Patient adherence and antiretroviral-related toxicity were assessed.

RESULTS: Cohort probabilities of retention in care were 0.86 at both M12 and M24. At survey, 71 (83%, M12) and 32 (78%, M24) children remained on therapy, and 84% participated in the survey. At ART start, 39 (45%) were female; median age was 5 years. Median initial CD4 percent was 11% [IQR 9-15] in children < 5 years old (n = 12); CD4 count was 151 cells/mm(3) [IQR 38-188] in those ≥ 5 years old (n = 26). At M12, median CD4 gains were 11% [IQR 10-14] in patients < 5 years old, and 206 cells/mm(3) [IQR 98-348] in ≥ 5 years old. At M24, median CD4 gains were 11% [IQR 5-17] and 132 cells/mm(3) [IQR 87-443], respectively. Viral suppression (< 400 copies/mL) was achieved in 59% (M12) and 33% (M24) of children. Antiretroviral resistance was found in 25% (M12) and 62% (M24) of children. Overall, 29% of patients had subtherapeutic NNRTI plasma concentrations.

CONCLUSIONS: After one year of therapy, satisfactory survival and immunological responses were observed, but nearly 1 in 4 children developed viral resistance and/or subtherapeutic plasma antiretroviral drug levels. Regular weight-adjustment dosing and strategies to reinforce and maintain ART adherence are essential to maximize duration of first-line therapy in children in resource-limited countries.

PMCID: PMC3176156 **Free PMC Article**
PMID: 21791095 [PubMed - indexed for MEDLINE]

76. **Transmitted antiretroviral drug resistance among newly HIV-1 diagnosed young individuals in Kampala.**

Ndembi N[1], Hamers RL, Sigaloff KC, Lyagoba F, Magambo B, Nanteza B, Watera C, Kaleebu P, Rinke de Wit TF.

AIDS. 2011 Apr 24;25(7):905-10. doi: 10.1097/QAD.0b013e328346260f.

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**ABSTRACT**

OBJECTIVE: To assess the emergence of transmitted HIV-1 drug resistance (TDR) in Kampala, Uganda, 10 years after the scale-up of antiretroviral treatment (ART) and to compare with a previous survey among antenatal clinic attendees in 2007 (reporting 0% TDR).

DESIGN: A cross-sectional survey was conducted among newly HIV-1 diagnosed, antiretroviral-naive young adults attending two large voluntary counseling and testing centers within the geographic area of Kampala.

METHODS: Proxy criteria for recent HIV-1 infection were used as defined by the WHO. Population sequencing of the pol gene was performed on plasma samples with HIV-1 RNA at least 1000 copies/ml. Surveillance drug resistance mutations (SDRMs) were identified according to the 2009 WHO list for surveillance of TDR. HIV-1 subtypes were designated using maximum likelihood phylogenetic reconstruction.

RESULTS: : Genotypic test results were obtained for 70 of 77 (90.9%) participants. SDRMs were identified in six samples yielding a prevalence of TDR of 8.6% (95% confidence interval 3.2-17.7%). Two had SDRMs to nucleoside reverse-transcriptase inhibitors (D67G and L210W), three had SDRMs to nonnucleoside reverse transcriptase inhibitors (G190A, G190S, and K101E), and one had SDRMs to protease inhibitors (N88D). Frequencies of HIV-1 subtypes were A (36/70, 51.4%), C ( two of 70; 2.9%), D (23/70, 32.9%), and unique recombinant forms (nine of 70, 12.9%).

CONCLUSION: This repeated survey suggests an increase in TDR in Kampala, compared with a previous survey. This finding justifies increased vigilance with respect to surveillance of TDR in areas in Africa where ART programs are rolled-out.
PMID: 21399479 [PubMed - indexed for MEDLINE]

77. **Prevalence of genotypic resistance to antiretroviral drugs in treatment-naive youths infected with diverse HIV type 1 subtypes and recombinant forms in Dar es Salaam, Tanzania.**

Mosha F[1], Urassa W, Aboud S, Lyamuya E, Sandstrom E, Bredell H, Williamson C.

AIDS Res Hum Retroviruses. 2011 Apr;27(4):377-82. doi: 10.1089/aid.2010.0113. Epub 2010 Oct 18.

[1]Field Epidemiology and Laboratory Training Programme, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania.

**ABSTRACT**

As human immunodeficiency virus (HIV) diversity may have an impact on both vaccine efficacy and drug resistance, it is important to have knowledge of circulating genetic variants. With widespread use of antiretroviral (ARV) drugs in Africa, one of the major potential challenges is the risk of emergence of ARV drug-resistant HIV strains. This study aimed to determine the circulating HIV subtypes and recombinant forms, as well as the prevalence of ARV drug resistance mutations, among 75 treatment-naive HIV-infected youths in Dar es Salaam, Tanzania. Gag (n = 48), partial pol (n = 44), and partial env (n = 35) sequencing was performed; all three regions were sequenced in 26 samples. Evidence of infection with recombinant viruses was found in 12 (46%) participants; AC recombinants were the most commonly detected and they were identified in six (23%) participants. Of individuals infected with nonrecombinant strains, subtype A was most commonly detected in seven (27%) participants, followed by subtype C detected in six (23%) participants and subtype D detected in one (4%) participant. Among the pol sequences from 44 individuals, three (7%) had resistance to nucleoside reverse transcriptase (RT) inhibitors and four (9%) had nonnucleoside RT inhibitor resistance mutations. Of these, three (7%) individuals were infected with viruses with cross-resistance mutations to both classes of RT inhibitors. These resistant mutations were all associated with drugs currently used in first-line therapy and in the prevention of vertical transmission. This high prevalence of resistance mutations is of considerable concern in apparently drug-naive populations as it may result in treatment failure and the spread of ARV-resistant strains.

PMID: 20954839 [PubMed - indexed for MEDLINE]

78. **Molecular characterization of the cervical and systemic B-cell repertoire: Unique, yet overlapping, immune compartments of an HIV-1 resistant individual.**

Gaudet RG[1], Breden F, Plummer F, Berry JD.

MAbs. 2011 Mar-Apr;3(2):181-91. Epub 2011 Mar 1.

[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada.

**ABSTRACT**

The cervical mucosa of women who are highly exposed to HIV-1, yet remain persistently seronegative (HEPS), presents a unique opportunity to study the dynamics of an immune compartment potentially capable of preventing HIV-1 infection. Herein, we provide a detailed characterization of the immunoglobulin repertoire of cervical and systemic B cells from one such HEPS individual from Nairobi,

Kenya. Analysis was done on 512 VH sequences that were RT-PCR amplified from B cells in a paired sample from the cervix and peripheral blood. The VH3 and DH repertoire of class switched cervical B cells differs significantly from that of systemic B cells indicating that the cervical environment affects local B cell populations and hence VH gene expression. Six networks of clonally related, heavily mutated B cells were identified that spanned the systemic and cervical B cell compartments. Analysis of somatic mutations suggests this is likely the result of systemic, class switched B cells homing to the cervical mucosa. Multiple networks of somatically mutated V-gene sequences, unique to the cervical mucosa, were also identified. This supports the notion that site specific responses occur and have unique regulation of tolerance and recruitment into local memory or blast B cell compartments. We conclude that while the nature of the cervical environment shapes the local B cell repertoire, the infusion of post germinal center B cells to the human cervix is a common occurrence, and represents a means by which systemic immunization could provide the local antibodies necessary to prevent HIV-1 at the site of initial contact.

PMCID: PMC3092619 **Free PMC Article**
PMID: 21293180 [PubMed - indexed for MEDLINE]

79. **HIV-1 drug resistance emergence among breastfeeding infants born to HIV-infected mothers during a single-arm trial of triple-antiretroviral prophylaxis for prevention of mother-to-child transmission: a secondary analysis.**

Zeh C[1], Weidle PJ, Nafisa L, Lwamba HM, Okonji J, Anyango E, Bondo P, Masaba R, Fowler MG, Nkengasong JN, Thigpen MC, Thomas T.

PLoS Med. 2011 Mar;8(3):e1000430. doi: 10.1371/journal.pmed.1000430. Epub 2011 Mar 29.

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**ABSTRACT**

BACKGROUND: Nevirapine and lamivudine given to mothers are transmitted to infants via breastfeeding in quantities sufficient to have biologic effects on the virus; this may lead to an increased risk of a breastfed infant's development of resistance to maternal antiretrovirals. The Kisumu Breastfeeding Study (KiBS), a single-arm open-label prevention of mother-to-child HIV transmission (PMTCT) trial, assessed the safety and efficacy of zidovudine, lamivudine, and either nevirapine or nelfinavir given to HIV-infected women from 34 wk gestation through 6 mo of breastfeeding. Here, we present findings from a KiBS trial secondary analysis that evaluated the emergence of maternal ARV-associated resistance among 32 HIV-infected breastfed infants.

METHODS AND FINDINGS: All infants in the cohort were tested for HIV infection using DNA PCR at multiple study visits during the 24 mo of the study, and plasma RNA viral load for all HIV-PCR-positive infants was evaluated retrospectively. Specimens from mothers and infants with viral load >1,000 copies/ml were tested for HIV drug resistance mutations. Overall, 32 infants were HIV infected by 24 mo of age, and of this group, 24 (75%) infants were HIV infected by 6 mo of age. Of the 24 infants infected by 6 mo, nine were born to mothers on a nelfinavir-based regimen, whereas the remaining 15 were born to mothers on a nevirapine-based regimen. All infants were also given single-dose nevirapine within 48 hours of birth. We detected genotypic resistance mutations in none of eight

infants who were HIV-PCR positive by 2 wk of age (specimens from six infants were not amplifiable), for 30% (6/20) at 6 wk, 63% (14/22) positive at 14 wk, and 67% (16/24) at 6 mo post partum. Among the 16 infants with resistance mutations by 6 mo post partum, the common mutations were M184V and K103N, conferring resistance to lamivudine and nevirapine, respectively. Genotypic resistance was detected among 9/9 (100%) and 7/15 (47%) infected infants whose mothers were on nelfinavir and nevirapine, respectively. No mutations were detected among the eight infants infected after the breastfeeding period (age 6 mo).

CONCLUSIONS: Emergence of HIV drug resistance mutations in HIV-infected infants occurred between 2 wk and 6 mo post partum, most likely because of exposure to maternal ARV drugs through breast milk. Our findings may impact the choice of regimen for ARV treatment of HIV-infected breastfeeding mothers and their infected infants.

TRIAL REGISTRATION: ClinicalTrials.gov NCT00146380.

PMCID: PMC3066134 **Free PMC Article**
PMID: 21468304 [PubMed - indexed for MEDLINE]

80. **HIV-1 drug resistance testing from dried blood spots collected in rural Tanzania using the ViroSeq HIV-1 Genotyping System.**

Johannessen A[1], Garrido C, Zahonero N, Naman E, de Mendoza C.

J Antimicrob Chemother. 2011 Feb;66(2):260-4. doi: 10.1093/jac/dkq433. Epub 2010 Nov 25.

[1]Department of Infectious Diseases, Oslo University Hospital, Ulleval, Oslo, Norway.

**ABSTRACT**

OBJECTIVES: To assess whether the commercial ViroSeq HIV-1 Genotyping System (Abbott Molecular, Des Plains, IL, USA) can be used in conjunction with dried blood spots (DBS) for clinical monitoring of drug resistance in patients who fail antiretroviral treatment (ART) in rural Tanzania.

PATIENTS AND METHODS: Patients at Haydom Lutheran Hospital with confirmed treatment failure (viral load >1000 copies/mL) of a first-line ART regimen were selected for resistance testing. DBS were stored with desiccant at -20 °C for a median of 126 days (range 0-203) and shipped at ambient temperature for 20 days. After manual extraction of nucleic acids, the ViroSeq kit was used for amplification and sequencing. DBS-derived genotypes were compared with those of a plasma-based assay.

RESULTS: Seventeen of 36 (47%) DBS specimens were successfully genotyped. Only 2 of 16 (13%) DBS with a viral load <10,000 copies/mL could be amplified, compared with 15 of 20 (75%) DBS with a viral load >10,000 copies/mL (P = 0.001). In samples that yielded a sequence, all 23 clinically significant reverse transcriptase (RT) mutations in plasma were also detected in DBS. One RT mutation was found in DBS only. In the protease region, 77 polymorphisms were found in plasma, of which 70 (91%) were

also detected in DBS. Sixteen of 17 (94%) patients had identical resistance profiles to antiretroviral drugs in plasma and DBS.

CONCLUSIONS: The ViroSeq kit performed well in patients with a high viral load, but failed to genotype most DBS with a viral load <10,000 copies/mL. In DBS that yielded a genotype, there was high concordance with a plasma-based assay.

PMCID: PMC3019084 **Free PMC Article**
PMID: 21115444 [PubMed - indexed for MEDLINE]

81. **Rates of anti-tuberculosis drug resistance in Kampala-Uganda are low and not associated with HIV infection.**

Lukoye D[1], Cobelens FG, Ezati N, Kirimunda S, Adatu FE, Lule JK, Nuwaha F, Joloba ML.

PLoS One. 2011 Jan 10;6(1):e16130. doi: 10.1371/journal.pone.0016130.

[1]Public Health Department, Kampala City Council, Kampala, Uganda.

**ABSTRACT**

BACKGROUND: Drug resistance among tuberculosis patients in sub-Saharan Africa is increasing, possibly due to association with HIV infection. We studied drug resistance and HIV infection in a representative sample of 533 smear-positive tuberculosis patients diagnosed in Kampala, Uganda.

METHODS/PRINCIPAL FINDINGS: Among 473 new patients, multidrug resistance was found in 5 (1.1%, 95% CI 0.3-2.5) and resistance to any drug in 57 (12.1%, 9.3-15.3). Among 60 previously treated patients this was 7 (11.7%, 4.8-22.6) and 17 (28.3%; 17.5-41.4), respectively. Of 517 patients with HIV results, 165 (31.9%, 27.9-36.1) tested positive. Neither multidrug (adjusted odds ratio (OR(adj)) 0.7; 95% CI 0.19-2.6) nor any resistance (OR(adj) 0.7; 0.43-1.3) was associated with HIV status. Primary resistance to any drug was more common among patients who had worked in health care (OR(adj) 3.5; 1.0-12.0).

CONCLUSION/SIGNIFICANCE: Anti-tuberculosis drug resistance rates in Kampala are low and not associated with HIV infection, but may be associated with exposure during health care.

PMCID: PMC3018425 **Free PMC Article**
PMID: 21249225 [PubMed - indexed for MEDLINE]

82. **Minor drug-resistant HIV type-1 variants in breast milk and plasma of HIV type-1-infected Ugandan women after nevirapine single-dose prophylaxis.**

Pilger D[1], Hauser A, Kuecherer C, Mugenyi K, Kabasinguzi R, Somogyi S, Harms G, Kunz A.

Antivir Ther. 2011;16(1):109-13. doi: 10.3851/IMP1698.

[1]Institute of Tropical Medicine and International Health, Charité - Universitätsmedizin Berlin, Berlin, Germany.

**ABSTRACT**

BACKGROUND: Nevirapine single-dose (NVP-SD) reduces mother-to-child transmission of HIV type-1 (HIV-1), but frequently induces resistance mutations in the HIV-1 genome. Little is known about drug-resistant HIV-1 variants in the breast milk of women who have taken NVP-SD.

METHODS: Blood and breast milk samples of 39 HIV-1-infected Ugandan women were taken 6-12 weeks after NVP-SD intake. Samples were analysed by population sequencing and allele-specific real-time PCR (AS-PCR) with detection limits for NVP-resistant HIV-1 variants (K103N and Y181C) of < 1% of the total viral population.

RESULTS: AS-PCR results for both plasma and breast milk were obtained for 19 women who constituted the final study group (HIV-1 subtype frequencies were A1 n = 11, D n = 5, G n = 2 and C n = 1). A total of 7 (37%) and 10 (53%) women carried NVP-resistant virus in breast milk and plasma, respectively. Overall, 71% (5/7) women with NVP-resistant HIV-1 in breast milk displayed >1 drug-resistant variant. Resistance in breast milk was higher at week 6 (6/13 samples [46%]) compared with week 12 (1/6 samples [17%]). In total, 10 drug-resistant populations harbouring the K103N and/or Y181C mutation were detected in the 19 breast milk samples; 7 (70%) were caused by resistant minorities (< 5% of the total HIV-1 population). In the four women with drug-resistant virus in both plasma and breast milk, the mutation patterns differed between the two compartments.

CONCLUSIONS: Minor populations of drug-resistant HIV-1 were frequently found in breast milk of Ugandan women after exposure to NVP-SD. Further studies need to explore the role of minor drug-resistant variants in the postnatal transmission of (resistant) HIV-1.
PMID: 21311114 [PubMed - indexed for MEDLINE]

83. **Reverse transcriptase inhibitors drug resistance mutations in drug-naive HIV type 1 positive Kenyans.**

Nyamache AK, Waihenya R, Ng'ang'a ZW, Muigai AW, Khamadi SA.

East Afr Med J. 2011 Jan;88(1):4-8.

**ABSTRACT**

OBJECTIVE: To evaluate the extent of HIV-1 drug resistance among drug naive Kenyan individuals.

DESIGN: Cross-sectional study.

SETTING: Kenya Medical Research Institute HIV laboratory Nairobi, Kenya.

SUBJECTS: A total of seventy eight HIV-1 positive drug naive subjects randomised from five Kenyan provincial hospitals between April and June 2004.

RESULTS: A major non-nucleoside reverse transcriptase (NNRTI) an associated mutation was found in one patient (1.3%). NNRTI associated resistance mutations were present at amino acid codon sites G98A (2.56%); K103E (1.3%) and L100F (3.57%) prevalences. Baseline resistance may compromise the response to standard NNRTI-based first-line ART in 1.3 % of the study subjects.

CONCLUSION: This indicates in general, that drug resistance among HIV-1 positive drug naive individual is at low thresholds (1.3%) but the problem could be more serious than reported here. Continuous resistance monitoring is therefore warranted to maintain individual and population-level ART effectiveness.
PMID: 24968596 [PubMed - indexed for MEDLINE]

84. **Early virologic failure and the development of antiretroviral drug resistance mutations in HIV-infected Ugandan children.**

Ruel TD[1], Kamya MR, Li P, Pasutti W, Charlebois ED, Liegler T, Dorsey G, Rosenthal PJ, Havlir DV, Wong JK, Achan J.

J Acquir Immune Defic Syndr. 2011 Jan 1;56(1):44-50. doi: 10.1097/QAI.0b013e3181fbcbf7.

[1]Department of Pediatrics, School of Medicine, University of California, San Francisco, San Francisco, CA 94143-0136, USA. ruelt@peds.ucsf.edu

**ABSTRACT**

BACKGROUND: Without virologic testing, HIV-infected African children starting antiretroviral (ARV) therapy are at risk for undetected virologic failure and the development of ARV resistance. We sought to determine the prevalence of early virologic failure (EVF), to characterize the evolution of ARV-resistance mutations and to predict the impact on second-line therapy.

METHODS: The prevalence of EVF (HIV RNA >400 copies/mL on sequential visits after 6 months of therapy) was identified among 120 HIV-infected Ugandan children starting ARV therapy. ARV mutations were identified by population sequencing of HIV-1 pol in sequential archived specimens. Composite discrete genotypic susceptibility scores were determined for second-line ARV regimens.

RESULTS: EVF occurred in 16 children (13%) and persisted throughout a median (interquartile ratio) 938 (760-1066) days of follow-up. M184V and nonnucleoside reverse transcriptase inhibitor-associated mutations emerged within 6 months of EVF; thymidine-analog-mutations arose after 12 months. Worse discrete genotypic susceptibility scores correlated with increasing duration of failure (Spearman R = -

0.47; P = 0.001). Only 1 child met World Health Organization CD4 criteria for ARV failure at the time of EVF or during the follow-up period.

CONCLUSIONS: A significant portion of HIV-infected African children experience EVF that would be undetected using CD4/clinical monitoring and resulted in the accumulation of ARV mutations that could compromise second-line therapy options.

PMCID: PMC3078046 **Free PMC Article**
PMID: 21099693 [PubMed - indexed for MEDLINE]

85. **Herpes simplex virus type 2 suppressive therapy with acyclovir or valacyclovir does not select for specific HIV-1 resistance in HIV-1/HSV-2 dually infected persons.**

Baeten JM[1], Lingappa J, Beck I, Frenkel LM, Pepper G, Celum C, Wald A, Fife KH, Were E, Mugo N, Sanchez J, Essex M, Makhema J, Kiarie J, Farquhar C, Corey L.

J Infect Dis. 2011 Jan 1;203(1):117-21. doi: 10.1093/infdis/jiq013.

[1]Department of Global Health, University of Washington, Seattle, Washington, USA.

**ABSTRACT**

Recent in vitro studies suggest that acyclovir may directly inhibit HIV-1 replication and can select for a specific HIV-1 reverse transcriptase mutation (V75I) with concomitant loss of an anti-HIV-1 effect. We tested for HIV-1 genotypic resistance at reverse transcriptase codon 75 in plasma from 168 HIV-1-infected persons from Botswana, Kenya, Peru, and the United States taking daily acyclovir or valacyclovir for between 8 weeks and 24 months. No V75I cases were detected (95% confidence interval, 0%-2.2%). These prospective in vivo studies suggest that standard-dose acyclovir or valacyclovir does not select for HIV-1 resistance.

PMCID: PMC3024584 **Free PMC Article**
PMID: 21148504 [PubMed - indexed for MEDLINE]

86. **HIV drug resistance (HIVDR) in antiretroviral therapy-naïve patients in Tanzania not eligible for WHO threshold HIVDR survey is dramatically high.**

Kasang C[1], Kalluvya S, Majinge C, Stich A, Bodem J, Kongola G, Jacobs GB, Mlewa M, Mildner M, Hensel I, Horn A, Preiser W, van Zyl G, Klinker H, Koutsilieri E, Rethwilm A, Scheller C, Weissbrich B.

PLoS One. 2011;6(8):e23091. doi: 10.1371/journal.pone.0023091. Epub 2011 Aug 19.

[1]Institute of Virology and Immunobiology, University of Würzburg, Würzburg, Germany.

**ABSTRACT**

BACKGROUND: The World Health Organization (WHO) has recommended guidelines for a HIV drug resistance (HIVDR) survey for resource-limited countries. Eligibility criteria for patients include age

below 25 years in order to focus on the prevalence of transmitted HIVDR (tHIVDR) in newly-infected individuals. Most of the participating sites across Africa have so far reported tHIVDR prevalences of below 5%. In this study we investigated whether the rate of HIVDR in patients <25 years is representative for HIVDR in the rest of the therapy-naïve population.

METHODS AND FINDINGS: HIVDR was determined in 88 sequentially enrolled ART-naïve patients from Mwanza, Tanzania (mean age 35.4 years). Twenty patients were aged <25 years and 68 patients were aged 25-63 years. The frequency of HIVDR in the study population was 14.8% (95%; CI 0.072-0.223) and independent of NVP-resistance induced by prevention of mother-to-child transmission programs. Patients >25 years had a significantly higher HIVDR frequency than younger patients (19.1%; 95% CI 0.095-0.28) versus 0%, P = 0.0344). In 2 out of the 16 patients with HIVDR we found traces of antiretrovirals (ARVs) in plasma.

CONCLUSIONS: ART-naïve patients aged over 25 years exhibited significantly higher HIVDR than younger patients. Detection of traces of ARVs in individuals with HIVDR suggests that besides transmission, undisclosed misuse of ARVs may constitute a significant factor in the generation of the observed high HIVDR rate. The current WHO tHIVDR survey that is solely focused on the transmission of HIVDR and that excludes patients over 25 years of age may therefore result in substantial underestimation of the prevalence of HIVDR in the therapy-naïve population. Similar studies should be performed also in other areas to test whether the so far reported optimistic picture of low HIVDR prevalence in young individuals is really representative for the rest of the ART-naïve HIV-infected population.

PMCID: PMC3158766 **Free PMC Article**
PMID: 21886779 [PubMed - indexed for MEDLINE]

87. **Emergence and persistence of minor drug-resistant HIV-1 variants in Ugandan women after nevirapine single-dose prophylaxis.**

Hauser A[1], Mugenyi K, Kabasinguzi R, Kuecherer C, Harms G, Kunz A.

PLoS One. 2011;6(5):e20357. doi: 10.1371/journal.pone.0020357. Epub 2011 May 31.

[1]Institute of Tropical Medicine and International Health, Charité-Universitätsmedizin Berlin, Berlin, Germany.

**ABSTRACT**

BACKGROUND: Nevirapine (NVP) single-dose is still a widely used antiretroviral prophylaxis for the prevention of vertical HIV-1 transmission in resource-limited settings. However, the main disadvantage of the Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI) NVP is the rapid selection of NVP-resistant virus with negative implications for subsequent NNRTI-based long-term antiretroviral therapy (ART). Here, we analysed the emergence of drug-resistant HIV-1 including minor variants in the early phase after NVP single-dose prophylaxis and the persistence of drug-resistant virus over time.

METHODS AND FINDINGS: NVP-resistant HIV-1 harbouring the K103N and/or Y181C resistance mutations in the HIV-1 reverse transcriptase gene was measured from 1 week up to 18 months after NVP single-dose prophylaxis in 29 Ugandan women using allele-specific PCR assays capable of detecting

drug-resistant variants representing less than 1% of the whole viral population. In total, drug-resistant HIV-1 was identified in 18/29 (62%) women; rates increased from 18% to 38% and 44% at week 1, 2, 6, respectively, and decreased to 18%, 25%, 13% and 4% at month 3, 6, 12 and 18, respectively. The proportion of NVP-resistant virus of the total viral population was significantly higher in women infected with subtype D (median 40.5%) as compared to subtype A (median 1.3%; p = 0.032, Mann-Whitney U test). 33% of resistant virus was not detectable at week 2 but was for the first time measurable 6-12 weeks after NVP single-dose prophylaxis. Three (10%) women harboured resistant virus in proportions >10% still at month 6.

CONCLUSIONS: Current WHO guidelines recommend an additional postnatal intake of AZT and 3TC for one week to avoid NVP resistance formation. Our findings indicate that a 1-week medication might be too short to impede the emergence of NVP resistance in a substantial proportion of women. Furthermore, subsequent NNRTI-based ART should not be started earlier than 12 months after NVP single-dose prophylaxis.

PMCID: PMC3105030 **Free PMC Article**
PMID: 21655245 [PubMed - indexed for MEDLINE]

88. **Antiretroviral adherence and development of drug resistance are the strongest predictors of genital HIV-1 shedding among women initiating treatment.**

Graham SM[1], Masese L, Gitau R, Jalalian-Lechak Z, Richardson BA, Peshu N, Mandaliya K, Kiarie JN, Jaoko W, Ndinya-Achola J, Overbaugh J, McClelland RS.

J Infect Dis. 2010 Nov 15;202(10):1538-42. doi: 10.1086/656790. Epub 2010 Oct 5.

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**ABSTRACT**

Persistent genital human immunodeficiency virus type 1 (HIV-1) shedding among women receiving antiretroviral therapy (ART) may present a transmission risk. We investigated the associations between genital HIV-1 suppression after ART initiation and adherence, resistance, pretreatment CD4 cell count, and hormonal contraceptive use. First-line ART was initiated in 102 women. Plasma and genital HIV-1 RNA levels were measured at months 0, 3, and 6. Adherence was a strong and consistent predictor of genital HIV-1 suppression (P <.001), whereas genotypic resistance was associated with higher vaginal HIV-1 RNA level at month 6 (P =.04). These results emphasize the importance of adherence to optimize the potential benefits of ART for reducing HIV-1 transmission risk.

PMCID: PMC2957525 **Free PMC Article**
PMID: 20923373 [PubMed - indexed for MEDLINE]

89. **Drug resistance is widespread among children who receive long-term antiretroviral treatment at a rural Tanzanian hospital.**

Bratholm C[1], Johannessen A, Naman E, Gundersen SG, Kivuyo SL, Holberg-Petersen M, Ormaasen V, Bruun JN.

J Antimicrob Chemother. 2010 Sep;65(9):1996-2000. doi: 10.1093/jac/dkq234. Epub 2010 Jun 24.

[1]Department of Infectious Diseases, Oslo University Hospital, Ulleval, Oslo, Norway.

**Comment in:**

- Comment on: Drug resistance is widespread among children who receive long-term antiretroviral treatment at a rural Tanzanian hospital. [J Antimicrob Chemother. 2011]

**ABSTRACT**

OBJECTIVES: To assess long-term virological efficacy and the emergence of drug resistance in children who receive antiretroviral treatment (ART) in rural Tanzania.

PATIENTS AND METHODS: Haydom Lutheran Hospital has provided ART to HIV-infected individuals since 2003. From February through May 2009, a cross-sectional virological efficacy survey was conducted among children (<15 years) who had completed >or=6 months of first-line non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART. Genotypic resistance was determined in those with a viral load of >200 copies/mL.

RESULTS: Virological response was measured in 19 of 23 eligible children; 8 of 19 were girls and median age at ART initiation was 5 years (range 2-14 years). Median duration of ART at the time of the survey was 40 months (range 11-61 months). Only 8 children were virologically suppressed (<or=40 copies/mL), whereas 11 children had clinically relevant resistance mutations in the reverse transcriptase gene. The most frequent mutations were M184V (n = 11), conferring resistance to lamivudine and emtricitabine, and Y181C (n = 4), G190A/S (n = 4) and K103N (n = 4), conferring resistance to NNRTIs. Of concern, three children had thymidine analogue mutations, associated with cross-resistance to all nucleoside reverse transcriptase inhibitors. Despite widespread resistance, however, only one child experienced a new WHO stage 4 event and none had a CD4 cell count of <200 cells/mm(3).

CONCLUSIONS: Among children on long-term ART in rural Tanzania, >50% harboured drug resistance. Results for children were markedly poorer than for adults attending the same programme, underscoring the need for improved treatment strategies for children in resource-limited settings.

PMCID: PMC2920178 **Free PMC Article**
PMID: 20576637 [PubMed - indexed for MEDLINE]

90. **Development and application of a broadly sensitive dried-blood-spot-based genotyping assay for global surveillance of HIV-1 drug resistance.**

Yang C[1], McNulty A, Diallo K, Zhang J, Titanji B, Kassim S, Wadonda-Kabondo N, Aberle-Grasse J, Kibuka T, Ndumbe PM, Vedapuri S, Zhou Z, Chilima B, Nkengasong JN.

J Clin Microbiol. 2010 Sep;48(9):3158-64. doi: 10.1128/JCM.00564-10. Epub 2010 Jul 21.

[1]Division of Global AIDS, International Laboratory Branch, DGHA, NCHHSTP, CDC, Mail Stop A-11, 1600 Clifton Road, Atlanta, GA 30333, USA. cxy0@cdc.gov

**ABSTRACT**

As antiretroviral therapy (ART) is scaled up in resource-limited countries, surveillance for HIV drug resistance (DR) is vital to ensure sustained effectiveness of first-line ART. We have developed and applied a broadly sensitive dried-blood-spot (DBS)-based genotyping assay for surveillance of HIV-1 DR in international settings. In 2005 and 2006, 171 DBS samples were collected under field conditions from newly diagnosed HIV-1-infected individuals from Malawi (n = 58), Tanzania (n = 60), and China (n =53). In addition, 30 DBS and 40 plasma specimens collected from ART patients in China and Cameroon, respectively, were also tested. Of the 171 DBS analyzed at the protease and RT regions, 149 (87.1%) could be genotyped, including 49 (81.7%) from Tanzania, 47 (88.7%) from China, and 53 (91.4%) from Malawi. Among the 70 ART patient samples analyzed, 100% (30/30) of the Chinese DBS and 90% (36/40) of the Cameroonian plasma specimens were genotyped, including 8 samples with a viral load of <400 copies/ml. The results of phylogenetic analyses indicated that the subtype, circulating recombinant form (CRF), and unique recombinant form (URF) distribution was as follows: 73 strains were subtype C (34%), 37 were subtype B (17.2%), 24 each were CRF01_AE or CRF02_AG (11.2% each), 22 were subtype A1 (10.2%), and 9 were unclassifiable (UC) (4.2%). The remaining samples were minor strains comprised of 6 that were CRF07_BC (2.8%), 5 that were CRF10_CD (2.3%), 3 each that were URF_A1C and CRF08_BC (1.4%), 2 each that were G, URF_BC, and URF_D/UC (0.9%), and 1 each that were subtype F1, subtype F2, and URF_A1D (0.5%). Our results indicate that this broadly sensitive genotyping assay can be used to genotype DBS collected from areas with diverse HIV-1 group M subtypes and CRFs. Thus, the assay is likely to become a useful screening tool in the global resistance surveillance and monitoring of HIV-1 where multiple subtypes and CRFs are found.

PMCID: PMC2937690 **Free PMC Article**
PMID: 20660209 [PubMed - indexed for MEDLINE]

91. **Effect of trimethoprim-sulphamethoxazole on the risk of malaria in HIV-infected Ugandan children living in an area of widespread antifolate resistance.**

Gasasira AF[1], Kamya MR, Ochong EO, Vora N, Achan J, Charlebois E, Ruel T, Kateera F, Meya DN, Havlir D, Rosenthal PJ, Dorsey G.

Malar J. 2010 Jun 23;9:177. doi: 10.1186/1475-2875-9-177.

[1]School of Medicine, Makerere University Kampala, Uganda. agasasira@gmail.com

**ABSTRACT**

BACKGROUND: Daily trimethoprim-sulfamethoxazole (TS) protects against malaria, but efficacy may be diminished as anti-folate resistance increases. This study assessed the incidence of falciparum malaria and the prevalence of resistance-conferring Plasmodium falciparum mutations in HIV-infected children receiving daily TS and HIV-uninfected children not taking TS.

MATERIALS AND METHODS: Subjects were 292 HIV-infected and 517 uninfected children from two cohort studies in Kampala, Uganda observed from August 2006 to December 2008. Daily TS was given to HIV-infected, but not HIV-uninfected children and all participants were provided an insecticide-treated bed net. Standardized protocols were used to measure the incidence of malaria and identify markers of antifolate resistance.

RESULTS: Sixty-five episodes of falciparum malaria occurred in HIV-infected and 491 episodes in uninfected children during the observation period. TS was associated with a protective efficacy of 80% (0.10 vs. 0.45 episodes per person year, $p < 0.001$), and efficacy did not vary over three consecutive 9.5 month periods (81%, 74%, 80% respectively, $p = 0.506$). The prevalences of dhfr 51I, 108N, and 59R and dhps 437G and 540E mutations were each over 90% among parasites infecting both HIV-infected and uninfected children. Prevalence of the dhfr 164L mutation, which is associated with high-level resistance, was significantly higher in parasites from HIV-infected compared to uninfected children (8% vs. 1%, $p = 0.001$). Sequencing of the dhfr and dhps genes identified only one additional polymorphism, dhps 581G, in 2 of 30 samples from HIV-infected and 0 of 54 samples from uninfected children.

CONCLUSION: Despite high prevalence of known anti-folate resistance-mediating mutations, TS prophylaxis was highly effective against malaria, but was associated with presence of dhfr 164L mutation.

PMCID: PMC2903607 **Free PMC Article**
PMID: 20573194 [PubMed - indexed for MEDLINE]

92. **Septicaemia in a population-based HIV clinical cohort in rural Uganda, 1996-2007: incidence, aetiology, antimicrobial drug resistance and impact of antiretroviral therapy.**

Mayanja BN[1], Todd J, Hughes P, Van der Paal L, Mugisha JO, Atuhumuza E, Tabuga P, Maher D, Grosskurth H

Trop Med Int Health. 2010 Jun;15(6):697-705. doi: 10.1111/j.1365-3156.2010.02528.x. Epub 2010 Apr 9.

[1]MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda. billy.mayanja@mrcuganda.org

**ABSTRACT**

OBJECTIVES: To describe the incidence and aetiology of septicaemia, and antimicrobial drug resistance in HIV-infected and uninfected individuals, and the impact of antiretroviral therapy (ART) on septicaemia.

METHODS: Between 1996 and 2007, we followed up a rural population-based cohort of HIV-infected and uninfected participants. The aetiology and incidence of septicaemia, and antimicrobial drug resistances were determined. ART became available in 2004, and its impact on the incidence of septicaemia was examined.

RESULTS: The overall septicaemia incidence (per 1000 pyrs) was 32.4 (95% CI 26.2-40.6) but was only 2.6 (95% CI 1.3-6.2) in HIV-negative patients and 67.1 (95% CI 53.4-85.4) in HIV-positive patients not on ART. Among those on ART, the overall incidence was 71.5 (95% CI 47.1-114.3), although it was 121.4 (95%CI 77.9-200.4) in the first year on ART and 37.4 (95%CI 18.9-85.2) in the subsequent period. Septicaemia incidence was significantly associated with lower CD4 counts. The commonest isolates were Streptococcus pneumoniae (SPN, n = 68) and Non-typhi salmonellae (NTS, n = 42). Most SPN isolates were susceptible to ceftriaxone and erythromycin, while resistance to cotrimoxazole and penicillin was common. All NTS isolates were susceptible to ciprofloxacin, but resistance to cotrimoxazole and chloramphenicol was common.

CONCLUSIONS: Septicaemia incidence was higher in HIV-infected than in HIV-uninfected participants, and it remained high for some time among those who started ART. Starting ART earlier at higher CD4 counts is likely to lead to lower septicaemia incidence. Both SPN and NTS, the commonest isolates, were resistant to most commonly available antimicrobials. Blood culture laboratory surveillance systems to monitor antibiotic susceptibility and inform treatment guidelines are needed in Africa.

**Free Article**

PMID: 20406428 [PubMed - indexed for MEDLINE]

93. **Analysis of drug resistance in children receiving antiretroviral therapy for treatment of HIV-1 infection in Uganda.**

Towler WI[1], Barlow-Mosha L, Church JD, Bagenda D, Ajuna P, Mubiru M, Musoke P, Eshleman SH.

AIDS Res Hum Retroviruses. 2010 May;26(5):563-8. doi: 10.1089/aid.2009.0164.

[1]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

**ABSTRACT**

We analyzed drug resistance in HIV-infected Ugandan children who received antiretroviral therapy in a prospective, observational study (2004-2006); some children had prior single-dose nevirapine (sdNVP) exposure. Children received stavudine (d4T), lamivudine (3TC), and nevirapine (NVP); treatment was continued if they were clinically and immunologically stable. Samples with >1,000 copies/ml HIV RNA were analyzed by using the ViroSeq HIV Genotyping System (ViroSeq). Subtype A and D pretreatment samples also were analyzed with the LigAmp assay (for K103N, Y181C, and G190A). ViroSeq results were obtained for 74 pretreatment samples (35 from sdNVP-exposed children (median age, 19 months) and 39 from sdNVP-unexposed children (median age, 84 months). This included 39 subtype A, 22 subtype D, 1 subtype C, and 12 inter-subtype recombinant samples. One sample had nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance, one had nucleoside reverse transcriptase inhibitor (NRTI) resistance, and three had protease inhibitor (PI) resistance. Y181C was detected by using LigAmp in five pretreatment samples [four (14.8%) of 37 samples from sdNVP-exposed children, one (4.2%) of

24 samples from children without prior sdNVP exposure; p = 0.35]. Among children who were not virally suppressed at 48 weeks of treatment, all 12 tested had NNRTI resistance, as well as resistance to 3TC and emtricitibine (FTC); three had resistance to other NRTIs. Seven of those children had a ViroSeq result at 96 weeks of treatment; four of the seven acquired resistance to additional NRTIs by 96 weeks. In Uganda, clinically and immunologically stable children receiving nonsuppressive antiretroviral treatment regimens are at risk for development of drug resistance.

PMCID: PMC2875950 **Free PMC Article**
PMID: 20455758 [PubMed - indexed for MEDLINE]

94. **Oxacillin resistant Staphylococcus aureus among HIV infected and non-infected Kenyan patients.**

Ouko TT[1], Ngeranwa JN, Orinda GO, Bii CC, Amukoye E, Lucy M, Wamae CN.

East Afr Med J. 2010 May;87(5):179-86.

[1]Centre for Microbiology Research, Kenya Medical Research Institute, P. O. Box 54840, Nairobi, Kenya.

**ABSTRACT**

BACKGROUND: Infections due to methicillin resistant S. aureus (MRSA) present global challenges to clinicians since therapeutic options are limited and suboptimal dosing contributes to heightened mortality and increased length of hospital stay particularly among the HIV infected patients.

OBJECTIVES: To assess the prevalence and relative risk of MRSA infections in HIV infected patients.

DESIGN: Cross sectional analytical study.

SETTING: Kenya Medical Research Institute, Opportunistic Infection Laboratories in Nairobi.

SUBJECTS: Four hundred and thirty six male and female patients aged one to 65 years, of whom 220 were HIV-infected and 216 were non-infected.

RESULTS: There was 436 male (57.1%) and female (42.9%) respondents. The prevalence of MRSA was 26.3% with majority infecting the HIV infected patients (P=0.046). Likewise, the overall Staphylococcal infections were more common in HIV patients (P <0.001). The common test for MRSA oxacillin disk diffusion had a sensitivity and specificity of 100% and 92%.

CONCLUSION: HIV is a predisposing factor to Staphylococcal infection and there are indications that treatment with beta-lactam antibiotics may no longer be relied on as sole empiric therapy for several ill HIV patients whose infections may be of MRSA in origin. There is need for an informed choice in administration of appropriate antibiotics in order to minimise treatment failures due to the multidrug resistance and Vanvomycin intermediate S. aureus (VISA) strains. Molecular epidemiology of MRSA strains in understanding new and emerging trends is recommended.
PMID: 23057279 [PubMed - indexed for MEDLINE]

95. **Reduced HIV-1 long terminal repeat transcription in subjects with protective interferon regulatory factor-1 genotype: a potential mechanism mediating resistance to infection by HIV-1.**

Ji H[1], Ball TB, Ao Z, Kimani J, Yao X, Plummer FA.

Scand J Infect Dis. 2010 May;42(5):389-94. doi: 10.3109/00365540903496536.

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Manitoba, Canada. hezhao_ji@phac-aspc.gc.ca

**ABSTRACT**

We previously described the polymorphism in the interferon regulatory factor-1 (IRF-1) gene as a novel correlate of resistance to HIV-1 infection in a Kenyan female sex worker cohort. However, the underlying mechanisms likely mediating this association remained to be elucidated. The initiation of HIV-1 long terminal repeat (LTR) transcription in peripheral blood mononuclear cells (PBMCs) from subjects with different IRF-1 haplotypes, representing protective, intermediate and the least protective IRF-1 allele combinations, were investigated here. A single-cycle pseudovirus construct expressing vesicular stomatitis virus envelop G-protein (VSV-G) and having an HIV-1 pNL4.3 backbone with luciferase insert was used to infect PBMCs with different IRF-1 haplotypes. The efficiency of early HIV-1 LTR transcription was monitored using a luciferase assay. IRF-1 protein levels induced by the infection were measured by quantitative Western blot. Our results showed that PBMCs with the protective IRF-1 genotype demonstrated significantly lower HIV-1 LTR transcription during the initial stages of infection compared to PBMCs with other haplotypes, which correlated with the kinetics of IRF-1 responsiveness to HIV-1 infection in the cells. It suggests that IRF-1 genotypes alter the efficiency of early HIV-1 LTR transcription, likely via modulating expression of IRF-1. This may represent one mechanism mediating the association between IRF-1 polymorphisms and resistance to HIV-1 infection.
PMID: 20100115 [PubMed - indexed for MEDLINE]

96. **Emergence and persistence of nevirapine resistance in breast milk after single-dose nevirapine administration.**

Hudelson SE[1], McConnell MS, Bagenda D, Piwowar-Manning E, Parsons TL, Nolan ML, Bakaki PM, Thigpen MC, Mubiru M, Fowler MG, Eshleman SH.

AIDS. 2010 Feb 20;24(4):557-61. doi: 10.1097/QAD.0b013e3283346e60.

[1]Department of Pathology, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205, USA.

**ABSTRACT**

OBJECTIVE: Single-dose nevirapine (NVP) (sdNVP) can reduce the risk of HIV vertical transmission. We assessed risk factors for NVP resistance in plasma and breast milk from sdNVP-exposed Ugandan women.

METHODS: Samples were analyzed using the Roche AMPLICOR HIV-1 Monitor Test Kit, version 1.5, and the ViroSeq HIV-1 Genotyping System. NVP concentrations were determined by liquid chromatography with tandem mass spectroscopy.

RESULTS: HIV genotypes (plasma and breast milk) were obtained for 30 women 4 weeks after sdNVP (HIV subtypes: 15A, 1C, 12D, two recombinant). NVP resistance was detected in 12 (40%) of 30 breast milk samples. There was a nonsignificant trend between detection of NVP resistance in breast milk and plasma (P = 0.06). There was no association of HIV resistance in breast milk with median maternal pre-NVP viral load or CD4 cell count, median breast milk viral load at 4 weeks, breast milk sodium more than 10 mmol/l, HIV subtype, or concentration of NVP in breast milk or plasma.

CONCLUSION: NVP resistance was frequently detected in breast milk 4 weeks after sdNVP exposure. In this study, we were unable to identify specific factors associated with breast milk NVP resistance.

PMCID: PMC3065236 **Free PMC Article**
PMID: 20057308 [PubMed - indexed for MEDLINE]

97. **HIV type-1 drug resistance testing on dried blood spots is feasible and reliable in patients who fail antiretroviral therapy in rural Tanzania.**

Johannessen A[1], Holberg-Petersen M, Lövgaarden G, Naman E, Ormaasen V, Matee MI, Gundersen SG, Bruun JN.

Antivir Ther. 2010;15(7):1003-9. doi: 10.3851/IMP1660.

[1]Department of Infectious Diseases, Oslo University Hospital, Ullevål, Oslo, Norway.
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**ABSTRACT**

BACKGROUND: HIV type-1 (HIV-1) drug resistance testing is rarely available in resource-limited settings because of high costs and stringent requirements for storage and transport of plasma. Dried blood spots (DBS) can be a convenient alternative to plasma, but the use of DBS needs validation under field conditions. We assessed the performance of DBS in genotypic resistance testing of patients who failed first-line antiretroviral therapy (ART) in rural Tanzania.

METHODS: A total of 36 ART-experienced patients with viral loads >1,000 copies/ml (median 15,180 copies/ml [range 1,350-3,683,000]) and with various HIV-1 subtypes were selected for resistance testing. DBS were stored with desiccant at ambient temperature for a median of 29 days (range 8-89). Samples were amplified using an in-house reverse transcriptase-nested PCR method and sequenced using the ViroSeq™ assay (Abbott Molecular, Des Plaines, IL, USA). DBS-derived genotypes were compared with genotypes from plasma.

RESULTS: Overall, 34 of 36 (94%) DBS specimens were successfully genotyped. In the protease region, of 142 polymorphisms found in plasma, 132 (93%) were also detected in DBS. In the reverse transcriptase region, of 57 clinically relevant mutations present in plasma, 51 (89%) were also detected

in DBS. A total of 30 of 34 (88%) patients had identical resistance profiles to antiretroviral drugs in plasma and DBS.

CONCLUSIONS: Genotyping was successful in the vast majority of DBS specimens stored at ambient temperature for up to 3 months, and there was high concordance between mutations found in DBS and plasma. Our study suggests that DBS can be a feasible and reliable tool to monitor HIV-1 drug resistance in patients on ART in resource-limited settings.
PMID: 21041915 [PubMed - indexed for MEDLINE]

98. **Viral rebound and emergence of drug resistance in the absence of viral load testing: a randomized comparison between zidovudine-lamivudine plus Nevirapine and zidovudine-lamivudine plus Abacavir.**

Ndembi N, Goodall RL, Dunn DT, McCormick A, Burke A, Lyagoba F, Munderi P, Katundu P, Kityo C, Robertson V, Yirrell DL, Walker AS, Gibb DM, Gilks CF, Kaleebu P, Pillay D; Development of Antiretroviral Treatment in Africa Virology Group and Trial Team.

J Infect Dis. 2010 Jan 1;201(1):106-13. doi: 10.1086/648590.

Collaborators: (198)
Munderi P, Kityo C, Walker AS, Gilks C, Gibb DM, Ssali F, Babiker AG, Reid A, Bray D, Darbyshire JH, Grosskurth H, Mugyenyi P, Grosskurth H, Munderi P, Kabuye G, Nsibambi D, Kasirye R, Zalwango E, Nakazibwe M, Kikaire B, Nassuna G, Massa R, Fadhiru K, Namyalo M, Zalwango A, Generous L, Khauka P, Rutikarayo N, Nakahima W, Mugisha A, Todd J, Levin J, Muyingo S, Ruberantwari A, Kaleebu P, Yirrell D, Ndembi N, Lyagoba F, Hughes P, Aber M, Medina Lara A, Foster S, Amurwon J, Nyanzi Wakholi B, Mugyenyi P, Kityo C, Ssali F, Tumukunde D, Otim T, Kabanda J, Musana H, Akao J, Kyomugisha H, Byamukama A, Sabiiti J, Komugyena J, Wavamunno P, Mukiibi S, Drasiku A, Byaruhanga R, Labeja O, Katundu P, Tugume S, Awio P, Namazzi A, Bakeinyaga GT, Katabira H, Abaine D, Tukamushaba J, Anywar W, Ojiambo W, Angweng E, Murungi S, Haguma W, Atwiine S, Kigozi J, Latif A, Hakim J, Robertson V, Reid A, Chidziva E, Bulaya-Tembo R, Musoro G, Taziwa F, Chimbetete C, Chakonza L, Mawora A, Muvirimi C, Tinago G, Svovanapasis P, Simango M, Chirema O, Machingura J, Mutsai S, Phiri M, Bafana T, Chirara M, Muchabaiwa L, Muzambi M, Katabira E, Ronald A, Kambungu A, Lutwama F, Nanfuka A, Walusimbi J, Nabankema E, Nalumenya R, Namuli T, Kulume R, Namata I, Nyachwo L, Florence A, Kusiima A, Lubwama E, Nairuba R, Oketta F, Buluma E, Waita R, Ojiambo H, Sadik F, Wanyama J, Nabongo P, Ochai R, Muhweezi D, Gilks C, Boocock K, Puddephatt C, Winogron D, Bohannon J, Darbyshire J, Gibb DM, Burke A, Bray D, Babiker A, Walker AS, Wilkes H, Rauchenberger M, Sheehan S, Peto L, Taylor K, Spyer M, Ferrier A, Naidoo B, Dunn D, Goodall R, Nanfuka R, Mufuka-Kapuya C, Kaleebu P, Pillay D, Robertson V, Yirrell D, Tugume S, Chirara M, Katundu P, Ndembi N, Lyagoba F, Dunn D, Goodall R, McCormick A, Medina Lara A, Foster S, Amurwon J, Nyanzi Wakholi B, Kigozi J, Muchabaiwa L, Muzambi M, Weller I, Babiker A, Bahendeka S, Bassett M, Chogo Wapakhabulo A, Darbyshire J, Gazzard B, Gilks C, Grosskurth H, Hakim J, Latif A, Mapuchere C, Mugurungi O, Mugyenyi P, Burke C, Jones S, Newland C, Rahim S, Rooney J, Smith M, Snowden W, Steens JM, Breckenridge A, McLaren A, Hill C, Matenga J, Pozniak A, Serwadda D, Peto T, Palfreeman A, Borok M, Katabira E.

**Erratum in:**

- J Infect Dis. 2010 Apr 15;201(8):1278. Ndembi, Nicasie [corrected to Ndembi, Nicaise].

**ABSTRACT**

BACKGROUND: We investigated virological response and the emergence of resistance in the Nevirapine or Abacavir (NORA) substudy of the Development of Antiretroviral Treatment in Africa (DART) trial.

METHODS: Six hundred symptomatic antiretroviral-naive human immunodeficiency virus (HIV)-infected adults (CD4 cell count, <200 cells/mm(3)) from 2 Ugandan centers were randomized to receive zidovudine-lamivudine plus abacavir or nevirapine. Virology was performed retrospectively on stored plasma samples at selected time points. In patients with HIV RNA levels >1000 copies/mL, the residual activity of therapy was calculated as the reduction in HIV RNA level, compared with baseline.

RESULTS: Overall, HIV RNA levels were lower in the nevirapine group than in the abacavir group at 24 and 48 weeks (P <.001), although no differences were observed at weeks 4 and 12. Virological responses were similar in the 2 treatment groups for baseline HIV RNA level <100,000 copies/mL. The mean residual activity at week 48 was higher for abacavir in the presence of the typically observed resistance pattern of thymidine analogue mutations (TAMs) and M184V (1.47 log(10) copies/mL) than for nevirapine with M184V and nonnucleoside reverse-transcriptase inhibitor mutations, whether accompanied by TAMs (0.96 log(10) copies/mL) or not (1.18 log(10) copies/mL).

CONCLUSIONS: There was more extensive genotypic resistance in both treatment groups than is generally seen in resource-rich settings. However, significant residual activity was observed among patients with virological failure, particularly those receiving zidovudine-lamivudine plus abacavir.

**Free Article**

PMID: 19938977 [PubMed - indexed for MEDLINE]

99. **HIV type 1 subtype diversity and drug resistance among HIV type 1-infected Kenyan patients initiating antiretroviral therapy.**

Lihana RW[1], Khamadi SA, Lubano K, Lwembe R, Kiptoo MK, Lagat N, Kinyua JG, Okoth FA, Songok EM, Makokha EP, Ichimura H.

AIDS Res Hum Retroviruses. 2009 Dec;25(12):1211-7. doi: 10.1089/aid.2009.0007.

[1]Center for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya. Rlihana@kemri.org

**ABSTRACT**

The treatment of HIV-1 infection with antiretroviral drugs has greatly improved the survival of those who are infected. However, HIV-1 diversity and drug resistance are major challenges in patient management, especially in resource-poor countries. To evaluate HIV-1 genetic diversity and drug resistance-associated mutations among drug-naive patients in Kenya prior to antiretroviral therapy

(ART), a genetic analysis of HIV-1 pol-RT and env-gp41 was performed on samples collected from 53 (18 males and 35 females) consenting patients between April and June 2005. The average age, baseline CD4(+) T cell counts, and viral loads were 38 (range, 24-62) years, 475 (range, 203-799) cells/mm(3), and 4.7 (range, 3.4-5.9) log(10) copies/ml, respectively. Phylogenetic analysis revealed that 40 samples (75.5%) were concordant subtypes for the two genes and 13 (24.5%) were discordant, suggesting possible recombination and/or dual infections. Prevalent subtypes included A1/A1(pol-RT/env-gp41), 31 (58.5%); D/D, 9 (16.9%); A1/C, 2 (3.8%); A1/D, 4 (7.5%); G/A1, 2 (3.8%); A1/A2, 1 (1.9%); C/A1, 2 (3.8%); D/A1, 1(1.9%); and D/A2, 1 (1.9%). Major reverse transcriptase inhibitor (RTI) resistance-associated mutations were found in four patients (7.5%). Of these patients, three had nucleoside RTI resistance mutations, such as M184V, K65R, D67N, K70R, and K219Q. Nonnucleoside RTI resistance-associated mutations K103N and Y181C were detected in three patients and one patient, respectively. Multiple drug resistance mutations were observed in this drug-naive population. With increasing numbers of patients that require treatment and the rapid upscaling of ART in Kenya, HIV-1 drug resistance testing is recommended before starting treatment in order to achieve better clinical outcomes.

PMID: 19954302 [PubMed - indexed for MEDLINE]

100. **Relative HIV resistance in kenyan sex workers is not due to an altered prevalence or mucosal immune impact of herpes simplex virus type 2 infection.**

Baltzer H[1], Chege D, Rebbapragada A, Wachihi C, Shin LY, Kimani J, Ball TB, Jaoko W, Plummer FA, Kaul R.

Curr HIV Res. 2009 Sep;7(5):504-7.

[1]Department of Medicine, University of Toronto, Toronto, Ontario, Canada.

**ABSTRACT**

Chronic infection by herpes simplex virus type 2 (HSV-2) increases HIV susceptibility, perhaps due to HSV-2-associated increases in activated mucosal immune cells. A small number of Kenyan female sex workers (FSWs) exhibit relative HIV resistance. We examined whether relative HIV resistance was related to differences in the prevalence or mucosal immune impact of HSV-2. Participants were recruited from an open cohort of HIV-uninfected FSWs in Nairobi, Kenya. Women who had been practicing sex work in the cohort for >or=3 years without acquiring HIV were defined as relatively HIV resistant. HSV-2 diagnostics were performed, and cervical immune cell subsets were examined by flow cytometry in a subset of participants. The study population comprised 139 HIV-uninfected FSWs. HSV-2 seroprevalence was actually higher in FSWs meeting criteria for relative HIV resistance than in non-resistant FSWs (75/80, 94% vs 46/59, 78%; LR = 7.5; P = 0.006), likely due to the increased age and longer duration of sex work in the resistant subgroup. Late HIV acquisition was not associated with recent HSV-2 infection, and HSV-2 associated increases in HIV-susceptible cervical immune cell populations were similar in both groups. Relative HIV resistance in Kenyan FSWs was not due to a reduced prevalence or mucosal immune impact of HSV-2 infection.

PMID: 19925401 [PubMed - indexed for MEDLINE]

101. **Lower risk of resistance after short-course HAART compared with zidovudine/single-dose nevirapine used for prevention of HIV-1 mother-to-child transmission.**

Lehman DA[1], Chung MH, Mabuka JM, John-Stewart GC, Kiarie J, Kinuthia J, Overbaugh J.

J Acquir Immune Defic Syndr. 2009 Aug 15;51(5):522-9. doi: 10.1097/QAI.0b013e3181aa8a22.

[1]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA.

**ABSTRACT**

BACKGROUND: Antiretroviral resistance after short-course regimens used to prevent mother-to-child transmission has consequences for later treatment. Directly comparing the prevalence of resistance after short-course regimens of highly active antiretroviral therapy (HAART) and zidovudine plus single-dose nevirapine (ZDV/sdNVP) will provide critical information when assessing the relative merits of these antiretroviral interventions.

METHODS: In a clinical trial in Kenya, pregnant women were randomized to receive either ZDV/sdNVP or a short-course of HAART through 6 months of breastfeeding. Plasma samples were collected 3-12 months after treatment cessation, and resistance to reverse transcriptase inhibitors was assessed using both a sequencing assay and highly sensitive allele-specific polymerase chain reaction assays.

RESULTS: No mutations associated with resistance were detectable by sequencing in either the ZDV/sdNVP or HAART arms at 3 months posttreatment, indicating that resistant viruses were not present in >20% of virus. Using allele-specific polymerase chain reaction assays for K103N and Y181C, we detected low levels of resistant virus in 75% of women treated with ZDV/sdNVP and only 18% of women treated with HAART (P = 0.007). Y181C was more prevalent than K103N at 3 months and showed little evidence of decay by 12 months.

CONCLUSIONS: Our finding provides evidence that compared with ZDV/sdNVP, HAART reduces but does not eliminate nevirapine resistance.

PMCID: PMC2765911 **Free PMC Article**
PMID: 19502990 [PubMed - indexed for MEDLINE]

102. **Virological efficacy and emergence of drug resistance in adults on antiretroviral treatment in rural Tanzania.**

Johannessen A[1], Naman E, Kivuyo SL, Kasubi MJ, Holberg-Petersen M, Matee MI, Gundersen SG, Bruun JN.

BMC Infect Dis. 2009 Jul 7;9:108. doi: 10.1186/1471-2334-9-108.

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**ABSTRACT**

BACKGROUND: Virological response to antiretroviral treatment (ART) in rural Africa is poorly described. We examined virological efficacy and emergence of drug resistance in adults receiving first-line ART for up to 4 years in rural Tanzania.

METHODS: Haydom Lutheran Hospital has provided ART to HIV-infected patients since October 2003. A combination of stavudine or zidovudine with lamivudine and either nevirapine or efavirenz is the standard first-line regimen. Nested in a longitudinal cohort study of patients consecutively starting ART, we carried out a cross-sectional virological efficacy survey between November 2007 and June 2008. HIV viral load was measured in all adults who had completed at least 6 months first-line ART, and genotypic resistance was determined in patients with viral load >1000 copies/mL.

RESULTS: Virological response was measured in 212 patients, of whom 158 (74.5%) were women, and median age was 35 years (interquartile range [IQR] 29-43). Median follow-up time was 22.3 months (IQR 14.0-29.9). Virological suppression, defined as <400 copies/mL, was observed in 187 patients (88.2%). Overall, prevalence of > or =1 clinically significant resistance mutation was 3.9, 8.4, 16.7 and 12.5% in patients receiving ART for 1, 2, 3 and 4 years, respectively. Among those successfully genotyped, the most frequent mutations were M184I/V (64%), conferring resistance to lamivudine, and K103N (27%), Y181C (27%) and G190A (27%), conferring resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs), whereas 23% had thymidine analogue mutations (TAMs), associated with cross-resistance to all nucleoside reverse transcriptase inhibitors (NRTIs). Dual-class resistance, i.e. resistance to both NRTIs and NNRTIs, was found in 64%.

CONCLUSION: Virological suppression rates were good up to 4 years after initiating ART in a rural Tanzanian hospital. However, drug resistance increased with time, and dual-class resistance was common, raising concerns about exhaustion of future antiretroviral drug options. This study might provide a useful forecast of drug resistance and demand for second-line antiretroviral drugs in rural Africa in the coming years.

PMCID: PMC2713244 **Free PMC Article**
PMID: 19583845 [PubMed - indexed for MEDLINE]

103. **Comparison of laboratory methods for analysis of non-nucleoside reverse transcriptase inhibitor resistance in Ugandan infants.**

Church JD[1], Huang W, Parkin N, Marlowe N, Guay LA, Omer SB, Musoke P, Jackson JB, Eshleman SH.

AIDS Res Hum Retroviruses. 2009 Jul;25(7):657-63. doi: 10.1089/aid.2008.0235.

[1]Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

**ABSTRACT**

Detailed comparisons of HIV drug resistance assays are needed to identify the most useful assays for research studies, and to facilitate comparison of results from studies that use different methods. We analyzed nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance in 40 HIV-infected Ugandan infants who had received nevirapine (NVP)-based prophylaxis using the following assays: an FDA-

cleared HIV genotyping assay (the ViroSeq HIV-1 Genotyping System v2.0), a commercially available HIV genotyping assay (GeneSeq HIV), a commercially available HIV phenotyping assay (PhenoSense HIV), and a sensitive point mutation assay (LigAmp). ViroSeq and GeneSeq HIV results (NVP resistance yes/no) were similar for 38 (95%) of 40 samples. In 6 (15%) of 40 samples, GeneSeq HIV detected mutations in minor subpopulations that were not detected by ViroSeq, which identified two additional infants with NVP resistance. LigAmp detected low-level mutations in 12 samples that were not detected by ViroSeq; however, LigAmp testing identified only one additional infant with NVP resistance. GeneSeq HIV and PhenoSense HIV determinations of susceptibility differed for specific NNRTIs in 12 (31%) of the 39 samples containing mixtures at relevant mutation positions. PhenoSense HIV did not detect any infants with NVP resistance who were not identified with GeneSeq HIV testing. In this setting, population sequencing-based methods (ViroSeq and GeneSeq HIV) were the most informative and had concordant results for 95% of the samples. LigAmp was useful for the detection and quantification of minority variants. PhenoSense HIV provided a direct and quantitative measure of NNRTI susceptibility.

PMCID: PMC2799186 **Free PMC Article**
PMID: 19621988 [PubMed - indexed for MEDLINE]

104. **In utero HIV infection is associated with an increased risk of nevirapine resistance in ugandan infants who were exposed to perinatal single dose nevirapine.**

Church JD[1], Mwatha A, Bagenda D, Omer SB, Donnell D, Musoke P, Nakabiito C, Eure C, Bakaki P, Matovu F, Thigpen MC, Guay LA, McConnell M, Fowler MG, Jackson JB, Eshleman SH.

AIDS Res Hum Retroviruses. 2009 Jul;25(7):673-7. doi: 10.1089/aid.2009.0003.

[1]Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

**ABSTRACT**

Use of single dose nevirapine (sdNVP) to prevent HIV mother-to-child transmission is associated with the emergence of NVP resistance in many infants who are HIV infected despite prophylaxis. We combined results from four clinical trials to analyze predictors of NVP resistance in sdNVP-exposed Ugandan infants. Samples were tested with the ViroSeq HIV Genotyping System and a sensitive point mutation assay (LigAmp, for detection of K103N, Y181C, and G190A). NVP resistance was detected at 6-8 weeks in 36 (45.0%) of 80 infants using ViroSeq and 33 (45.8%) of 72 infants using LigAmp. NVP resistance was more frequent among infants who were infected in utero than among infants who were diagnosed with HIV infection after birth by 6-8 weeks of age. Detection of NVP resistance at 6-8 weeks was not associated with HIV subtype (A vs. D), pre-NVP maternal viral load or CD4 cell count, infant viral load at 6-8 weeks, or infant sex. NVP resistance was still detected in some infants 6-12 months after sdNVP exposure. In this study, in utero HIV infection was the only factor associated with detection of NVP resistance in infants 6-8 weeks after sdNVP exposure.

PMCID: PMC2752753 **Free PMC Article**
PMID: 19552593 [PubMed - indexed for MEDLINE]

105. **Identification of nevirapine-resistant HIV-1 in the latent reservoir after single-dose nevirapine to prevent mother-to-child transmission of HIV-1.**

Wind-Rotolo M[1], Durand C, Cranmer L, Reid A, Martinson N, Doherty M, Jilek BL, Kagaayi J, Kizza A, Pillay V, Laeyendecker O, Reynolds SJ, Eshleman SH, Lau B, Ray SC, Siliciano JD, Quinn TC, Siliciano RF.

J Infect Dis. 2009 May 1;199(9):1301-9. doi: 10.1086/597759.

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**ABSTRACT**

BACKGROUND: Intrapartum single-dose nevirapine decreases mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) but promotes nevirapine resistance. Although resistant viruses fade to undetectable levels in plasma, they may persist as stably integrated proviruses within the latent reservoir in resting CD4(+) T cells, potentially complicating future treatment.

METHODS: Blood samples were collected from 60 women from South Africa and Uganda >6 months after they had received single-dose nevirapine. To selectively analyze the stable latent form of HIV-1, resting CD4(+) T cells were isolated and activated in the presence of reverse-transcriptase inhibitors and integrase inhibitors, which allows for the specific isolation of viruses produced by cells with stably integrated proviral DNA. These viruses were then analyzed for nevirapine resistance.

RESULTS: Although only a small number of latently infected cells were present in each blood sample (mean, 162 cells), nevirapine resistance mutations (K103N and G190A) were detected in the latent reservoir of 4 (8%) of 50 evaluable women.

CONCLUSIONS: A single dose of nevirapine can establish antiretroviral resistance within the latent reservoir. This results in a potentially lifelong risk of reemergence of nevirapine-resistant virus and highlights the need for strategies to prevent transmission that do not compromise successful future treatment.

PMCID: PMC2703715 **Free PMC Article**
PMID: 19338474 [PubMed - indexed for MEDLINE]

106. **Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+)FOXP3(+) regulatory T cells.**

Card CM[1], McLaren PJ, Wachihi C, Kimani J, Plummer FA, Fowke KR.

J Infect Dis. 2009 May 1;199(9):1318-22. doi: 10.1086/597801.

[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada.

**ABSTRACT**

Human immunodeficiency virus (HIV)-resistant commercial sex workers provide a unique opportunity to study correlates of protection associated with natural resistance to HIV infection. Emerging data from studies of these individuals and other uninfected individuals who have been exposed to HIV suggest that low levels of immune activation may contribute to protection against infection. In the present study, HIV-resistant individuals were shown to have reduced frequencies of T cells expressing the activation marker CD69. They were also found to have elevated frequencies of regulatory T (T(reg)) cells, compared with HIV-negative control individuals. By controlling levels of T cell activation, T(reg) cells may contribute to HIV resistance by minimizing the pool of cells susceptible to infection.

**Free Article**

PMID: 19301980 [PubMed - indexed for MEDLINE]

107. **Daily trimethoprim-sulfamethoxazole prophylaxis rapidly induces corresponding resistance among intestinal Escherichia coli of HIV-infected adults in Kenya.**

Chiller TM[1], Polyak CS, Brooks JT, Williamson J, Ochieng B, Shi YP, Ouma P, Greene C, Hamel M, Vulule J, Bopp C, Slutsker L, Mintz E.

J Int Assoc Physicians AIDS Care (Chic). 2009 May-Jun;8(3):165-9. doi: 10.1177/1545109709333112. Epub 2009 Apr 8.

[1]Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

**ABSTRACT**

BACKGROUND: Trimethoprim-sulfamethoxazole (TMP-SMZ) has been recommended by World Health Organization (WHO) as daily prophylaxis for Africans with AIDS to prevent opportunistic infections. Daily TMP-SMZ may reduce its susceptibility to commensal intestinal Escherichia coli (E coli), increasing the burden of TMP-SMZ-resistant pathogens.

METHODS: Participants received either daily TMP-SMZ (CD4 <350 cells/mm(3)) or daily multivitamins (MVIs; CD4 > or =350 cells/mm(3)) for 6 months. Stool was collected at baseline, 2 weeks, 2 months, and 6 months. A random E coli was tested for susceptibility.

RESULTS: Baseline prevalence of TMP-SMZ resistance ranged from 71% to 81% and was not different across CD4 strata. At 2 weeks, prevalence of TMP-SMZ-resistant E coli increased significantly from 78% to 98% (P <.001) among persons taking daily TMP-SMZ and did not change among persons taking MVIs.

CONCLUSIONS: Daily prophylaxis with TMP-SMZ induced in vivo resistance to the drug after 2 weeks. Empiric therapy for diarrhea with agents other than TMP-SMZ should be considered for HIV-infected persons receiving daily TMP-SMZ prophylaxis.

PMID: 19357424 [PubMed - indexed for MEDLINE]

108. **Cervical HIV-specific IgA in a population of commercial sex workers correlates with repeated exposure but not resistance to HIV.**

Horton RE[1], Ball TB, Wachichi C, Jaoko W, Rutherford WJ, Mckinnon L, Kaul R, Rebbapragada A, Kimani J, Plummer FA.

AIDS Res Hum Retroviruses. 2009 Jan;25(1):83-92. doi: 10.1089/aid.2008.0207.

[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada. r.horton@griffith.edu.au

**ABSTRACT**

We conducted a comprehensive cross-sectional analysis of total and HIV-specific cervical antibody levels in HIV-1-resistant, uninfected, and infected women in order to examine the role of HIV-specific antibody responses in the female genital tract and examine the effect on antibody levels of various epidemiologic factors in this population. Cervical lavages were collected from 272 subjects of the Pumwani commercial sex worker cohort. Total and HIV-specific genital tract IgA and IgG levels were measured using an ELISA and correlated with behavioral and demographic factors. No significant difference was seen between cervical HIV-specific IgA levels in infected, uninfected, and resistant individuals, nor were any correlations between cervical HIV-specific IgA and neutralization capacity or viral shedding seen. We did, however, note increased HIV-specific IgA in HIV-negative women with four or more clients per day, and decreased HIV-specific IgA in both long-term nonprogressors and long-term survivors. These results show that there is not a strong cohort-wide correlation between HIV-specific cervical IgA levels and resistance to infection by HIV-1 as previously believed, but there is a correlation between exposure to HIV and HIV-specific cervical IgA. Our findings do not preclude the possibility that functional differences in the cervical IgA of HEPS women may play a role in resistance, but argue that HIV-specific responses may not be a universal protective factor. They also indicate that resistance to HIV is a complex condition related to more factors than exposure. Further studies of correlates of immune protection in these individuals would be beneficial to the field.

PMID: 19108692 [PubMed - indexed for MEDLINE]

109. **Evolution of drug resistance after virological failure of a first-line highly active antiretroviral therapy regimen in Uganda.**

Reynolds SJ[1], Kityo C, Mbamanya F, Dewar R, Ssali F, Quinn TC, Mugyenyi P, Dybul M.

Antivir Ther. 2009;14(2):293-7.

[1]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. sjr@jhmi.edu

**ABSTRACT**

BACKGROUND: This study aimed to determine the extent of viral resistance over time among non-clade B HIV type-1-infected patients in Uganda who were maintained on first-line highly active antiretroviral therapy (HAART) following virological failure.

METHODS: Genotyping was performed on 16 patients with virological failure who were enrolled in an open-label randomized clinical trial of short-cycle treatment interruption.

RESULTS: All patients receiving efavirenz-containing HAART had > or =1 efavirenz resistance mutation develop during follow-up. The majority (13/15, 86%) developed lamivudine resistance during follow-up, but no thymidine analogue mutations (TAMs) developed during a median duration of virological failure of 325.5 days.

CONCLUSIONS: Genotype resistance to both efavirenz and lamivudine developed early during the course of treatment after virological failure. TAMs did not emerge early despite moderate exposure time to thymidine analogues during virological failure.

PMCID: PMC2749943 **Free PMC Article**
PMID: 19430104 [PubMed - indexed for MEDLINE]

110. **Cotrimoxazole resistance of Streptococcus pneumoniae and commensal streptococci from Kampala, Uganda.**

Wilén M[1], Buwembo W, Sendagire H, Kironde F, Swedberg G.

Scand J Infect Dis. 2009;41(2):113-21. doi: 10.1080/00365540802651889.

[1]Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden.

**ABSTRACT**

Trimethoprim sulfamethoxazole (cotrimoxazole, CTX) is used frequently as part of standard medical care for people living with HIV/AIDS in Africa. The mechanisms of resistance to sulfonamides and trimethoprim in commensal streptococci from Uganda were determined and compared to S. pneumoniae. Commensal streptococci showing high-level resistance to cotrimoxazole were cultured and analysed for species identity and polymorphisms in the genes coding for dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR). Seven isolates of S. pneumoniae from blood and cerebrospinal fluid (CSF) were similarly examined. There was considerable polymorphism in both DHPS and DHFR. In DHFR, the mutations E20D and I100L were present in all sequenced isolates. Other mutations such as L135F, and different substitutions in D92, were frequent. The most common DHPS variants had 2 serine residues added after amino acid 60, or arginine and proline added after amino acid 59. In addition, 3 new insertions/substitutions were found. There were no obvious differences between the mutation patterns in S. pneumoniae and commensal streptococci, suggesting that the chromosomal mutations have been spread by transformational interchanges of DNA among related organisms.
PMID: 19140088 [PubMed - indexed for MEDLINE]

111. **Prevalence of nevirapine-associated resistance mutations after single dose prophylactic treatment among antenatal clinic attendees in north rift Kenya.**

Kiptoo M[1], Ichimura H, Wembe RL, Ng'Ang'a Z, Mueke J, Kinyua J, Lagat N, Okoth F, Songok EM.

AIDS Res Hum Retroviruses. 2008 Dec;24(12):1555-9. doi: 10.1089/aid.2008.0018.

[1]Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya.

**ABSTRACT**

The use of single dose nevirapine to prevent mother-to-child transmission of HIV has been reported to induce drug-resistant mutations and reduce options for antiretroviral treatment for HIV-infected mothers and their children. To explore the status of nevirapine-resistant HIV genotypes in rural hospitals in the North Rift Valley Province of Kenya, samples collected 3 months after single dose nevirapine from 36 mothers and their children were analyzed. Resistance mutations were genotypically evaluated through proviral DNA amplification, cloning, and sequencing. Ten mothers (27.8%) had antiretroviral-associated resistance mutations of whom four (11.1%) had specific nevirapine (NNRTI) resistance-associated mutations. Three mothers (8.3%) transmitted the infection to their infants. This presence of nevirapine mutations in rural antenatal clinic attendees confirms the importance of integrating antiretroviral resistance monitoring as a key component in programs geared to prevention of HIV mother-to-child transmission.
PMID: 19102687 [PubMed - indexed for MEDLINE]

112. **Identification of differentially expressed proteins in the cervical mucosa of HIV-1-resistant sex workers.**

Burgener A[1], Boutilier J, Wachihi C, Kimani J, Carpenter M, Westmacott G, Cheng K, Ball TB, Plummer F.

J Proteome Res. 2008 Oct;7(10):4446-54. doi: 10.1021/pr800406r. Epub 2008 Aug 16.

[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2. burgener@cc.umanitoba.ca

**ABSTRACT**

Novel tools are necessary to understand mechanisms of altered susceptibility to HIV-1 infection in women of the Pumwani Sex Worker cohort, Kenya. In this cohort, more than 140 of the 2000 participants have been characterized to be relatively resistant to HIV-1 infection. Given that sexual transmission of HIV-1 occurs through mucosal surfaces such as that in the cervicovaginal environment, our hypothesis is that innate immune factors in the genital tract may play a role in HIV-1 infection resistance. Understanding this mechanism may help develop microbicides and/or vaccines against HIV-1. A quantitative proteomics technique (2D-DIGE: two-dimensional difference in-gel electrophoresis) was used to examine cervical mucosa of HIV-1 resistant women ( n = 10) for biomarkers of HIV-1 resistance. Over 15 proteins were found to be differentially expressed between HIV-1-resistant women and control groups ( n = 29), some which show a greater than 8-fold change. HIV-1-resistant women overexpressed several antiproteases, including those from the serpin B family,

and also cystatin A, a known anti-HIV-1 factor. Immunoblotting for a selection of the identified proteins confirmed the DIGE volume differences. Validation of these results on a larger sample of individuals will provide further evidence these biomarkers are associated with HIV-1 resistance and could help aid in the development of effective microbicides against HIV-1.

PMID: 18707157 [PubMed - indexed for MEDLINE]

113. **Analysis of nevirapine resistance mutations in cloned HIV type 1 variants from HIV-infected Ugandan infants using a single-step amplification-sequencing method (AmpliSeq).**

Towler WI[1], Church JD, Eshleman JR, Fowler MG, Guay LA, Jackson JB, Eshleman SH.

AIDS Res Hum Retroviruses. 2008 Sep;24(9):1209-13. doi: 10.1089/aid.2008.0109.

[1]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

**ABSTRACT**

We analyzed the genetic linkage of nevirapine (NVP) resistance mutations and the genetic complexity of HIV-1 variants in Ugandan infants who were HIV infected despite single dose (SD) prophylaxis. Plasma samples were obtained from six HIV-infected infants who had two or more NVP resistance mutations detected by population sequencing (ViroSeq). ViroSeq PCR products were cloned and transformed, and a single-step amplification-sequencing reaction (AmpliSeq) was used to analyze NVP resistance mutations in cloned HIV-1 variants directly from bacterial colonies. Fifty clones were analyzed for each infant sample. This analysis revealed numerous NVP resistance mutations not detected by population sequencing, genetically linked NVP resistance mutations, and a high degree of genetic complexity at codons that influence NVP susceptibility.

PMCID: PMC2562759 **Free PMC Article**

PMID: 18788912 [PubMed - indexed for MEDLINE]

114. **Does cotrimoxazole prophylaxis for the prevention of HIV-associated opportunistic infections select for resistant pathogens in Kenyan adults?**

Hamel MJ[1], Greene C, Chiller T, Ouma P, Polyak C, Otieno K, Williamson J, Shi YP, Feikin DR, Marston B, Brooks JT, Poe A, Zhou Z, Ochieng B, Mintz E, Slutsker L.

Am J Trop Med Hyg. 2008 Sep;79(3):320-30.

[1]Centers for Disease Control and Prevention, Atlanta, Georgia, USA. mhamel@ke.cdc.gov

**ABSTRACT**

We assessed the effect of daily cotrimoxazole, essential for HIV care, on development of antifolate-resistant Plasmodium falciparum, naso-pharyngeal Streptococcus pneumoniae (pneumococcus), and commensal Escherichia coli. HIV-positive subjects with CD4 cell count < 350 cells/muL (lower-CD4; N = 692) received cotrimoxazole; HIV-positive with CD4 cell count > or = 350 cells/muL (higher-CD4; N =

336) and HIV-negative subjects (N = 132) received multivitamins. Specimens were collected at baseline, 2 weeks, monthly, and at sick visits during 6 months of follow-up to compare changes in resistance, with higher-CD4 as referent. P. falciparum parasitemia incidence density was 16 and 156/100 person-years in lower-CD4 and higher-CD4, respectively (adjusted rate ratio [ARR] = 0.11; 95% confidence interval [CI] = 0.06-0.15; P < 0.001) and 97/100 person-years in HIV-negative subjects (ARR = 0.62; 95% CI = 0.44-0.86; P = 005). Incidence density of triple and quintuple dihydrofolate-reductase/dihydropteroate-synthetase mutations was 90% reduced in lower-CD4 compared with referent. Overall, cotrimoxazole non-susceptibility was high among isolated pneumococcus (92%) and E. coli (76%) and increased significantly in lower-CD4 subjects by Week 2 (P < 0.005). Daily cotrimoxazole prevented malaria and reduced incidence of antifolate-resistant P. falciparum but contributed to increased pneumococcus and commensal Escherichia coli resistance.

**Free Article**

PMID: 18784222 [PubMed - indexed for MEDLINE]

115. **Transmitted antiretroviral drug resistance surveillance among newly HIV type 1-diagnosed women attending an antenatal clinic in Entebbe, Uganda.**

Ndembi N[1], Lyagoba F, Nanteza B, Kushemererwa G, Serwanga J, Katongole-Mbidde E, Grosskurth H, Kaleebu P; Uganda HIV Drug Resistance Working Group.

AIDS Res Hum Retroviruses. 2008 Jun;24(6):889-95. doi: 10.1089/aid.2007.0317.

[1]MRC/UVR1/Uganda Research Unit on AIDS, Entebbe, Uganda.

**ABSTRACT**

To evaluate transmitted HIV-1 drug resistance and study the natural polymorphism in pol of HIV-1 strains of newly diagnosed women attending an antenatal clinic in Uganda we sequenced the protease and reverse transcriptase genes for 46 HIV-1 strains from the threshold surveillance. Of the 46 sequences analyzed, 48.0% were subtype A1 (n 22), 39.0% subtype D (n 18), 2.0% subtype A2 (n 1), 2.0% subtype C (n 1), and 9.0% intersubtype recombinant A1/D (n 4). Overall, many minor mutations were identified in the protease sequences. None of the strains had major associated mutations to any RTI drug or drug class interest after genotyping 37 samples of our cohort. The HIV drug resistance prevalence estimate in Entebbe following the HIVDR-TS methodology is less than 5% as set out by WHO guidelines.

PMID: 18544019 [PubMed - indexed for MEDLINE]

116. **Associations of human leukocyte antigen DRB with resistance or susceptibility to HIV-1 infection in the Pumwani Sex Worker Cohort.**

Lacap PA[1], Huntington JD, Luo M, Nagelkerke NJ, Bielawny T, Kimani J, Wachihi C, Ngugi EN, Plummer FA.

AIDS. 2008 May 31;22(9):1029-38. doi: 10.1097/QAD.0b013e3282ffb3db.

[1]National Microbiology Laboratory, Winnipeg, Canada.

**ABSTRACT**

OBJECTIVE: A group of commercial sex workers in the Pumwani Sex Worker Cohort, established in 1985 in Nairobi, Kenya, remain HIV-1 uninfected despite heavy exposure to HIV-1 through active sex work. Previous studies showed that this resistance is associated with a strong CD4+ T-cell response, which suggested that human leukocyte antigen class II antigens are important in resistance/susceptibility to HIV-1 infection. DRB1 is the most polymorphic locus among class II genes and forms haplotypes with DRB3, DRB4 and DRB5. The aim of this study is to investigate the role of DRB alleles/haplotypes on resistance/susceptibility to HIV-1 infection.

DESIGN: In total, 1090 women enrolled in the Pumwani cohort were genotyped for DRB1, DRB3, DRB4 and DRB5 using a high-resolution sequence-based method. Allele/haplotype frequencies were compared between HIV-positive women and women who have remained HIV negative for more than 3 years despite frequent exposure.

METHODS: Human leukocyte antigen DRB genes were amplified, sequenced and genotyped using a two-step sequence-based method. Allele/haplotype frequencies were determined using PyPop32-0.6.0. Statistical analysis was conducted using SPSS 11.0 for Windows.

RESULTS: Three DRB1 alleles were associated with resistance: DRB1*010101 (P = 0.016; odd ratio (OR): 2.55; 95% confidence interval (CI): 1.16-5.61), DRB1*010201 (P = 0.019; OR: 1.86; 95% CI: 1.10-3.15), and DRB1*1102 (P = 0.025; OR: 1.72; 95% CI: 1.07-2.78). DRB1*030201 (P = 0.038; OR: 0.48; 95% CI: 0.23-0.98), DRB1*070101 (P = 0.035; OR: 0.54; 95% CI: 0.30-0.97), DRB1*1503 (P = 0.0004; OR: 0.34; 95% CI: 0.19-0.64), and DRB5*010101 (P = 0.001; OR: 0.37; 95% CI: 0.20-0.67) were associated with susceptibility. The haplotype DRB1*1102-DRB3*020201 was associated with HIV-1 resistance (P = 0.041; OR: 1.68; 95% CI: 1.02-2.78), whereas the haplotypes DRB1*070101-DRB4*01010101 (P = 0.041; OR: 0.52; 95% CI: 0.28-0.98) and DRB1*1503-DRB5*01010101 (P = 0.0002; OR: 0.30; 95% CI: 0.15-0.58) were associated with susceptibility. These associations with resistance/susceptibility to HIV-1 were independent of previously reported alleles HLA-DRB1*01 and HLA-A*2301.

CONCLUSION: Our findings indicate that human leukocyte antigen DRB-specific CD4+ T-cell responses are an important factor in resistance/susceptibility to HIV-1 infection.
PMID: 18520346 [PubMed - indexed for MEDLINE]

117. **Human leukocyte antigen-DQ alleles and haplotypes and their associations with resistance and susceptibility to HIV-1 infection.**

Hardie RA[1], Luo M, Bruneau B, Knight E, Nagelkerke NJ, Kimani J, Wachihi C, Ngugi EN, Plummer FA.

AIDS. 2008 Apr 23;22(7):807-16. doi: 10.1097/QAD.0b013e3282f51b71.

[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

**ABSTRACT**

OBJECTIVES: To determine the association of DQ antigens with resistance and susceptibility to HIV-1.

DESIGN: Despite repeated exposure to HIV-1, a subset of women in the Pumwani Sex Worker cohort established in Nairobi, Kenya in 1985 have remained HIV-1 negative for at least 3 years and are classified as resistant. Differential susceptibility to HIV-1 infection is associated with HIV-1 specific CD4 and CD8 T cell responses. As human leukocyte antigen-DQ antigens present viral peptides to CD4 cells, we genotyped human leukocyte antigen -DQ alleles for 978 women enrolled in the cohort and performed cross-sectional and longitudinal analyses to identify associations of human leukocyte antigen -DQ with resistance/susceptibility to HIV-1.

METHODS: DQA1 and DQB1 were genotyped using taxonomy-based sequence analysis. SPSS 13.0 was used to determine associations of DQ alleles/haplotypes with HIV-1 resistance, susceptibility, and seroconversion rates.

RESULTS: Several DQB1 alleles and DQ haplotypes were associated with resistance to HIV-1 infection. These included DQB1*050301 (P = 0.055, Odds Ratio = 12.77, 95% Confidence Interval = 1.44-112), DQB1*0603 and DQB1*0609 (P = 0.037, Odds Ratio = 3.25, 95% Confidence Interval = 1.12-9.47), and DQA1*010201-DQB1*0603 (P = 0.044, Odds Ratio = 17.33, 95% Confidence Interval = 1.79-168). Conversely, DQB1*0602 (P = 0.048, Odds Ratio = 0.68, 95% Confidence Interval = 0.44-1.05) and DQA1*010201-DQB1*0602 (P = 0.039, Odds Ratio = 0.64, 95% Confidence Interval = 0.41-1.03) were overrepresented in the HIV-1 infected population. DQA1*0504-DQB1*0201, DQA1*010201-DQB1*0201, DQA1*0402-DQB1*0402 and DQA1*0402-DQB1*030101 genotypes were only found in HIV-1 positive subjects (Odds Ratio = 0.30-0.31, 95% Confidence Interval = 0.03-3.70), and these women seroconverted rapidly. The associations of these DQ alleles and haplotypes with resistance and susceptibility to HIV-1 were independent of the previously reported human leukocyte antigen-DRB*01, human leukocyte antigen A2/6802, and human leukocyte antigen-A*2301.

CONCLUSION: The associations of DQ alleles and haplotypes with resistance and susceptibility to HIV-1 emphasize the importance of human leukocyte antigen-DQ and CD4 in anti-HIV-1 immunity.
PMID: 18427198 [PubMed - indexed for MEDLINE]

118. **Effect of trimethoprim-sulfamethoxazole prophylaxis on antimicrobial resistance of fecal Escherichia coli in HIV-infected patients in Tanzania.**

Morpeth SC[1], Thielman NM, Ramadhani HO, Hamilton JD, Ostermann J, Kisenge PR, Shao HJ, Reller LB, Itemba DK, Sam NE, Bartlett JA, Shao JF, Crump JA.

J Acquir Immune Defic Syndr. 2008 Apr 15;47(5):585-91. doi: 10.1097/QAI.0b013e31816856db.

[1]Division of Infectious Diseases and International Health, Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA.

**ABSTRACT**

BACKGROUND: Trimethoprim-sulfamethoxazole (SXT) reduces morbidity and mortality among HIV-infected persons in Africa, but its impact on antimicrobial resistance is of concern.

METHODS: HIV-uninfected (group A), HIV-infected but not requiring SXT (group B), and HIV-infected and eligible for SXT (group C) adults were recruited into a prospective observational cohort study in Moshi, Tanzania. Stool was examined for Escherichia coli nonsusceptible to SXT at baseline and at weeks 1, 2, 4, and 24. General estimating equation models were used to assess differences in susceptibility over time and cross-resistance to other antimicrobials.

RESULTS: Of 181 subjects, 118 (65.1%) were female and the median (range) age was 36 (20 to 72) years. At baseline, E. coli nonsusceptible to SXT was isolated from 23 (53.5%) of 43 patients in group A, 25 (67.6%) of 37 patients in group B, and 37 (64.9%) of 57 patients in group C. The odds ratios (P value) for SXT nonsusceptibility in group C at weeks 1, 2, 4, and 24 compared with baseline were 3.4 (0.013), 3.0 (0.019), 2.9 (0.030), and 1.5 (0.515), respectively. SXT nonsusceptibility was associated with nonsusceptibility to ampicillin, chloramphenicol, ciprofloxacin, and nalidixic acid (P <or= 0.006).

CONCLUSION: In Tanzania, carriage of fecal E. coli nonsusceptible to SXT is common before SXT prophylaxis. Initiation of SXT leads to further loss of susceptibility to SXT and to other antimicrobials.

PMCID: PMC2586846 **Free PMC Article**
PMID: 18285712 [PubMed - indexed for MEDLINE]

119. **A radiolabeled oligonucleotide ligation assay demonstrates the high frequency of nevirapine resistance mutations in HIV type 1 quasispecies of NVP-treated and untreated mother-infant pairs from Uganda.**

Troyer RM[1], Lalonde MS, Fraundorf E, Demers KR, Kyeyune F, Mugyenyi P, Syed A, Whalen CC, Bajunirwe F, Arts EJ.

AIDS Res Hum Retroviruses. 2008 Feb;24(2):235-50. doi: 10.1089/aid.2007.0138.

[1]Division of Infectious Diseases, Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, USA.

**ABSTRACT**

This study explores the levels of NVP and AZT resistance mutations in untreated, NVP- or AZT-treated mother-infant pairs in Uganda. PCR-amplified reverse transcriptase (RT) gene fragments derived from PBMC samples of 85 mothers (10 AZT treated, 35 NVP treated, and 40 untreated) and their 52 infected infants (5 AZT, 9 NVP, and 38 untreated) were classified as subtype A (59%), D (29%), C (3%), and recombinant forms (9%) by population sequencing. Only 16% of the NVP-treated infected mothers and infants harbored either the K103N or the Y181C at 6 weeks postdelivery. The majority of these samples (n = 107) were then analyzed using a radiolabeled oligonucleotide ligation assay (OLA) specific for K70R, K103N, and Y181C, using nonstandard bases to accommodate sequence heterogeneity. By OLA, 43% of the NVP-treated group had K103N and/or Y181C mutations in their HIV-1 population, using >0.6% cutoff based on a comparative clonal analysis of clinical isolates. Surprisingly, an equal fraction of the untreated and NVP-treated mother-infant group had the K103N mutation in their HIV-1 population in the range of 0.6-5%. These findings suggest a relatively high frequency of K103N mutation in the drug-naive, subtype A and D infected Ugandan population as compared to the very low frequency of the Y181C and K70R mutation (<0.6%). The prevalence of the K103N mutations may be related to its low fitness cost and high genetic stability. The persistence of these mutations may reduce the effectiveness of subsequent NVP use in treatment or prevention of perinatal transmission.

PMID: 18284323 [PubMed - indexed for MEDLINE]

120. **Surveillance of transmitted HIV drug resistance among women attending antenatal clinics in Dar es Salaam, Tanzania.**

Somi GR[1], Kibuka T, Diallo K, Tuhuma T, Bennett DE, Yang C, Kagoma C, Lyamuya EF, Swai RO, Kassim S.

Antivir Ther. 2008;13 Suppl 2:77-82.

[1]National AIDS Control Program, Dar es Salaam, Tanzania.

**ABSTRACT**

BACKGROUND: In resource-limited settings where antiretroviral treatment (ART) access is being scaled-up, the World Health Organization (WHO) recommends surveillance of transmitted HIV drug resistance (HIVDR). We used the WHO HIVDR threshold survey method to assess transmitted HIVDR in Dar es Salaam where ART was introduced in 1995 and where approximately 11,000 people are currently on ART.

METHODS: From November 2005 to February 2006, dried blood spot (DBS) specimens were made from remnant specimens collected during the national HIV serosurvey from 60 primagravidas <25 years old attending six antenatal clinics for routine syphilis testing. Genotyping was performed at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Protease and reverse transcriptase drug resistance mutations were identified using the Stanford University HIV drug resistance database. We used the National Institutes of Health genotyping tool for HIV-1 subtyping. HIVDR prevalence categorization was based on the WHO threshold survey binomial sequential sampling method.

RESULTS: Among the 60 eligible specimens collected, 50 DBS were successfully amplified using RT-PCR. Sequencing was performed on the first 39 specimens: 13 (33.3%) were subtype A1, 13 (33.3%)

subtype C, and 4 (10.3%) subtype D, the remainder differed in the closest subtype based on protease versus reverse transcriptase. No resistance mutations were seen; HIVDR to all drug classes was categorized as <5%.

CONCLUSIONS: Our survey indicates that prevalence of transmitted HIVDR among recently infected pregnant women in Dar es Salaam is low (<5/%). The survey should be repeated during the next HIV sentinel survey in Dar es Salaam and extended to other regions where ART is being scaled up.
PMID: 18575194 [PubMed - indexed for MEDLINE]

121. **Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania.**

Ramadhani HO[1], Thielman NM, Landman KZ, Ndosi EM, Gao F, Kirchherr JL, Shah R, Shao HJ, Morpeth SC, McNeill JD, Shao JF, Bartlett JA, Crump JA.

Clin Infect Dis. 2007 Dec 1;45(11):1492-8. Epub 2007 Oct 22.

[1]Kilimanjaro Christian Medical Centre, Tumaini University, Moshi, Tanzania.

**ABSTRACT**

BACKGROUND: Access to antiretroviral therapy is rapidly expanding in sub-Saharan Africa. Identifying the predictors of incomplete adherence, virologic failure, and antiviral drug resistance is essential to achieving long-term success.

METHODS: A total of 150 subjects who had received antiretroviral therapy for at least 6 months completed a structured questionnaire and adherence assessment, and plasma human immunodeficiency virus (HIV) RNA levels were measured. Virologic failure was defined as an HIV RNA level >400 copies/mL; for patients with an HIV RNA level >1000 copies/mL, genotypic antiviral drug resistance testing was performed. Predictors were analyzed using bivariable and multivariable logistic regression models.

RESULTS: A total of 23 (16%) of 150 subjects reported incomplete adherence. Sacrificing health care for other necessities (adjusted odds ratio [AOR], 19.8; P<.01) and the proportion of months receiving self-funded treatment (AOR, 23.5; P=.04) were associated with incomplete adherence. Virologic failure was identified in 48 (32%) of 150 subjects and was associated with incomplete adherence (AOR, 3.6; P=.03) and the proportion of months receiving self-funded antiretroviral therapy (AOR, 13.0; P=.02). Disclosure of HIV infection status to family members or others was protective against virologic failure (AOR, 0.10; P=.04).

CONCLUSIONS: Self-funded treatment was associated with incomplete adherence and virologic failure, and disclosure of HIV infection status was protective against virologic failure. Efforts to provide free antiretroviral therapy and to promote social coping may enhance adherence and reduce rates of virologic failure.

**Free Article**

PMID: 17990233 [PubMed - indexed for MEDLINE]

122. **Etiology and resistance patterns of respiratory isolates in Kenyan adults with AIDS from slum population.**

Krcmery V[1], Benca J, Liskova A, Mitterpachova E, Kolenova A, Sladeckova V, Horvathova D, Kiwou M. Neuro Endocrinol Lett. 2007 Nov;28 Suppl 3:37-9.

[1]Mary Immaculate Clinic Nairobi, Kenya.

**ABSTRACT**

We investigated regularly swabs of adults dispenzarised at Mary Immaculate Clinic of Trnava University in Nairobi providing free health care for about 50 000 population of Mukuru Slums. 20 patients who were treated for AIDS by our clinic (those who started HAART before Free National AIDS Cooperation Programme - NASCOP) were assessed after 1, 2 and 3 years (18 of 20 completed the survey, other 2 loss of follow up, probably died. Exposure to other molecules can select resistant mutants. Previous exposure to TMP/SMX was similar in both groups and therefore was not responsible for the difference between resistance patterns.

PMID: 18030279 [PubMed - indexed for MEDLINE]

123. **Independent associations of insulin resistance with high whole-body intermuscular and low leg subcutaneous adipose tissue distribution in obese HIV-infected women.**

Albu JB[1], Kenya S, He Q, Wainwright M, Berk ES, Heshka S, Kotler DP, Engelson ES.

Am J Clin Nutr. 2007 Jul;86(1):100-6.

[1]Endocrine, Diabetes and Nutrition Division, the New York Obesity Research Center, St Luke's Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, NY 10025, USA. jba1@columbia.edu

**ABSTRACT**

BACKGROUND: Obesity and insulin resistance are growing problems in HIV-positive (HIV+) women receiving highly active antiretroviral therapy (HAART).

OBJECTIVE: The objective was to determine the contribution of adipose tissue (AT) enlargement and distribution to the presence of insulin resistance in obese HIV+ women.

DESIGN: Whole-body intermuscular AT (IMAT), visceral AT (VAT), subcutaneous AT (SAT), and SAT distribution (leg versus upper body) were measured by whole-body magnetic resonance imaging. Insulin sensitivity (S(I)) was measured with an intravenous glucose tolerance test in obese HIV+ women recruited because of their desire to lose weight (n=17) and in obese healthy controls (n=32).

RESULTS: The HIV+ women had relatively less whole-body SAT and more VAT and IMAT than did the controls ($P<0.05$ for all). A significant interaction by HIV status was observed for the relation of total SAT with S(I) ($P<0.001$ for the regression's slope interactions after adjustment for age, height, and

weight). However, relations of IMAT, VAT, and SAT distribution (leg SAT as a percentage of total SAT; leg SAT%) with S(I) did not differ significantly between groups. For both groups combined, the best model predicting a low S(I) included significant contributions by both high IMAT and low leg SAT%, independent of age, height, and weight, and no interaction between groups was observed (overall r(2)=0.44, P=0.0003).

CONCLUSION: In obese HIV+ women, high whole-body IMAT and low leg SAT% distribution are independently associated with insulin resistance.

PMCID: PMC2670485 **Free PMC Article**
PMID: 17616768 [PubMed - indexed for MEDLINE]

124. **Anti-retroviral drug resistance-associated mutations among non-subtype B HIV-1-infected Kenyan children with treatment failure.**

Lwembe R[1], Ochieng W, Panikulam A, Mongoina CO, Palakudy T, Koizumi Y, Kageyama S, Yamamoto N, Shioda T, Musoke R, Owens M, Songok EM, Okoth FA, Ichimura H.
J Med Virol. 2007 Jul;79(7):865-72.
[1]Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya.

**ABSTRACT**

Recently increased availability of anti-retroviral therapy (ART) has mitigated HIV-1/AIDS prognoses especially in resource poor settings. The emergence of ART resistance-associated mutations from non-suppressive ART has been implicated as a major cause of ART failure. Reverse transcriptase inhibitor (RTI)-resistance mutations among 12 non-subtype B HIV-1-infected children with treatment failure were evaluated by genotypically analyzing HIV-1 strains isolated from plasma obtained between 2001 and 2004. A region of pol-RT gene was amplified and at least five clones per sample were analyzed. Phylogenetic analysis revealed HIV-1 subtype A1 (n = 7), subtype C (n = 1), subtype D (n = 3), and CRF02_AG (n = 1). Before treatment, 4 of 12 (33.3%) children had primary RTI-resistance mutations, K103N (n = 3, ages 5-7 years) and Y181C (n = 1, age 1 year). In one child, K103N was found as a minor population (1/5 clones) before treatment and became major (7/7 clones) 8 months after RTI treatment. In 7 of 12 children, M184V appeared with one thymidine-analogue-associated mutation (TAM) as the first mutation, while the remaining 5 children had only TAMs appearing either individually (n = 2), or as TAMs 1 (M41L, L210W, and T215Y) and 2 (D67N, K70R, and K219Q/E/R) appearing together (n = 3). These results suggest that "vertically transmitted" primary RTI-resistance mutations, K103N and Y181C, can persist over the years even in the absence of drug pressure and impact RTI treatment negatively, and that appearing patterns of RTI-resistance mutations among non-subtype B HIV-1-infected children could possibly be different from those reported in subtype B-infected children.
PMID: 17516531 [PubMed - indexed for MEDLINE]

125. **Polymorphisms in IRF-1 associated with resistance to HIV-1 infection in highly exposed uninfected Kenyan sex workers.**

Ball TB[1], Ji H, Kimani J, McLaren P, Marlin C, Hill AV, Plummer FA.

AIDS. 2007 May 31;21(9):1091-101.

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, Canada. tball@cc.umanitoba.ca

**ABSTRACT**

OBJECTIVE: To determine the correlation between polymorphisms in the IL-4 gene cluster and resistance to HIV-1 infection.

DESIGN: : A cross-sectional genetic analysis of polymorphisms within the IL-4 gene cluster was conducted in a well-described female sex worker cohort from Nairobi, Kenya, known to exhibit differential susceptibility to HIV-1 infection.

METHODS: Microsatellite genotyping was used to screen six microsatellite markers in the IL-4 gene cluster for associations with HIV-1 resistance. Further analysis of the interferon regulatory factor 1 (IRF-1) gene was conducted by genomic sequencing. Associations between IRF-1 gene polymorphisms and the HIV-1 resistance phenotype were determined using the chi-square test and Kaplan-Meier survival analysis. The functional consequence of IRF-1 polymorphism was conducted by quantitative Western blot.

RESULTS: Three polymorphisms in IRF-1, located at 619, the microsatellite region and 6516 of the gene, showed associations with resistance to HIV-1 infection. The 619A, 179 at IRF-1 microsatellite and 6516G alleles were associated with the HIV-1-resistant phenotype and a reduced likelihood of seroconversion. Peripheral blood mononuclear cells from patients with protective IRF-1 genotypes exhibited significantly lower basal IRF-1 expression and reduced responsiveness to exogenous IFN-gamma stimulation.

CONCLUSION: Polymorphisms in the IRF-1 gene are associated with resistance to infection by HIV-1 and a lowered level of IRF-1 protein expression. This study adds IRF-1, a transcriptional immunoregulatory gene, to the list of genetic correlates of altered susceptibility to HIV-1. This is the first report suggesting that a viral transcriptional regulator might contribute to resistance to HIV-1. Further functional analysis on the role of IRF-1 polymorphisms and HIV-1 resistance is underway.
PMID: 17502719 [PubMed - indexed for MEDLINE]

126. **M. tuberculosis genotypic diversity and drug susceptibility pattern in HIV-infected and non-HIV-infected patients in northern Tanzania.**

Kibiki GS[1], Mulder B, Dolmans WM, de Beer JL, Boeree M, Sam N, van Soolingen D, Sola C, van der Zanden AG.

BMC Microbiol. 2007 May 31;7:51.

[1]Department of Internal Medicine, Endoscopy Unit, Kilimanjaro Christian Medical Centre, Tumaini University, Moshi, Tanzania. gkibiki@gmail.com

**ABSTRACT**

BACKGROUND: Tuberculosis (TB) is a major health problem and HIV is the major cause of the increase in TB. Sub-Saharan Africa is endemic for both TB and HIV infection. Determination of the prevalence of M. tuberculosis strains and their drug susceptibility is important for TB control.TB positive culture, BAL fluid or sputum samples from 130 patients were collected and genotyped. The spoligotypes were correlated with anti-tuberculous drug susceptibility in HIV-infected and non-HIV patients from Tanzania.

RESULTS: One-third of patients were TB/HIV co-infected. Forty-seven spoligotypes were identified. Fourteen isolates (10.8%) had new and unique spoligotypes while 116 isolates (89.2%) belonged to 33 known spoligotypes. The major spoligotypes contained nine clusters: CAS1-Kili 30.0%, LAM11- ZWE 14.6%, ND 9.2%, EAI 6.2%, Beijing 5.4%, T-undefined 4.6%, CAS1-Delhi 3.8%, T1 3.8% and LAM9 3.8%. Twelve (10.8%) of the 111 phenotypically tested strains were resistant to anti-TB drugs. Eight (7.2%) were monoresistant strains: 7 to isoniazid (INH) and one to streptomycin. Four strains (3.5%) were resistant to multiple drugs: one (0.9%) was resistant to INH and streptomycin and the other three (2.7%) were MDR strains: one was resistant to INH, rifampicin and ethambutol and two were resistant to all four anti-TB drugs. Mutation in the katG gene codon 315 and the rpoB hotspot region showed a low and high sensitivity, respectively, as predictor of phenotypic drug resistance.

CONCLUSION: CAS1-Kili and LAM11-ZWE were the most common families. Strains of the Beijing family and CAS1-Kili were not or least often associated with resistance, respectively. HIV status was not associated with spoligotypes, resistance or previous TB treatment.

PMCID: PMC1913919 **Free PMC Article**
PMID: 17540031 [PubMed - indexed for MEDLINE]

127. **Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study.**

Blomberg B[1], Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, Msangi V, Tellevik MG, Holberg-Petersen M, Harthug S, Maselle SY, Langeland N.

BMC Infect Dis. 2007 May 22;7:43.

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**ABSTRACT**

BACKGROUND: Bloodstream infection is a common cause of hospitalization, morbidity and death in children. The impact of antimicrobial resistance and HIV infection on outcome is not firmly established.

METHODS: We assessed the incidence of bloodstream infection and risk factors for fatal outcome in a prospective cohort study of 1828 consecutive admissions of children aged zero to seven years with signs of systemic infection. Blood was obtained for culture, malaria microscopy, HIV antibody test and, when necessary, HIV PCR. We recorded data on clinical features, underlying diseases, antimicrobial drug use and patients' outcome.

RESULTS: The incidence of laboratory-confirmed bloodstream infection was 13.9% (255/1828) of admissions, despite two thirds of the study population having received antimicrobial therapy prior to blood culture. The most frequent isolates were klebsiella, salmonellae, Escherichia coli, enterococci and Staphylococcus aureus. Furthermore, 21.6% had malaria and 16.8% HIV infection. One third (34.9%) of the children with laboratory-confirmed bloodstream infection died. The mortality rate from Gram-negative bloodstream infection (43.5%) was more than double that of malaria (20.2%) and Gram-positive bloodstream infection (16.7%). Significant risk factors for death by logistic regression modeling were inappropriate treatment due to antimicrobial resistance, HIV infection, other underlying infectious diseases, malnutrition and bloodstream infection caused by Enterobacteriaceae, other Gram-negatives and candida.

CONCLUSION: Bloodstream infection was less common than malaria, but caused more deaths. The frequent use of antimicrobials prior to blood culture may have hampered the detection of organisms susceptible to commonly used antimicrobials, including pneumococci, and thus the study probably underestimates the incidence of bloodstream infection. The finding that antimicrobial resistance, HIV-infection and malnutrition predict fatal outcome calls for renewed efforts to curb the further emergence of resistance, improve HIV care and nutrition for children.
PMCID: PMC1891109 Free PMC Article
PMID: 17519011 [PubMed - indexed for MEDLINE]

128. **Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda.**

Oyugi JH[1], Byakika-Tusiime J, Ragland K, Laeyendecker O, Mugerwa R, Kityo C, Mugyenyi P, Quinn TC, Bangsberg DR.

AIDS. 2007 May 11;21(8):965-71.

[1]Infectious Diseases Institute, Kampala, Uganda.

**ABSTRACT**

OBJECTIVE: To evaluate adherence, treatment interruptions, and outcomes in patients purchasing antiretroviral fixed-dose combination (FDC) therapy.

DESIGN: Ninety-seven participants were recruited into a prospective 24-week observational cohort study of HIV-positive, antiretroviral-naive individuals initiating self-pay Triomune or Maxivir therapy in Kampala, Uganda. Adherence was measured by monthly structured interview, unannounced home pill count, and electronic medication monitors (EMM). Treatment interruptions were measured as continuous intervals greater than 48 h without opening the EMM. The primary outcomes were survival with viral suppression below 400 copies/ml, CD4 cell increases, and genotypic drug resistance at 24 weeks.

RESULTS: The median baseline CD4 cell count was 56 cells/microl and median log10 copies RNA/ml was 5.54; mean adherence ranged from 82 to 95% for all measures but declined significantly over time. In an intent-to-treat analysis, 70 (72%) patients had an undetectable plasma HIV-RNA level at week 24. Sixty-two of 95 (65%) individuals with continuous EMM data had a treatment interruption of greater than 48 h. Treatment interruptions accounted for 90% of missed doses. None of 33 participants who did not interrupt treatment for over 48 h had drug resistance, whereas eight of 62 (13%) participants who did interrupt therapy experienced drug resistance. Antiretroviral resistance was seen in 8% of individuals and overall mortality was 10% at 24 weeks.

CONCLUSION: HIV-positive individuals purchasing generic FDC antiretroviral therapy have high rates of adherence and viral suppression, low rates of antiretroviral resistance, and robust CD4 cell responses. Adherence is an important predictor of survival with full viral suppression. Treatment interruptions are an important predictor of drug resistance.
PMID: 17457090 [PubMed - indexed for MEDLINE]

129. **HIV type 1 diversity and antiretroviral drug resistance mutations in Burundi.**

Vidal N[1], Niyongabo T, Nduwimana J, Butel C, Ndayiragije A, Wakana J, Nduwimana M, Delaporte E, Peeters M.
AIDS Res Hum Retroviruses. 2007 Jan;23(1):175-80.
[1]UMR145, Institut de Recherche pour le Développement and University of Montpellier 1, Montpellier, France.

**ABSTRACT**

In 2002, an HIV surveillance study was performed among more than 5500 individuals representing the general population of urban and rural districts in Burundi. In this report, we genetically characterized a subset of the HIV-1-positive samples identified during this survey, including all the HIV-positive samples from Bujumbura, the capital city, and samples from one semiurban and one rural district. One hundred and nineteen samples were genetically characterized in the V3-V5 region of the env gene and/or in the protease and reverse transcriptase region of the pol gene. Phylogenetic analysis of 101 env/pol sequences revealed that the HIV-1 epidemic in Burundi was driven by subtype C (81.2%), followed by subtype A (7.9 %) and polC/envA recombinants (5.9%). One major mutation associated with resistance to antiretroviral drugs (ARVs) in the pol gene, as defined by the International AIDS Society Resistance Testing-USA panel, was observed in one individual, but many minor resistance-associated mutations were also present in the majority of the samples.

PMID: 17263648 [PubMed - indexed for MEDLINE]

130. **Quantitative analysis of HIV-1 variants with the K103N resistance mutation after single-dose nevirapine in women with HIV-1 subtypes A, C, and D.**

Flys TS[1], Chen S, Jones DC, Hoover DR, Church JD, Fiscus SA, Mwatha A, Guay LA, Mmiro F, Musoke P, Kumwenda N, Taha TE, Jackson JB, Eshleman SH.

J Acquir Immune Defic Syndr. 2006 Aug 15;42(5):610-3.

[1]Department of Pathology, The Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.

**ABSTRACT**

INTRODUCTION: We used a sensitive point mutation assay, LigAmp, to detect and quantify K103N-containing variants in African women who received single-dose nevirapine (NVP) to prevent mother-to-child HIV-1 transmission.

METHODS: Plasma for testing was collected 6 to 8 weeks postpartum from 301 women (144 subtype A, 63 subtype C, and 94 subtype D).

RESULTS: The portion of women with 0.5% or more K103N-containing variants was lowest for subtype A (60/144, 41.7%) and highest for subtype C (44/63, 69.8%; $P < 0.0001$). K103N was rarely detected in pre-NVP samples. In a multivariate model, K103N detection was associated with HIV-1 subtype (C > A), after adjusting for log10 delivery viral load, the number of days between NVP dosing and sample collection, age, and parity. Among women with K103N detected: (1) the median %K103N was lower for subtype A (2.2%) than C (11.7%, $P = 0.0001$) or D (5.5%, $P = 0.04$), and (2) in a multivariate linear model, higher log10 (%K103N) was associated with HIV subtype (C > A, $P = 0.0001$; D > A, $P = 0.01$; and C vs D, no difference), but not other factors.

CONCLUSIONS: After administration of single-dose NVP, K103N was detected more frequently and at higher levels in women with subtypes C and D than A. Further studies are needed to evaluate the clinical significance of NVP-resistant variants in this setting.

PMID: 16773030 [PubMed - indexed for MEDLINE]

131. **Elevated T cell counts and RANTES expression in the genital mucosa of HIV-1-resistant Kenyan commercial sex workers.**

Iqbal SM[1], Ball TB, Kimani J, Kiama P, Thottingal P, Embree JE, Fowke KR, Plummer FA.

J Infect Dis. 2005 Sep 1;192(5):728-38. Epub 2005 Jul 27.

[1]Department of Medical Microbiology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

**ABSTRACT**

The initial site of exposure to human immunodeficiency virus (HIV)-1 during heterosexual transmission occurs in the genital tract. Although the majority of immunological studies have focused on the immune response to HIV-1 at the systemic level, our understanding of tissue-specific immunity is deficient. The goal of the present study was to characterize T cell populations found in the cervix of women shown to be resistant to infection by HIV-1. Levels of both systemic and cervical mucosal lymphocytes were compared between HIV-1-resistant, HIV-1-uninfected, and HIV-1-infected commercial sex workers (CSWs) as well as HIV-1-uninfected non-CSW control subjects at low risk for exposure. The HIV-1-resistant CSWs had increased cervical CD4+ and CD8+ T cell counts, compared with the HIV-1-uninfected CSWs; importantly, these increases were not reflected in the systemic lymphocyte compartment. There was a 2-fold increase in CD4+ T cell counts in the HIV-1-resistant CSWs, compared with both the HIV-1-infected and the HIV-1-uninfected CSWs. Expression of the HIV-1 coreceptors CCR5 and CXCR4 was also determined, and cytokine and beta chemokine levels in the genital mucosa were assessed. The HIV-1-resistant CSWs had a 10-fold increase in RANTES expression, compared with the HIV-1-uninfected CSWs. This is the first study to show elevated levels of beta chemokines and CD4+ T cells in the genital tracts of women who are exposed to HIV-1 and yet are uninfected.

**Free Article**

PMID: 16088822 [PubMed - indexed for MEDLINE]

132. **Genetic linkage of nevirapine resistance mutations in HIV type 1 seven days after single-dose nevirapine.**

Jones D[1], Parkin N, Hudelson SE, Guay LA, Musoke P, Mmiro F, Jackson JB, Eshleman SH.

AIDS Res Hum Retroviruses. 2005 Apr;21(4):319-24.

[1]Johns Hopkins Medical Institutions, Baltimore, Maryland 21205, USA.

**ABSTRACT**

The HIVNET 012 trial in Uganda demonstrated that a regimen of single-dose nevirapine (NVP) can prevent HIV-1 mother-to-child transmission. Previous studies show that HIV-1 with one or more NVP resistance (NVPR) mutations can be selected in many women as early as 7 days after single-dose NVP. We evaluated the genetic linkage of NVPR mutations in plasma from women in HIVNET 012 collected 7 days after single-dose NVP administration. The HIV-1 pol region was amplified and cloned from 20 plasma samples (16 with NVPR mutations detected by population sequencing and 4 with no NVPR mutations detected), and 10 clones from each sample were sequenced. Up to five different NVPR mutations were detected in clones from a single sample. K103N and Y181C were the most common mutations detected. Clones with two genetically linked mutations were detected in four samples. Different combinations of NVPR mutations were linked in individual clones, but none of the clones contained both K103N and Y181C. Further studies are needed to evaluate whether selection of minority variants with one or more NVPR mutations after single-dose NVP is clinically relevant.
PMID: 15943576 [PubMed - indexed for MEDLINE]

133. **Novel antibiotic-resistant pneumococcal strains recovered from the upper respiratory tracts of HIV-infected adults and their children in Kisumu, Kenya.**

Medina MJ[1], Greene CM, Gertz RE, Facklam RR, Jagero G, Hamel M, Shi YP, Slutsker L, Feikin DR, Beall B.

Microb Drug Resist. 2005 Spring;11(1):9-17.

[1]Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.

**ABSTRACT**

In a survey of genetic diversity within penicillin-nonsusceptible pneumococcal isolates in Kenya, we examined 162 upper respiratory isolates from 104 human immunodeficiency virus (HIV)-infected adults and 46 children in a cotrimoxazole prophylaxis study. Antibiotic resistance levels were high; 152 (94.4%) were cotrimoxazole nonsusceptible (134 fully resistant) and 124 (77%) were intermediately penicillin resistant. Isolates nonsusceptible to penicillin and cotrimoxazole (PNCNP) were found among 24 of the 29 serotypes encountered, 15 of which have rarely or never had documented nonsusceptibility to penicillin. These included serotypes 3, 4, 7C, 7F, 10A, 11A, 13, 15A, 15B, 16F, 17F, 19B, 21, 35A, and 35B. Segments of pbp2b genes from 9 PNCNP (serotypes 3, 13, 15A, 16F, 20, and 35A) were typical of resistance-conferring alleles in that they were highly divergent and contained two substitutions thought to be critical for resistance. Similarly, the dhfr genes from 3 PNCNP were divergent and contained a substitution required for cotrimoxazole resistance. Multilocus sequence typing (MLST) of 48 PNCNP revealed 33 sequence types (STs), none of which were previously recorded at http://www.mlst.net. Comparisons with all known STs revealed that 23 of these STs were unrelated to other known STs, whereas 10 STs were highly related to STs from internationally disseminated strains, including 2 of the 26 antibiotic-resistant clones recognized by the Pneumococcal Molecular Epidemiology Network. Based upon differing serotypes expressed by strains of identical or closely similar genotypes, there has been an extensive history of capsular switching within seven genetic clusters represented by these 10 STs and related STs described at http://www.mlst.net.
PMID: 15770088 [PubMed - indexed for MEDLINE]

134. **Drug resistance testing provides evidence of the globalization of HIV type 1: a new circulating recombinant form.**

Gómez-Carrillo M[1], Quarleri JF, Rubio AE, Carobene MG, Dilernia D, Carr JK, Salomón H.

AIDS Res Hum Retroviruses. 2004 Aug;20(8):885-8.

[1]Centro Nacional de Referencia para el SIDA, Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, C1121ABG Buenos Aires, Argentina. mcarrill@fmed.uba.ar

**ABSTRACT**

To monitor HIV-1 diversity in Argentina, a phylogenetic-based analysis of HIV-1 partial pol sequences obtained for resistance testing in 587 treatment failure patients was performed in Buenos Aires city between 2001 and 2003. HIV-1 RNA was isolated from plasma samples and partial pol fragments amplified by RT-PCR. Sequences were obtained by automated sequencing. Phylogenetic analysis was performed and recombination patterns characterized. A total of 299 sequences grouped into clade B (50.94%) and 284 were B/F recombinants (48.38%). Four sequences were grouped into clades A, C, and F (0.68%). The clade C sample, 96105, was found to be a BC recombinant and samples 103396 and 104575 showed the same mosaic pattern with Kisii5009 from Kenya and 97KR004 from Korea, previously described as A2D recombinants. With the presence of two full-length genomes, one from Kenya and one from Korea, and now two partial genomes from Argentina, this recombinant is designated CRF16_A2D. Its presence on three continents shows that CRF16_A2D has a global distribution.
PMID: 15320992 [PubMed - indexed for MEDLINE]

135. **Comparison of nevirapine (NVP) resistance in Ugandan women 7 days vs. 6-8 weeks after single-dose nvp prophylaxis: HIVNET 012.**

Eshleman SH[1], Guay LA, Mwatha A, Cunningham SP, Brown ER, Musoke P, Mmiro F, Jackson JB.
AIDS Res Hum Retroviruses. 2004 Jun;20(6):595-9.

[1]Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205, USA. seshlem@jhmi.edu

**ABSTRACT**

We compared nevirapine (NVP) resistance (NVPR) mutations in maternal plasma 7 days vs. 6-8 weeks after single-dose NVP prophylaxis. In the HIVNET 012 trial, Ugandan women received a single dose of NVP in labor for prevention of HIV-1 mother-to-child transmission. NVPR mutations were detected in 70 (25%) of 279 women 6-8 weeks after NVP. Samples collected 7 days after NVP were analyzed from a subset of those 279 women. Genotyping was performed with the ViroSeq HIV-1 Genotyping System. NVPR was analyzed using paired samples from 7 days and 6-8 weeks after NVP. Sixty-five women had genotyping results obtained for samples collected at both 7 days and 6-8 weeks post-NVP. Twenty-one (32%) of those women had NVPR mutations detected in one or both samples. This included three women with NVPR at 7 days only, seven with NVPR at 6-8 weeks only, and 11 with NVPR at both time points. Eight women had >1 NVPR mutation detected 7 days after NVP. Y181C was the most common NVPR mutation detected at 7 days, whereas K103N was the most common NVPR mutation detected

at 6-8 weeks. We conclude that NVPR may be detected in women as early as 7 days after single-dose NVP. Complex patterns of NVPR are detected in some women. The Y181C NVPR mutation often fades from detection by 6-8 weeks. In contrast, the K103N mutation emerges more slowly, but often remains detectable 6-8 weeks after NVP.

PMID: 15242535 [PubMed - indexed for MEDLINE]

136. **High prevalence of antiretroviral resistance in treated Ugandans infected with non-subtype B human immunodeficiency virus type 1.**

Richard N[1], Juntilla M, Abraha A, Demers K, Paxinos E, Galovich J, Petropoulos C, Whalen CC, Kyeyune F, Atwine D, Kityo C, Mugyenyi P, Arts EJ.

AIDS Res Hum Retroviruses. 2004 Apr;20(4):355-64.

[1]Division of Infectious Diseases, Department of Medicine, Case Western Reserve University, Cleveland, Ohio 44106, USA. eja3@po.cwru.edu

**ABSTRACT**

This study examined the emergence and prevalence of drug-resistant mutations in reverse transcriptase and protease coding regions in human immunodeficiency virus type 1 (HIV-1)-infected Ugandans treated with antiretroviral drugs (ARV). Genotypic resistance testing was performed on 50 and 16 participants who were enrolled in a cross-sectional and longitudinal observational cohort, respectively. The majority of the 113 HIV-1 PR-RT sequences were classified as subtypes A and D. Drug resistance mutations were prevalent in 52% of ARV-experienced individuals, and 17 of 27 ARV-resistant isolates had three mutations or more in reverse transcriptase. Resistance mutations in protease were less prevalent but only 17 of the 50 patients were receiving a protease inhibitor upon sample collection. Mutations conferring drug resistance were also selected in 3 of 16 participants in the longitudinal cohort, i.e., less than 8 months after the initiation of ARV treatment. Rapid emergence of ARV resistance was associated with poor adherence to treatment regimens, which was related to treatment costs. ARV resistance did, however, appear at a slightly higher prevalence in HIV-1 subtype D (21 of 33) than subtype A (7 of 25) infected individuals. Overall, this observational study suggests that ARV-resistant HIV-1 isolates are emerging rapidly in ARV-treated individual in Uganda and possibly other developing countries.

PMID: 15157354 [PubMed - indexed for MEDLINE]

137. **HIV type 1 pol gene diversity and archived nevirapine resistance mutation in pregnant women in Rwanda.**

Servais J[1], Lambert C, Karita E, Vanhove D, Fischer A, Baurith T, Schmit JC, Schneider F, Hemmer R, Arendt V.

AIDS Res Hum Retroviruses. 2004 Mar;20(3):279-83.

[1]Treatment Research AIDS Center, Kigali, Rwanda. rwa21@rwandal.com

**ABSTRACT**

This study aimed to find out whether genetic polymorphisms were present in positions potentially affecting susceptibility to antiretrovirals in non-B subtypes from HIV-1-infected patients in Rwanda. Viral pol gene diversity was investigated by direct sequencing in 43 treatment-naive women. In addition, 10 DNA sequences from uncultured peripheral blood mononuclear cells were analyzed 6 weeks after a single dose of nevirapine (prevention of mother-to-child transmission program). Phylogenetic analyses have shown 34 subtype A1, 6 subtype C, and 2 subtype D strains. In addition, an A/C recombinant between the protease (PR) (subtype A1) and the reverse transcriptase (RT) (subtype C) was identified. In the PR coding region, high numbers of polymorphisms were found, including substitutions in secondary PR resistance sites. PR 35D, 36I, and 37N were always present within subtype A as were PR 93L in subtype C strains. PR 10I/V, 20R, 33F, and 77V were found in subtype A whereas PR 36I was highly prevalent in subtype C strains. The A/C recombinant displayed substitutions related to resistance (PR 10, 33, 36 and RT 118). One nevirapine resistance mutation (RT 181Y/C) was found in proviral DNA after 6 weeks. In conclusion, subtypes A and C are predominant in this cohort in Rwanda. Substitutions similar to secondary protease inhibitor resistance mutations are common before treatment whereas major resistance mutation may be archived after a single dose of nevirapine. Accordingly, the hypothesis of a genetic background effect in non-B strains has to be further addressed in programs of introduction of antivirals in Africa.

PMID: 15117451 [PubMed - indexed for MEDLINE]

138. **Development of phenotypic and genotypic resistance to antiretroviral therapy in the UNAIDS HIV Drug Access Initiative--Uganda.**

Weidle PJ[1], Downing R, Sozi C, Mwebaze R, Rukundo G, Malamba S, Respess R, Hertogs K, Larder B, Ochola D, Mermin J, Samb B, Lackritz E.

AIDS. 2003 Jul;17 Suppl 3:S39-48.

[1]Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention (NCHSTP), Centers for Disease Control and Prevention (CDC), Atlanta, Georgia 30333, USA. pweidle@cdc.gov

**ABSTRACT**

OBJECTIVE: We describe phenotypic drug resistance, response to therapy, and genotypic mutations among HIV-infected patients in Uganda taking antiretroviral medications for > or = 90 days who had a viral load > or = 1000 copies/ml.

METHODS: HIV-1 group and subtype, virologic and immunologic responses to antiretroviral therapy, phenotypic resistance to antiretroviral drugs, and associated genotypic mutations among patients at three treatment centers in Uganda between June 1999 and August 2000 were assessed. Therapy was two nucleoside reverse transcriptase inhibitors (NRTIs) or highly active antiretroviral therapy (HAART).

RESULTS: All HIV identified was HIV-1, group M, subtypes A, C, and D. Sixty-one (65%) of 94 patients with a phenotypic resistance result had evidence of phenotypic resistance including resistance to a NRTI for 51 of 92 (55%) taking NRTIs, to a non-nucleoside reverse transcriptase inhibitor (NNRTI) for nine of 16 (56%) taking NNRTIs, and to a protease inhibitor (PI) for eight of 37 (22%) taking PIs. At the time of the first specimen with resistance, the median change from baseline viral load was -0.56 log

copies/ml [interquartile range (IQR), -1.47 to +0.29] and CD4+ cell count was +35 x 10(6) cells/l (IQR, -18 to +87). Genotypic resistance mutations, matched with phenotypic resistance assay results and drug history, were generally consistent with those seen for HIV-1, group M, subtype B infections in industrialized countries.

CONCLUSION: Initial phenotypic resistance and corresponding genotypic mutations among patients treated in Uganda were similar to those with subtype B infections in North America and Europe. These data support policies that promote the use of HAART regimens against HIV-1, group M, non-B subtypes in a manner consistent with that used for subtype B infections.
PMID: 14565608 [PubMed - indexed for MEDLINE]

139. **Resistance to reinfection with Schistosoma mansoni in occupationally exposed adults and effect of HIV-1 co-infection on susceptibility to schistosomiasis: a longitudinal study.**

Karanja DM[1], Hightower AW, Colley DG, Mwinzi PN, Galil K, Andove J, Secor WE.

Lancet. 2002 Aug 24;360(9333):592-6.

[1]Centre for Vector Biology and Control Research, Kenya Medical Research Institute, Kisumu, Kenya.

**ABSTRACT**

BACKGROUND: Previous studies have reported age-dependent development of resistance to reinfection by schistosomes and identified immunological correlates of this resistance. However, whether resistance exists that is independent of age effects has been questioned. We did a longitudinal investigation of reinfection by Schistosoma mansoni in an adult population with high occupational exposure.

METHODS: We monitored a cohort of 96 male car washers working along the shores of Lake Victoria, Kenya during 349.7 person-years for frequency of water contact and infection with S mansoni. Patients were treated with praziquantel upon study entry and after reinfection with S mansoni. Bivariate analyses and a multivariate proportional hazards model were used to assess the effects of water contact, previous infections, and HIV-1 on S mansoni reinfection rates.

FINDINGS: 13 car washers did not get reinfected or only became reinfected after an extended time (91 weeks). 47 initially had a short time to reinfection (15 weeks) but on subsequent treatments showed increased time to reinfection (29-38 weeks). 36 consistently displayed short times to reinfection (<15 weeks) despite multiple reinfection and treatment cycles. Decreased CD4 T-cell counts in HIV-1-positive individuals corresponded to increased susceptibility to S mansoni reinfection.

INTERPRETATION: Adults similarly exposed to schistosomiasis are either resistant to reinfection; susceptible, but develop resistance to reinfection after multiple treatments; or remain susceptible to reinfection. Thus, immunological resistance to reinfection with S mansoni exists or can develop independent of age effects. The consequence of HIV-1 co-infection suggests that CD4 T cells contribute to this resistance.
PMID: 12241930 [PubMed - indexed for MEDLINE]

140. **Nevirapine resistance after single dose prophylaxis.**

Eshleman SH[1], Jackson JB.

AIDS Rev. 2002 Apr-Jun;4(2):59-63.

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**ABSTRACT**

Nevirapine (NVP) is a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase. In 1999, the HIVNET 012 trial in Uganda demonstrated that a simple regimen of NVP prophylaxis can dramatically reduce the rate of HIV-1 mother-to-child transmission (MTCT). In the HIVNET 012 regimen, women received a single dose of NVP in labor, and infants received a single dose of NVP within 72 h of birth. The simplicity, efficacy, and low cost of the HIVNET 012 regimen are attractive for prevention of MTCT in resource-poor settings. Plans are underway to implement this regimen in several resource-poor countries. Single mutations in HIV-1 RT can cause high level NVP-resistance and are likely to exist in most HIV-1 infected patients at low levels prior to antiretroviral drug exposure. This favors emergence of NVP-resistant HIV-1 following NVP exposure. NVP-resistant HIV-1 has been shown to emerge in some women and infants following single dose NVP. Emergence of NVP-resistant HIV-1 in this setting is more common among women with high baseline viral loads and low baseline CD4 cell counts. The rate of NVP-resistance in women receiving single dose NVP prophylaxis may also be influenced by HIV-1 subtype. The NVP-resistant HIV-1 typically fades from detection in women and infants over time. We review studies examining the emergence and fading of NVP-resistant HIV-1 in women and infants who received single dose NVP prophylaxis, and discuss the potential clinical relevance of NVP-resistance in this setting.
PMID: 12152519 [PubMed - indexed for MEDLINE]

141. **Sequence and peptide-binding motif for a variant of HLA-A*0214 (A*02142) in an HIV-1-resistant individual from the Nairobi Sex Worker cohort.**

Luscher MA[1], MacDonald KS, Bwayo JJ, Plummer FA, Barber BH.

Immunogenetics. 2001 Feb;53(1):10-4.

[1]Department of Medicine, University of Toronto, Canada.

**Erratum in:**

- Immunogenetics 2001 Oct;53(8):717.

**ABSTRACT**

As part of the ongoing study of natural HIV-1 resistance in the women of the Nairobi Sex Workers' study, we have examined a resistance-associated HLA class I allele at the molecular level. Typing by polymerase chain reaction using sequence-specific primers determined that this molecule is closely

related to HLA-A*0214, one of a family of HLA-A2 supertype alleles which correlate with HIV-1 resistance in this population. Direct nucleotide sequencing shows that this molecule differs from A*0214, having a silent nucleotide substitution. We therefore propose to designate it HLA-A*02142. We have determined the peptide-binding motif of HLA-A*0214/02142 by peptide elution and bulk Edman degradative sequencing. The resulting motif, X-[Q,V]-X-X-X-K-X-X-[V,L], includes lysine as an anchor at position 6. The data complement available information on the peptide-binding characteristics of this molecule, and will be of use in identifying antigenic peptides from HIV-1 and other pathogens.

PMID: 11261925 [PubMed - indexed for MEDLINE]

142. **Late seroconversion in HIV-resistant Nairobi prostitutes despite pre-existing HIV-specific CD8+ responses.**

Kaul R[1], Rowland-Jones SL, Kimani J, Dong T, Yang HB, Kiama P, Rostron T, Njagi E, Bwayo JJ, MacDonald KS, McMichael AJ, Plummer FA.

J Clin Invest. 2001 Feb;107(3):341-9.

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**ABSTRACT**

Resistance to HIV infection in a small group of Kenyan sex workers is associated with CD8+-lymphocyte responses to HIV cytotoxic T-lymphocyte (CTL) epitopes. Eleven prostitutes meeting criteria for HIV resistance seroconverted between 1996 and 1999. The occurrence and specificity of preexisting HIV-1 epitope-specific responses were examined using the IFN-gamma enzyme-linked immunospot assay, and any epitopes recognized were cloned and sequenced from the infecting viral isolate. Immunologic and behavioral variables were compared between late seroconverters and persistently uninfected sex worker controls. HIV-1 CTL epitope responses were present in four of six cases, 5-18 months before seroconversion, and their presence was confirmed by bulk CTL culture. A possible viral escape mutation was found in one of six epitopes. The key epidemiologic correlate of late seroconversion was a reduction in sex work over the preceding year. In persistently uninfected controls, a break from sex work was associated with a loss of HIV-specific CD8+ responses. Late seroconversion may occur in HIV-1-resistant sex workers despite preceding HIV-specific CD8+ responses. Seroconversion generally occurs in the absence of detectable CTL escape mutations and may relate to the waning of HIV-specific CD8+ responses due to reduced antigenic exposure.

PMCID: PMC199193 **Free PMC Article**
PMID: 11160158 [PubMed - indexed for MEDLINE]

143. **Identification of the K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission.**

Jackson JB[1], Becker-Pergola G, Guay LA, Musoke P, Mracna M, Fowler MG, Mofenson LM, Mirochnick M, Mmiro F, Eshleman SH.

AIDS. 2000 Jul 28;14(11):F111-5.

[1]Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205, USA.

**ABSTRACT**

OBJECTIVE: A recent trial in Uganda demonstrated that a simple, inexpensive regimen of nevirapine (NVP) prophylaxis can dramatically reduce HIV-1 vertical transmission risk. In this regimen, women receive a single dose of NVP at the onset of labor and infants receive a single dose of NVP within 72 h of birth. The objective of this study was to determine whether HIV-1 variants with NVP resistance mutations were selected in Ugandan women who received this regimen in the Phase I/II trial HIVNET 006.

METHODS: Reverse transcriptase (RT) sequences from plasma HIV-1 were analyzed from 15 women 6 weeks after NVP dosing. RT sequences from plasma collected prior to NVP dosing were also analyzed.

RESULTS: The K103N NVP resistance mutation was detected 6 weeks after NVP administration in three (20%) out of 15 women (95% confidence interval, 0-40%). Pre-dose samples were available from two of the three women; both pre-dose samples lacked the mutation. Other NVP resistance mutations were absent from all 15 women. Women with the K103N mutation had a longer median NVP elimination half-life, decreased median oral clearance, and increased median area under the concentration time curve than those without the mutation. An evaluable sample was obtained from one of these three women 33 months after delivery; the K103N mutation was not detected in that sample.

CONCLUSIONS: This preliminary study demonstrates that HIV-1 with the RT K103N mutation can be detected in some Ugandan women following a single dose of NVP. This suggests that non-nucleoside RT inhibitor resistance may be selected in some people by single dose NVP prophylaxis. Pharmacokinetic data suggested that a more prolonged exposure to NVP after dosing may favor selection of NVP-resistant HIV-1.
PMID: 10983633 [PubMed - indexed for MEDLINE]

144. **HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi.**

Kaul R[1], Plummer FA, Kimani J, Dong T, Kiama P, Rostron T, Njagi E, MacDonald KS, Bwayo JJ, McMichael AJ, Rowland-Jones SL.

J Immunol. 2000 Feb 1;164(3):1602-11.

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**ABSTRACT**

Understanding how individuals with a high degree of HIV exposure avoid persistent infection is paramount to HIV vaccine design. Evidence suggests that mucosal immunity, particularly virus-specific CTL, could be critically important in protection against sexually acquired HIV infection. Therefore, we have looked for the presence of HIV-specific CD8+ T cells in cervical mononuclear cells from a subgroup of highly HIV-exposed but persistently seronegative female sex workers in Nairobi. An enzyme-linked immunospot assay was used to measure IFN-gamma release in response to known class I HLA-restricted CTL epitope peptides using effector cells from the blood and cervix of HIV-1-resistant and -infected sex workers and from lower-risk uninfected controls. Eleven of 16 resistant sex workers had HIV-specific CD8+ T cells in the cervix, and a similar number had detectable responses in blood. Where both blood and cervical responses were detected in the same individual, the specificity of the responses was similar. Neither cervical nor blood responses were detected in lower-risk control donors. HIV-specific CD8+ T cell frequencies in the cervix of HIV-resistant sex workers were slightly higher than in blood, while in HIV-infected donor cervical response frequencies were markedly lower than blood, so that there was relative enrichment of cervical responses in HIV-resistant compared with HIV-infected donors. HIV-specific CD8+ T cell responses in the absence of detectable HIV infection in the genital mucosa of HIV-1-resistant sex workers may be playing an important part in protective immunity against heterosexual HIV-1 transmission.

**Free Article**
PMID: 10640781 [PubMed - indexed for MEDLINE]

145. **Resistance to HIV-1 infection among highly exposed sex workers in Nairobi: what mediates protection and why does it develop?**

Plummer FA[1], Ball TB, Kimani J, Fowke KR.

Immunol Lett. 1999 Mar;66(1-3):27-34.

[1]Department of Medical Microbiology, University of Nairobi, Kenya. plummer@form-net.com

**ABSTRACT**

Variability in susceptibility to infection and disease caused by infectious agents is a characteristic of all populations. Among susceptible individuals exposed to an infection, not all become infected and among infected individuals, not all develop disease. It seems logical that variability in susceptibility to infection and disease would apply to infection and disease with human immunodeficiency viruses.

However, until recently, it has been generally held that there is no natural immunity to HIV-1 and that once infected, all individuals would ultimately succumb to AIDS.
PMID: 10203031 [PubMed - indexed for MEDLINE]

146. **HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers.**

Kaul R[1], Trabattoni D, Bwayo JJ, Arienti D, Zagliani A, Mwangi FM, Kariuki C, Ngugi EN, MacDonald KS, Ball TB, Clerici M, Plummer FA.

AIDS. 1999 Jan 14;13(1):23-9.

[1]Department of Medical Microbiology and Community Health, University of Nairobi, Kenya.

**ABSTRACT**

OBJECTIVES: Most HIV-1 transmission is sexual; therefore, immune responses in the genital mucosa may be important in mediating protection against HIV infection. This study examined HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan female sex workers.

METHODS: HIV-1-specific immune responses were compared in HIV-1-resistant and HIV-1-infected sex workers, and in lower risk uninfected women. Cervical and vaginal samples from each group were tested for HIV-1-specific IgA and IgG by enzyme immunoassay. Systemic T-helper lymphocyte cell responses to HIV-1 envelope peptide epitopes were assayed using an interleukin 2 bioassay. HIV-1 risk-taking behaviours were assessed using standardized questionnaires.

RESULTS: HIV-1-specific IgA was present in the genital tract of 16 out of 21 (76%) HIV-1-resistant sex workers, five out of 19 (26%) infected women, and three out of 28 (11%) lower risk women ($P < 0.0001$). Among lower risk women, the presence of HIV-1-specific IgA was associated with HIV-1 risk-taking behaviour. Systemic T-helper lymphocyte responses to HIV-1 envelope peptides were present in 11 out of 20 (55%) HIV-1-resistant women, four out of 18 (22%) infected women, and one out of 25 (4%) lower risk women ($P < 0.001$). T-helper lymphocyte responses did not correlate with the presence or titre of virus-specific mucosal IgA in any study group.

CONCLUSIONS: HIV-1-specific IgA is present in the genital tract of most HIV-1-resistant Kenyan sex workers, and of a minority of lower risk uninfected women, where it is associated with risk-taking behaviour. These data suggest a role for mucosal HIV-1-specific IgA responses in HIV-1 resistance, independent of host cellular responses.
PMID: 10207541 [PubMed - indexed for MEDLINE]

147. **HIV type 1 resistance in Kenyan sex workers is not associated with altered cellular susceptibility to HIV type 1 infection or enhanced beta-chemokine production.**

Fowke KR[1], Dong T, Rowland-Jones SL, Oyugi J, Rutherford WJ, Kimani J, Krausa P, Bwayo J, Simonsen JN, Shearer GM, Plummer FA.

AIDS Res Hum Retroviruses. 1998 Nov 20;14(17):1521-30.

[1]Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA. fowkek@exchange.nih.gov

**ABSTRACT**

A small group of women (n = 80) within the Nairobi-based Pumwani Sex Workers Cohort demonstrates epidemiologic resistance to HIV-1 infection. Chemokine receptor polymorphisms and beta-chemokine overproduction have been among the mechanisms suggested to be responsible for resistance to HIV-1 infection. This study attempts to determine if any of those mechanisms are protecting the HIV-1-resistant women. Genetic analysis of CCR5 and CCR3 from the resistant women demonstrated no polymorphisms associated with resistance. Expression levels of CCR5 among the resistant women were shown to be equivalent to that found in low-risk seronegative (negative) controls, while CXCR4 expression was greater among some of the resistant women. In vitro infection experiments showed that phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) from resistant women were as susceptible to infection to T cell- and macrophage-tropic North American and Kenyan HIV-1 isolates as were the PBMCs from negative controls. No significant difference in circulating plasma levels of MIP-1alpha and MIP-1beta were found between the resistant women and negative or HIV-1-infected controls. In vitro cultures of media and PHA-stimulated PBMCs indicated that the resistant women produced significantly less MIP-1alpha and MIP-1beta than did negative controls and no significant difference in RANTES levels were observed. In contrast to studies in Caucasian cohorts, these data indicate that CCR5 polymorphisms, altered CCR5 and CXCR4 expression levels, cellular resistance to in vitro HIV-1 infection, and increased levels of beta-chemokine production do not account for the resistance to HIV-1 infection observed among the women of the Pumwani Sex Workers Cohort.

PMID: 9840285 [PubMed - indexed for MEDLINE]

148. **Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi.**

Rowland-Jones SL[1], Dong T, Fowke KR, Kimani J, Krausa P, Newell H, Blanchard T, Ariyoshi K, Oyugi J, Ngugi E, Bwayo J, MacDonald KS, McMichael AJ, Plummer FA.

J Clin Invest. 1998 Nov 1;102(9):1758-65.

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**ABSTRACT**

Many people who remain persistently seronegative despite frequent HIV exposure have HIV-specific immune responses. The study of these may provide information about mechanisms of natural

protective immunity to HIV-1. We describe the specificity of cytotoxic T lymphocyte responses to HIV in seronegative prostitutes in Nairobi who are apparently resistant to HIV infection. These women have had frequent exposure to a range of African HIV-1 variants, primarily clades A, C, and D, for up to 12 yr without becoming infected. Nearly half of them have CTL directed towards epitopes previously defined for B clade virus, which are largely conserved in the A and D clade sequences. Stronger responses are frequently elicited using the A or D clade version of an epitope to stimulate CTL, suggesting that they were originally primed by exposure to these virus strains. CTL responses have been defined to novel epitopes presented by HLA class I molecules associated with resistance to infection in the cohort, HLA-A*6802 and HLA-B18. Estimates using a modified interferon-gamma Elispot assay indicate a circulating frequency of CTL to individual epitopes of between 1:3,200 and 1:50,000. Thus, HIV-specific immune responses-particularly cross-clade CTL activity- may be responsible for protection against persistent HIV infection in these African women.

PMCID: PMC509124 **Free PMC Article**
PMID: 9802890 [PubMed - indexed for MEDLINE]

149. **Naturally occurring IgG anti-HLA alloantibody does not correlate with HIV type 1 resistance in Nairobi prostitutes.**

Luscher MA[1], Choy G, Njagi E, Bwayo JJ, Anzala AO, Ndinya-Achola JO, Ball TB, Wade JA, Plummer FA, Barber BH, MacDonald KS.

AIDS Res Hum Retroviruses. 1998 Jan 20;14(2):109-15.

[1]Department of Immunology, University of Toronto, The Toronto Hospital, Canada.

**ABSTRACT**

In an effort to identify an immunological basis for natural resistance to HIV-1 infection, we have examined serum antibody responses to HLA class I antigens in female prostitutes of the Nairobi Sex Workers Study. Anti-HLA antibodies are known to block HIV infectivity in vitro and can be protective against SIV challenge in macaques immunized with purified class I HLA. Thus, it was postulated that broadly cross-reactive alloantibodies recognizing common HLA alleles in the client population might contribute to the prevention of heterosexual transmission of HIV. In fact, 12% of the women were found to have serum IgG antibodies against class I alloantigens. However, this alloantibody did not correlate with the HIV status of the women and was found in a similar proportion of HIV-positive and HIV-resistant women. The observed levels of alloantibody did not increase with HIV infection in susceptible individuals, suggesting that potential antigenic mimicry between HIV and host HLA class I antigens does not significantly increase levels of anti-class I antibodies. The lack of correlation between serum anti-allo-class I HLA antibodies and the risk of sexual transmission indicates that this humoral immune response is unlikely to be the natural mechanism behind the HIV-resistance phenotype of persistently HIV-seronegative women. This result, however, does not preclude the further investigation of alloimmunization as an artificial HIV immunization strategy.
PMID: 9462920 [PubMed - indexed for MEDLINE]

150. **Royal Society of Tropical Medicine and Hygiene Meeting at Manson House, London, 12 December 1996. HIV and pneumococcal infection in Africa. Microbiological aspects.**

Paul J[1].

Trans R Soc Trop Med Hyg. 1997 Nov-Dec;91(6):632-7.

[1]Brighton Public Health Laboratory, Royal Sussex County Hospital, UK.

**ABSTRACT**

By the 1930s several studies had shown that Streptococcus pneumoniae was an important pathogen in Nairobi (Kenya) and various risk factors for infection were recognized, including seasonally cold conditions, overcrowding and recent arrival in the city. Research into pneumococcal disease declined with the arrival of penicillin but recently interest has been rekindled by recognition of the pneumococcus as a human immunodeficiency virus (HIV)-associated pathogen and by the emergence of antibiotic resistance. The pneumococcus and its association with HIV were studied during the course of the Wellcome Trust/Kenya Medical Research Institute HIV Programme in Nairobi (1988-1993). There were generally high rates of pneumococcal disease. The pneumococcus (with tuberculosis and salmonellosis) was a major HIV-related pathogen. One study showed HIV seropositivity to confer a relative risk of 17.8 for pneumococcal infection. Recurrent infection accounted for a large proportion (25%) of disease episodes in a longitudinally studied cohort of HIV patients. There were higher pneumococcal carriage rates in HIV-positive than in HIV-negative patients (28% vs. 16%, P = 0.003). High rates of resistance were found to penicillin (25%). Molecular characterization of penicillin-resistant strains identified 11 separate clones, showing great genetic diversity in a small sample of isolates, and there was evidence of horizontal spread of penicillin-binding protein genes between separate lineages. Molecular characterization of isolates from patients with recurrent disease suggested that both relapse and reinfection might occur. There was molecular evidence of transfer of capsular genes between clones (serotype switching). The overall spectrum of serotypes resembled those reported elsewhere, most serotypes being included in the 23-valent vaccine. Higher numbered serotypes were associated with respiratory tract source and antibiotic resistance. Various methods were used to show 82% concordance between pernasal and blood isolates in pneumonia cases. HIV-seropositive patients were more prone to infection with penicillin- and tetracycline-resistant organisms than seronegative patients (penicillin, 27% vs. 7%; tetracycline, 40% vs. 17%), a difference reflected by different serotype profiles in the 2 groups. These studies highlight the importance of the pneumococcus as an HIV-related pathogen in one part of Africa. The high rates of antibiotic resistance are a cause of concern. There should be continued monitoring of resistance patterns, and assessments of the significance of pneumococcal disease made elsewhere in Africa are to be encouraged.

PIP: Pneumococcus has been recognized as an important HIV-related pathogen in Africa and may cause significant excess mortality in severely immunocompromised HIV patients. This relationship was investigated in depth in Nairobi, Kenya, in 1988-93 through the linkage of a clinical HIV project sponsored by Wellcome Trust/Kenya Medical Research Institute with facilities for microbiological research. Kenyan HIV patients were prone to higher rates of colonization and invasive disease than seronegative patients and HIV infection was associated with a different pattern of serotypes and higher rates of antibiotic resistance. In one study, HIV infection was associated with a relative risk of 17.8 for pneumococcal infection. The pneumococcal carriage rate was 28% in HIV-positive patients compared with 16% in HIV-negative individuals. In a longitudinal study of a cohort of HIV patients,

25% were resistant to penicillin; 11 resistant strains were identified, with evidence of horizontal spread of penicillin-binding protein genes between separate lineages. Molecular characterization of isolates from patients with recurrent pneumococcal disease suggested the occurrence of both relapse and reinfection. There was an 82% concurrence between pernasal and blood isolates in pneumonia cases. Compared with HIV-negative persons, HIV-positive patients were significantly more prone to infection with penicillin-resistant (7% and 27%, respectively) and tetracycline-resistant (17% and 40%, respectively) organisms. Continued monitoring of resistance patterns and assessments of the significance of pneumococcal disease in other parts of Africa are recommended.
PMID: 9509167 [PubMed - indexed for MEDLINE]

151. **Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya.**

Fowke KR[1], Nagelkerke NJ, Kimani J, Simonsen JN, Anzala AO, Bwayo JJ, MacDonald KS, Ngugi EN, Plummer FA.

Lancet. 1996 Nov 16;348(9038):1347-51.

[1]Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada.

**ABSTRACT**

BACKGROUND: There is indirect evidence that HIV-1 exposure does not inevitably lead to persistent infection. Heterogeneity in susceptibility to infection could be due to protective immunity. The objective of this study was to find out whether in highly HIV-1-exposed populations some individuals are resistant to infection.

METHODS: We did an observational cohort study of incident HIV-1 infection-among 424 initially HIV-1-seronegative prostitutes in Nairobi, Kenya, between 1985 and 1994. 239 women seroconverted to HIV-1 during the study period. Exponential, Weibull, and mixture survival models were used to examine the effect of the duration of follow-up on incidence of HIV-1 infection. The influence of the duration of exposure to HIV-1 through prostitution on seroconversion risk was examined by Cox proportional hazards modelling, with control for other known or suspected risk factors for incident HIV-1 infection. HIV-1 PCR with env, nef, and vif gene primers was done on 43 persistently seronegative prostitutes who remained seronegative after 3 or more years of follow-up.

FINDINGS: Modelling of the time to HIV-1 seroconversion showed that the incidence of HIV-1 seroconversion decreased with increasing duration of exposure, which indicates that there is heterogeneity in HIV-1 susceptibility or acquired immunity to HIV-1. Each weighted year of exposure through prostitution resulted in a 1.2-fold reduction in HIV-1 seroconversion risk (hazard ratio 0.83 [95% CI 0.79-0.88], $p < 0.0001$). Analyses of epidemiological and laboratory data, show that persistent seronegativity is not explained by seronegative HIV-1 infection or by differences in risk factors for HIV-1 infection such as safer sexual behaviours or the incidence of other sexually transmitted infections.

INTERPRETATION: We conclude that a small proportion of highly exposed individuals, who may have natural protective immunity to HIV-1, are resistant to HIV-1.

PIP: A cohort study conducted in 1985-94 among 424 prostitutes from Nairobi, Kenya, who were initially human immunodeficiency virus (HIV)-1 seronegative, tended to provide support for the

observation that some individuals in highly exposed populations may be resistant to infection. During the 10-year study period, 239 of these women seroconverted. The overall HIV-1 incidence was 42/100 person-years. After the first 2 years of follow up, in which the majority of seroconversions occurred, HIV-1 prevalence reached a plateau and then began a steep decline. To determine whether the risk of HIV-1 infection declined over time as a result of the selection of resistance, incidence rates among women with less than 3 years' versus more than 3 years' duration of prostitution were compared for 1989-93. An increasing protective effect for each seronegative year of exposure was observed. The estimated cumulative protective effect for women practicing prostitution from 1984-93 and remaining seronegative, compared to women who entered prostitution in 1994, was over 100-fold. To rule out the possibility that the decrease in seroconversion with duration of exposure reflected differences in sexual behavior or immunity to sexually transmitted diseases that facilitate HIV transmission, Cox proportional hazards modelling was performed. The weighted duration of prostitution was independently associated with a decreased risk of seroconversion. Each weighted year of exposure resulted in a 1.2-fold decrease in risk. Women who seroconverted were more likely to report 1 or more regular partners and to use condoms with these partners than their counterparts who remained seronegative. Elucidation of the protective mechanisms and the factors mediating the development of immunity against HIV-1 could be important to HIV-1 vaccine research.

PMID: 8918278 [PubMed - indexed for MEDLINE]

152. **Nasopharyngeal carriage of Staphylococcus aureus and carriage of tetracycline-resistant strains associated with HIV-seropositivity.**

Amir M[1], Paul J, Batchelor B, Kariuki S, Ojoo J, Waiyaki P, Gilks C.

Eur J Clin Microbiol Infect Dis. 1995 Jan;14(1):34-40.

[1]Wellcome Trust Research Laboratories, Kilifi, Kenya.

**ABSTRACT**

The aim of this prospective study was to investigate the relationship between carriage of antibiotic-resistant Staphylococcus aureus and infection with the human immunodeficiency virus (HIV). A total of 554 pernasal swabs was taken during a six-month period from 554 adult patients attending three outpatient clinics and from inpatients from a hospital in Nairobi, Kenya. Overall, 121 swabs (22%) yielded Staphylococcus aureus, there being significantly higher carriage in HIV-positive patients (71/264, 27%) than in HIV-negative patients (50/290, 17%); p = 0.008. Antimicrobial resistance rates were determined for 110 isolates and were high for penicillin (91%) and tetracycline (72%) and low for erythromycin (8%), methicillin (3%), gentamicin (5%) and chloramphenicol (0%). Genetic analysis showed plasmids in the range of 24-42 MDa to be associated with beta-lactamase production and plasmids in the range of 3-5 MDa to be associated with resistance to tetracycline, erythromycin and trimethoprim. All nine erythromycin-resistant strains were from HIV-positive patients (p = 0.02). There was a significant association of tetracycline resistance with HIV seropositivity (p = 0.002). The association of HIV seropositivity with Staphylococcus aureus carriage and carriage of antibiotic-resistant strains against the background of the HIV epidemic are of relevance in individual patient care and raise concern for public health.

PIP: The authors report findings from a prospective study conducted to investigate the relationship between the carriage of antibiotic-resistant Staphylococcus aureus and infection with HIV. 554

pernasal swabs were taken during a six-month period from 554 adult patients attending three outpatient clinics and from inpatients in a hospital in Nairobi, Kenya. 22% of swabs yielded Staphylococcus aureus, with significantly higher carriage in HIV-positive patients than in HIV-negative patients: 27% and 17%, respectively. Antimicrobial resistance rates determined for 110 isolates were 91% for penicillin, 72% for tetracycline, 8% for erythromycin, 3% for methicillin, 5% for gentamicin, and 0% for chloramphenicol. Genetic analysis identified plasmids in the range of 24-42 MDa associated with B-lactamase production and plasmids in the range of 3-5 MDa associated with resistance to tetracycline, erythromycin, and trimethoprim. All nine erythromycin-resistant strains were from HIV-positive patients. There was a significant association of tetracycline resistance with HIV seropositivity.

PMID: 7729450 [PubMed - indexed for MEDLINE]

153. **Multiresistant Shigella species from African AIDS patients: antibacterial resistance patterns and application of the E-test for determination of minimum inhibitory concentration.**

Kruse H[1], Kariuki S, Søli N, Olsvik O.

Scand J Infect Dis. 1992;24(6):733-9.

[1]Department of Microbiology and Immunology, Norwegian College of Veterinary Medicine, Oslo.

**ABSTRACT**

The antibacterial resistance pattern and minimum inhibitory concentrations (MIC) of 25 Shigella flexneri, 5 S. boydii, 8 S. sonnei, and 3 strains of S. dysenteriae type 2 isolated from Kenyan prostitutes with bacillary dysentery and AIDS were determined, and the applicability of the E-test for MIC determination evaluated. All strains were resistant to > or = 3 of 9 different antibacterial agents tested. All strains were resistant to tetracycline and erythromycin, 95% to trimethoprim/sulfonamide, 93% to streptomycin, 54% to ampicillin, 39% to chloramphenicol, 2% to nalidixic acid and none to gentamicin and ciprofloxacin. Six different resistance patterns were observed. The most common pattern was resistance to tetracycline, erythromycin, trimethoprim/sulfa and streptomycin (39%). The E-test was shown to be well-suited for susceptibility testing of multiresistant Shigella spp.; the reproducibility was excellent and the correlation with the microtiter dilution method and the disk diffusion method were 98% in both instances. The MIC measured with E-test and the microdilution method were within +/- 1 dilution step for 94.4% of the combinations tested.

PMID: 1287807 [PubMed - indexed for MEDLINE]

# INSECTICIDE DRUG RESISTANCE

## 57 Citations

(sorted newest to oldest)

# INSECTICIDE RESISTANCE

1. **Exposure to deltamethrin affects development of Plasmodium falciparum inside wild pyrethroid resistant Anopheles gambiae s.s. mosquitoes in Uganda.**

Kristan M[1], Lines J[2], Nuwa A[3], Ntege C[4], Meek SR[5], Abeku TA[6].

Parasit Vectors. 2016 Feb 24;9:100. doi: 10.1186/s13071-016-1384-x.

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**ABSTRACT**

BACKGROUND: Pyrethroid resistance in African vector mosquitoes is a threat to malaria control. Resistant mosquitoes can survive insecticide doses that would normally be lethal. We studied effects of such doses on Plasmodium falciparum development inside kdr-resistant Anopheles gambiae s.s. in Uganda.

METHODS: We collected An. gambiae s.s. homozygous for kdr-L1014S mutation, fed them on blood samples from 42 P. falciparum-infected local patients, then exposed them either to nets treated with sub-lethal doses of deltamethrin or to untreated nets. After seven days, we dissected 692 mosquitoes and examined their midguts for oocysts. Prevalence (proportion infected) and intensity of infection (number of oocysts per infected mosquito) were recorded for each group.

RESULTS: Both prevalence and intensity of infection were significantly reduced in deltamethrin-exposed mosquitoes, compared to those exposed to untreated nets. With low doses (2.5-5.0 mg/m(2)), prevalence was reduced by 59% (95% CI = 22%-78%) and intensity by 41% (95% CI = 25%-54%). With high doses (10-16.7 mg/m(2)), prevalence was reduced by 80% (95% CI = 67%-88 %) and intensity by 34 % (95 % CI = 20%-46%).

CONCLUSIONS: We showed that, with locally-sampled parasites and mosquitoes, doses of pyrethroids that are sub-lethal for resistant mosquitoes can interfere with parasite development inside mosquitoes.

This mechanism could enable pyrethroid-treated nets to prevent malaria transmission despite increasing vector resistance.

PMCID: PMC4765236 **Free PMC Article**
PMID: 26911550 [PubMed - indexed for MEDLINE]

2. **Pyrethroid and DDT Resistance and Organophosphate Susceptibility among Anopheles spp. Mosquitoes, Western Kenya.**

Wanjala CL, Mbugi JP, Ototo E, Gesuge M, Afrane YA, Atieli HE, Zhou G, Githeko AK, Yan G.

Emerg Infect Dis. 2015 Dec;21(12):2178-81. doi: 10.3201/eid2112.150814.

**ABSTRACT**

We conducted standard insecticide susceptibility testing across western Kenya and found that the Anopheles gambiae mosquito has acquired high resistance to pyrethroids and DDT, patchy resistance to carbamates, but no resistance to organophosphates. Use of non-pyrethroid-based vector control tools may be preferable for malaria prevention in this region.

PMCID: PMC4672417 **Free PMC Article**
PMID: 26583525 [PubMed - indexed for MEDLINE]

3. **Presence of the knockdown resistance mutation, Vgsc-1014F in Anopheles gambiae and An. arabiensis in western Kenya.**

Ochomo E[1,2], Subramaniam K[3], Kemei B[4], Rippon E[5], Bayoh NM[6], Kamau L[7], Atieli F[8], Vulule JM[9], Ouma C[10,11], Gimnig J[12], Donnelly MJ[13,14], Mbogo C[15,16]

Parasit Vectors. 2015 Dec 1;8:616. doi: 10.1186/s13071-015-1223-5.

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**ABSTRACT**

INTRODUCTION: The voltage gated sodium channel mutation Vgsc-1014S (kdr-east) was first reported in Kenya in 2000 and has since been observed to occur at high frequencies in the local Anopheles gambiae s.s.

POPULATION: The mutation Vgsc-1014F has never been reported from An. gambiae Complex complex mosquitoes in Kenya.

FINDINGS: Molecularly confirmed An. gambiae s.s. (hereafter An. gambiae) and An. arabiensis collected from 4 different parts of western Kenya were genotyped for kdr from 2011 to 2013. Vgsc-1014F was observed to have emerged, apparently, simultaneously in both An. gambiae and An. arabiensis in 2012. A portion of the samples were submitted for sequencing in order to confirm the Vgsc-1014F genotyping results. The resulting sequence data were deposited in GenBank (Accession numbers: KR867642-KR867651, KT758295-KT758303). A single Vgsc-1014F haplotype was observed suggesting, a common origin in both species.

CONCLUSION: This is the first report of Vgsc-1014F in Kenya. Based on our samples, the mutation is present in low frequencies in both An. gambiae and An. arabiensis. It is important that we start monitoring relative frequencies of the two kdr genes so that we can determine their relative importance in an area of high insecticide treated net ownership.

PMCID: PMC4666190 **Free PMC Article**
PMID: 26626424 [PubMed - indexed for MEDLINE]

4. **Electrostatic coating enhances bioavailability of insecticides and breaks pyrethroid resistance in mosquitoes.**

Andriessen R[1], Snetselaar J[1], Suer RA[1], Osinga AJ[1], Deschietere J[2], Lyimo IN[3], Mnyone LL[3], Brooke BD[4], Ranson H[5], Knols BG[1], Farenhorst M[6].

Proc Natl Acad Sci U S A. 2015 Sep 29;112(39):12081-6. doi: 10.1073/pnas.1510801112. Epub 2015 Aug 31.

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**ABSTRACT**

Insecticide resistance poses a significant and increasing threat to the control of malaria and other mosquito-borne diseases. We present a novel method of insecticide application based on netting treated with an electrostatic coating that binds insecticidal particles through polarity. Electrostatic netting can hold small amounts of insecticides effectively and results in enhanced bioavailability upon contact by the insect. Six pyrethroid-resistant Anopheles mosquito strains from across Africa were exposed to similar concentrations of deltamethrin on electrostatic netting or a standard long-lasting deltamethrin-coated bednet (PermaNet 2.0). Standard WHO exposure bioassays showed that electrostatic netting induced significantly higher mortality rates than the PermaNet, thereby effectively breaking mosquito resistance. Electrostatic netting also induced high mortality in resistant mosquito strains when a 15-fold lower dose of deltamethrin was applied and when the exposure time was reduced to only 5 s. Because different types of particles adhere to electrostatic netting, it is also possible to apply nonpyrethroid insecticides. Three insecticide classes were effective against strains of Aedes and Culex mosquitoes, demonstrating that electrostatic netting can be used to deploy a wide range of active insecticides against all major groups of disease-transmitting mosquitoes. Promising applications include the use of electrostatic coating on walls or eave curtains and in trapping/contamination devices. We conclude that application of electrostatically adhered particles boosts the efficacy of WHO-recommended insecticides even against resistant mosquitoes. This innovative technique has potential to

support the use of unconventional insecticide classes or combinations thereof, potentially offering a significant step forward in managing insecticide resistance in vector-control operations.

PMCID: PMC4593083 **Free PMC Article**
PMID: 26324912 [PubMed - indexed for MEDLINE]

5. **RNA-seq analyses of changes in the Anopheles gambiae transcriptome associated with resistance to pyrethroids in Kenya: identification of candidate-resistance genes and candidate-resistance SNPs.**

Bonizzoni M[1,2], Ochomo E[3], Dunn WA[4], Britton M[5], Afrane Y[6], Zhou G[7], Hartsel J[8], Lee MC[9], Xu J[10], Githeko A[11], Fass J[12], Yan G[13].

Parasit Vectors. 2015 Sep 17;8:474. doi: 10.1186/s13071-015-1083-z.

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**ABSTRACT**

BACKGROUND: The extensive use of pyrethroids for control of malaria vectors, driven by their cost, efficacy and safety, has led to widespread resistance. To favor their sustainable use, the World Health Organization (WHO) formulated an insecticide resistance management plan, which includes the identification of the mechanisms of resistance and resistance surveillance. Recognized physiological mechanisms of resistance include target site mutations in the para voltage-gated sodium channel, metabolic detoxification and penetration resistance. Such understanding of resistance mechanisms has

allowed the development of resistance monitoring tools, including genotyping of the kdr mutation L1014F/S in the para gene.

METHODS: The sequence-based technique RNA-seq was applied to study changes in the transcriptome of deltamethrin-resistant and -susceptible Anopheles gambiae mosquitoes from the Western Province of Kenya. The resulting gene expression profiles were compared to data in the most recent literature to derive a list of candidate resistance genes. RNA-seq data were analyzed also to identify sequence polymorphisms linked to resistance.

RESULTS: A total of five candidate-resistance genes (AGAP04177, AGAP004572, AGAP008840, AGAP007530 and AGAP013036) were identified with altered expression between resistant and susceptible mosquitoes from West and East Africa. A change from G to C at position 36043997 of chromosome 3R resulting in A101G of the sulfotransferase gene AGAP009551 was significantly associated with the resistance phenotype (odds ratio: 5.10). The kdr L1014S mutation was detected at similar frequencies in both phenotypically resistant and susceptible mosquitoes, suggesting it is no longer fully predictive of the resistant phenotype.

CONCLUSIONS: Overall, these results support the conclusion that resistance to pyrethroids is a complex and evolving phenotype, dependent on multiple gene functions including, but not limited to, metabolic detoxification. Functional convergence among metabolic detoxification genes may exist, with the role of each gene being modulated by the life history and selection pressure on mosquito populations. As a consequence, biochemical assays that quantify overall enzyme activity may be a more suitable method for predicting metabolic resistance than gene-based assays.

PMCID: PMC4574070 **Free PMC Article**
PMID: 26381877 [PubMed - indexed for MEDLINE]

6. **Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation.**

Kleinschmidt I[1,2], Mnzava AP[3], Kafy HT[4], Mbogo C[5], Bashir AI[6,7], Bigoga J[8], Adechoubou A[9], Raghavendra K[10], Knox TB[11], Malik EM[12], Nkuni ZJ[13], Bayoh N[14], Ochomo E[15], Fondjo E[16], Kouambeng C[17], Awono-Ambene HP[18], Etang J[19,20], Akogbeto M[21], Bhatt R[22], Swain DK[23], Kinyari T[24], Njagi K[25], Muthami L[26], Subramaniam K[27], Bradley J[28], West P[29], Massougbodji A[30], Okê-Sopoh M[31], Hounto A[32], Elmardi K[33], Valecha N[34], Kamau L[35], Mathenge E[36], Donnelly MJ[37,38].

Malar J. 2015 Jul 22;14:282. doi: 10.1186/s12936-015-0782-4.

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**ABSTRACT**

BACKGROUND: Progress in reducing the malaria disease burden through the substantial scale up of insecticide-based vector control in recent years could be reversed by the widespread emergence of insecticide resistance. The impact of insecticide resistance on the protective effectiveness of insecticide-treated nets (ITN) and indoor residual spraying (IRS) is not known. A multi-country study was undertaken in Sudan, Kenya, India, Cameroon and Benin to quantify the potential loss of epidemiological effectiveness of ITNs and IRS due to decreased susceptibility of malaria vectors to insecticides. The design of the study is described in this paper.

METHODS: Malaria disease incidence rates by active case detection in cohorts of children, and indicators of insecticide resistance in local vectors were monitored in each of approximately 300 separate locations (clusters) with high coverage of malaria vector control over multiple malaria seasons. Phenotypic and genotypic resistance was assessed annually. In two countries, Sudan and India, clusters were randomly assigned to receive universal coverage of ITNs only, or universal coverage of ITNs combined with high coverage of IRS. Association between malaria incidence and insecticide resistance, and protective effectiveness of vector control methods and insecticide resistance were estimated, respectively.

RESULTS: Cohorts have been set up in all five countries, and phenotypic resistance data have been collected in all clusters. In Sudan, Kenya, Cameroon and Benin data collection is due to be completed in 2015. In India data collection will be completed in 2016.

DISCUSSION: The paper discusses challenges faced in the design and execution of the study, the analysis plan, the strengths and weaknesses, and the possible alternatives to the chosen study design.

PMCID: PMC4508808 **Free PMC Article**
PMID: 26194648 [PubMed - indexed for MEDLINE]

7. **Implementation of the global plan for insecticide resistance management in malaria vectors: progress, challenges and the way forward.**

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Malar J. 2015 Apr 23;14:173. doi: 10.1186/s12936-015-0693-4.

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**ABSTRACT**

In recent years, there has been an increase in resistance of malaria vectors to insecticides, particularly to pyrethroids which are widely used in insecticide-treated nets. The Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM), released in May 2012, is a collective strategy for the malaria community to tackle this challenge. This review outlines progress made to date and the challenges experienced in the implementation of GPIRM, and outlines focus areas requiring urgent attention. Whilst there has been some advancement, uptake of GPIRM at the national level has generally been poor for various reasons, including limited availability of vector control tools with new mechanisms of action as well as critical financial, human and infrastructural resource deficiencies. There is an urgent need for a global response plan to address these deficits and ensure the correct and efficient use of available tools in order to maintain the effectiveness of current vector control efforts whilst novel vector control tools are under development. Emphasis must be placed on enhancing national capacities (such as human and infrastructural resources) to enable efficient monitoring and management of insecticide resistance, and to support availability and accessibility of appropriate new vector control products. Lack of action by the global community to address the threat of insecticide resistance is unacceptable and deprives affected communities of their basic right of universal access to effective malaria prevention.

Aligning efforts and assigning the needed resources will ensure the optimal implementation of GPIRM with the ultimate goal of maintaining effective malaria vector control.

PMCID: PMC4423491 **Free PMC Article**
PMID: 25899397 [PubMed - indexed for MEDLINE]

8. **Early biting and insecticide resistance in the malaria vector Anopheles might compromise the effectiveness of vector control intervention in Southwestern Uganda.**

Ojuka P[1], Boum Y 2nd[2,3], Denoeud-Ndam L[4], Nabasumba C[5], Muller Y[6], Okia M[7], Mwanga-Amumpaire J[8,9], De Beaudrap P[10], Protopopoff N[11], Etard JF[12,13].

Malar J. 2015 Apr 9;14:148. doi: 10.1186/s12936-015-0653-z.

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**ABSTRACT**

BACKGROUND: Southwestern Uganda has high malaria heterogeneity despite moderate vector control and other interventions. Moreover, the early biting transmission and increased resistance to insecticides might compromise strategies relying on vector control. Consequently, monitoring of vector behaviour and insecticide efficacy is needed to assess the effectiveness of strategies aiming at malaria control. This eventually led to an entomological survey in two villages with high malaria prevalence in this region.

METHODS: During rainy, 2011 and dry season 2012, mosquitoes were collected in Engari and Kigorogoro, Kazo subcounty, using human landing collection, morning indoor resting collection,

pyrethrum spray collection and larval collection. Circumsporozoite protein of Plasmodium falciparum sporozoites in female Anopheles mosquitoes was detected using ELISA assay. Bioassays to monitor Anopheles resistance to insecticides were performed.

RESULTS: Of the 1,021 female Anopheles species captured, 62% (632) were Anopheles funestus and 36% (371) were Anopheles gambiae s.l. The most common species were Anopheles gambiae s.l. in Engari (75%) and A. funestus in Kigorogoro (83%). Overall, P. falciparum prevalence was 2.9% by ELISA. The daily entomological inoculation rates were estimated at 0.17 and 0.58 infected bites/person/night during rainy and dry season respectively in Engari, and 0.81 infected bites/person/night in Kigorogoro during dry season. In both areas and seasons, an unusually early evening biting peak was observed between 6 - 8 p.m. In Engari, insecticide bioassays showed 85%, 34% and 12% resistance to DDT during the rainy season, dry season and to deltamethrin during the dry season, respectively. In Kigorogoro, 13% resistance to DDT and to deltamethrin was recorded. There was no resistance observed to bendiocarb and pirimiphos methyl.

CONCLUSIONS: The heterogeneity of mosquito distribution, entomological indicators and resistance to insecticides in villages with high malaria prevalence highlight the need for a long-term vector control programme and monitoring of insecticide resistance in Uganda. The early evening biting habits of Anopheles combined with resistance to DDT and deltamethrin observed in this study suggest that use of impregnated bed nets alone is insufficient as a malaria control strategy, urging the need for additional interventions in this area of high transmission.

PMCID: PMC4416237 **Free PMC Article**
PMID: 25879539 [PubMed - indexed for MEDLINE]

9. **Trends in the selection of insecticide resistance in Anopheles gambiae s.l. mosquitoes in northwest Tanzania during a community randomized trial of longlasting insecticidal nets and indoor residual spraying.**

Matowo J[1], Kitau J, Kaaya R, Kavishe R, Wright A, Kisinza W, Kleinschmidt I, Mosha F, Rowland M, Protopopoff N.

Med Vet Entomol. 2015 Mar;29(1):51-9. doi: 10.1111/mve.12090. Epub 2014 Dec 24.

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**ABSTRACT**

Anopheles gambiae s.l. (Diptera: Culicidae) in Muleba, Tanzania has developed high levels of resistance to most insecticides currently advocated for malaria control. The kdr mutation has almost reached fixation in An. gambiae s.s. in Muleba. This change has the potential to jeopardize malaria control interventions carried out in the region. Trends in insecticide resistance were monitored in two intervention villages using World Health Organization (WHO) susceptibility test kits. Additional

mechanisms contributing to observed phenotypic resistance were investigated using Centers for Disease Control (CDC) bottle bioassays with piperonylbutoxide (PBO) and S,S,S-tributyl phosphorotrithioate (DEF) synergists. Resistance genotyping for kdr and Ace-1 alleles was conducted using quantitative polymerase chain reaction (qPCR). In both study villages, high phenotypic resistance to several pyrethroids and DDT was observed, with mortality in the range of 12-23%. There was a sharp decrease in mortality in An. gambiae s.l. exposed to bendiocarb (carbamate) from 84% in November 2011 to 31% in December 2012 after two rounds of bendiocarb-based indoor residual spraying (IRS). Anopheles gambiae s.l. remained susceptible to pirimiphos-methyl (organophosphate). Bendiocarb-based IRS did not lead to the reversion of pyrethroid resistance. There was no evidence for selection for Ace-1 resistance alleles. The need to investigate the operational impact of the observed resistance selection on the effectiveness of longlasting insecticidal nets and IRS for malaria control is urgent.

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PMID: 25537754 [PubMed - indexed for MEDLINE]

10. **Investigating mosquito net durability for malaria control in Tanzania - attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): study protocol.**

Lorenz LM, Overgaard HJ[1], Massue DJ, Mageni ZD, Bradley J, Moore JD, Mandike R, Kramer K, Kisinza W, Moore SJ.

BMC Public Health. 2014 Dec 13;14:1266. doi: 10.1186/1471-2458-14-1266.

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**ABSTRACT**

BACKGROUND: Long-Lasting Insecticidal Nets (LLINs) are one of the major malaria vector control tools, with most countries adopting free or subsidised universal coverage campaigns of populations at-risk from malaria. It is essential to understand LLIN durability so that public health policy makers can select the most cost effective nets that last for the longest time, and estimate the optimal timing of repeated distribution campaigns. However, there is limited knowledge from few countries of the durability of LLINs under user conditions.

METHODS/DESIGN: This study investigates LLIN durability in eight districts of Tanzania, selected for their demographic, geographic and ecological representativeness of the country as a whole. We use a two-stage approach: First, LLINs from recent national net campaigns will be evaluated retrospectively in 3,420 households. Those households will receive one of three leading LLIN products at random (Olyset®, PermaNet®2.0 or Netprotect®) and will be followed up for three years in a prospective study to compare their performance under user conditions. LLIN durability will be evaluated by measuring Attrition (the rate at which nets are discarded by households), Bioefficacy (the insecticidal efficacy of

the nets measured by knock-down and mortality of mosquitoes), Chemical content (g/kg of insecticide available in net fibres) and physical Degradation (size and location of holes). In addition, we will extend the current national mosquito insecticide Resistance monitoring program to additional districts and use these data sets to provide GIS maps for use in health surveillance and decision making by the National Malaria Control Program (NMCP).

DISCUSSION: The data will be of importance to policy makers and vector control specialists both in Tanzania and the SSA region to inform best practice for the maintenance of high and cost-effective coverage and to maximise current health gains in malaria control.

PMCID: PMC4301422 **Free PMC Article**
PMID: 25495268 [PubMed - indexed for MEDLINE]

11. **Islands and stepping-stones: comparative population structure of Anopheles gambiae sensu stricto and Anopheles arabiensis in Tanzania and implications for the spread of insecticide resistance.**

Maliti D[1], Ranson H[2], Magesa S[3], Kisinza W[4], Mcha J[5], Haji K[5], Killeen G[6], Weetman D[2].

PLoS One. 2014 Oct 29;9(10):e110910. doi: 10.1371/journal.pone.0110910. eCollection 2014.

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**ABSTRACT**

Population genetic structures of the two major malaria vectors Anopheles gambiae s.s. and An. arabiensis, differ markedly across Sub-Saharan Africa, which could reflect differences in historical demographies or in contemporary gene flow. Elucidation of the degree and cause of population structure is important for predicting the spread of genetic traits such as insecticide resistance genes or artificially engineered genes. Here the population genetics of An. gambiae s.s. and An. arabiensis in the central, eastern and island regions of Tanzania were compared. Microsatellite markers were screened in 33 collections of female An. gambiae s.l., originating from 22 geographical locations, four of which

were sampled in two or three years between 2008 and 2010. An. gambiae were sampled from six sites, An. arabiensis from 14 sites, and both species from two sites, with an additional colonised insectary sample of each species. Frequencies of the knock-down resistance (kdr) alleles 1014S and 1014F were also determined. An. gambiae exhibited relatively high genetic differentiation (average pairwise FST = 0.131), significant even between nearby samples, but without clear geographical patterning. In contrast, An. arabiensis exhibited limited differentiation (average FST = 0.015), but strong isolation-by-distance (Mantel test r = 0.46, p = 0.0008). Most time-series samples of An. arabiensis were homogeneous, suggesting general temporal stability of the genetic structure. An. gambiae populations from Dar es Salaam and Bagamoyo were found to have high frequencies of kdr 1014S (around 70%), with almost 50% homozygote but was at much lower frequency on Unguja Island, with no. An. gambiae population genetic differentiation was consistent with an island model of genetic structuring with highly restricted gene flow, contrary to An. arabiensis which was consistent with a stepping-stone model of extensive, but geographically-restricted gene flow.

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PMID: 25353688 [PubMed - indexed for MEDLINE]

12. **Impact of agriculture on the selection of insecticide resistance in the malaria vector Anopheles gambiae: a multigenerational study in controlled conditions.**

Nkya TE[1,2,3], Poupardin R[4], Laporte F[5,6], Akhouayri I[7,8], Mosha F[9], Magesa S[10,11], Kisinza W[12], David JP[13,14].

Parasit Vectors. 2014 Oct 16;7:480. doi: 10.1186/s13071-014-0480-z.

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**ABSTRACT**

BACKGROUND: Resistance of mosquitoes to insecticides is mainly attributed to their adaptation to vector control interventions. Although pesticides used in agriculture have been frequently mentioned as an additional force driving the selection of resistance, only a few studies were dedicated to validate this hypothesis and characterise the underlying mechanisms. While insecticide resistance is rising dramatically in Africa, deciphering how agriculture affects resistance is crucial for improving resistance management strategies. In this context, the multigenerational effect of agricultural pollutants on the selection of insecticide resistance was examined in Anopheles gambiae.

METHODS: An urban Tanzanian An. gambiae population displaying a low resistance level was used as a parental strain for a selection experiment across 20 generations. At each generation larvae were selected with a mixture containing pesticides and herbicides classically used in agriculture in Africa. The resistance levels of adults to deltamethrin, DDT and bendiocarb were compared between the selected and non-selected strains across the selection process together with the frequency of kdr mutations. A microarray approach was used for pinpointing transcription level variations selected by the agricultural pesticide mixture at the adult stage.

RESULTS: A gradual increase of adult resistance to all insecticides was observed across the selection process. The frequency of the L1014S kdr mutation rose from 1.6% to 12.5% after 20 generations of selection. Microarray analysis identified 90 transcripts over-transcribed in the selected strain as compared to the parental and the non-selected strains. Genes encoding cuticle proteins, detoxification enzymes, proteins linked to neurotransmitter activity and transcription regulators were mainly affected. RT-qPCR transcription profiling of candidate genes across multiple generations supported their link with insecticide resistance.

CONCLUSIONS: This study confirms the potency of agriculture in selecting for insecticide resistance in malaria vectors. We demonstrated that the recurrent exposure of larvae to agricultural pollutants can select for resistance mechanisms to vector control insecticides at the adult stage. Our data suggest that in addition to selected target-site resistance mutations, agricultural pollutants may also favor cuticle, metabolic and synaptic transmission-based resistance mechanisms. These results emphasize the need for integrated resistance management strategies taking into account agriculture activities.

PMCID: PMC4201709 **Free PMC Article**
PMID: 25318645 [PubMed - indexed for MEDLINE]

13. **Widespread pyrethroid and DDT resistance in the major malaria vector Anopheles funestus in East Africa is driven by metabolic resistance mechanisms.**

Mulamba C[1], Riveron JM[2], Ibrahim SS[2], Irving H[2], Barnes KG[2], Mukwaya LG[3], Birungi J[3], Wondji CS[2].

PLoS One. 2014 Oct 15;9(10):e110058. doi: 10.1371/journal.pone.0110058. eCollection 2014.

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[3]Uganda Virus Research Institute, Entebbe, Uganda.

**ABSTRACT**

BACKGROUND: Establishing the extent, geographical distribution and mechanisms of insecticide resistance in malaria vectors is a prerequisite for resistance management. Here, we report a widespread distribution of insecticide resistance in the major malaria vector An. funestus across Uganda and western Kenya under the control of metabolic resistance mechanisms.

METHODOLOGY/PRINCIPAL FINDINGS: Female An. funestus collected throughout Uganda and western Kenya exhibited a Plasmodium infection rate between 4.2 to 10.4%. Widespread resistance against both type I (permethrin) and II (deltamethrin) pyrethroids and DDT was observed across Uganda and western Kenya. All populations remain highly susceptible to carbamate, organophosphate and dieldrin insecticides. Knockdown resistance plays no role in the pyrethroid and DDT resistance as no kdr mutation associated with resistance was detected despite the presence of a F1021C replacement. Additionally, no signature of selection was observed on the sodium channel gene. Synergist assays and qRT-PCR indicated that metabolic resistance plays a major role notably through elevated expression of cytochrome P450s. DDT resistance mechanisms differ from West Africa as the L119F-GSTe2 mutation only explains a small proportion of the genetic variance to DDT resistance.

CONCLUSION: The extensive distribution of pyrethroid and DDT resistance in East African An. funestus populations represents a challenge to the control of this vector. However, the observed carbamate and organophosphate susceptibility offers alternative solutions for resistance management.

PMCID: PMC4198208 **Free PMC Article**
PMID: 25333491 [PubMed - indexed for MEDLINE]

14. **Distribution and spread of pyrethroid and DDT resistance among the Anopheles gambiae complex in Tanzania.**

Kabula B[1], Tungu P, Malima R, Rowland M, Minja J, Wililo R, Ramsan M, McElroy PD, Kafuko J, Kulkarni M, Protopopoff N, Magesa S, Mosha F, Kisinza W.

Med Vet Entomol. 2014 Sep;28(3):244-52. doi: 10.1111/mve.12036. Epub 2013 Nov 5.

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**ABSTRACT**

The development of insecticide resistance is a threat to the control of malaria in Africa. We report the findings of a national survey carried out in Tanzania in 2011 to monitor the susceptibility of malaria vectors to pyrethroid, organophosphate, carbamate and DDT insecticides, and compare these findings with those identified in 2004 and 2010. Standard World Health Organization (WHO) methods were used to detect knock-down and mortality rates in wild female Anopheles gambiae s.l. (Diptera: Culicidae) collected from 14 sentinel districts. Diagnostic doses of the pyrethroids deltamethrin, lambdacyhalothrin and permethrin, the carbamate propoxur, the organophosphate fenitrothion and the organochlorine DDT were used. Anopheles gambiae s.l. was resistant to permethrin in Muleba, where a mortality rate of 11% [95% confidence interval (CI) 6-19%] was recorded, Muheza (mortality rate of 75%, 95% CI 66-83%), Moshi and Arumeru (mortality rates of 74% in both). Similarly, resistance was reported to lambdacyhalothrin in Muleba, Muheza, Moshi and Arumeru (mortality rates of 31-82%), and to deltamethrin in Muleba, Moshi and Muheza (mortality rates of 28-75%). Resistance to DDT was reported in Muleba. No resistance to the carbamate propoxur or the organophosphate fenitrothion was observed. Anopheles gambiae s.l. is becoming resistant to pyrethoids and DDT in several parts of Tanzania. This has coincided with the scaling up of vector control measures. Resistance may impair the effectiveness of these interventions and therefore demands close monitoring and the adoption of a resistance management strategy.

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PMID: 24192019 [PubMed - indexed for MEDLINE]

15. **Preventive effect of permethrin-impregnated long-lasting insecticidal nets on the blood feeding of three major pyrethroid-resistant malaria vectors in western Kenya.**

Kawada H[1], Ohashi K, Dida GO, Sonye G, Njenga SM, Mwandawiro C, Minakawa N.

Parasit Vectors. 2014 Aug 20;7:383. doi: 10.1186/1756-3305-7-383.

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**ABSTRACT**

BACKGROUND: Since the World Health Organization (WHO) adopted the use of long-lasting insecticidal nets (LLINs) as a principal strategy for effective malaria prevention and control, pyrethroids have been the only class of insecticides used for LLINs. The dramatic success of insecticide-treated nets (ITNs) and LLINs in African countries, however, has been threatened by the rapid development of pyrethroid resistance in vector mosquitoes. ITNs and LLINs are still used as effective self-protection measures, but there have been few studies on the effectiveness of ITNs and LLINs in areas where vector mosquitoes are pyrethroid-resistant.

METHODS: To investigate the behavioral pattern of mosquitoes in the houses where LLINs were used, indoor mosquito trappings of Anopheles gambiae s.s., An. arabiensis, and An. funestus s.s. were performed with Centers for Disease Control and Prevention (CDC) miniature light trap equipped with a collection bottle rotator at 2-hour intervals between 4:00 pm and 8:00 am. The trapped female mosquitoes were identified and classified as unfed, blood fed, and gravid. The abdominal contents of fed female mosquitoes were used for DNA extractions to identify the blood source.

RESULTS: A large proportion of human blood feeding of An. arabiensis and An. funestus s.s. (but not An. gambiae s.s.) took place during the time people were active outside LLINs. However, during the hours when people were beneath LLINs, these provided protective efficacy as indicated by reduced human blood feeding rates.

CONCLUSION: LLINs provided effective protection against pyrethroid-resistant malaria vector populations during bedtime hours. However, protection of LLINs was insufficient during the hours when people were active outside of the bed nets. Such limitation of LLINs will need to be intensively addressed in African countries in the near future.

PMCID: PMC4150967 **Free PMC Article**
PMID: 25141947 [PubMed - indexed for MEDLINE]

16. **Pyrethroid susceptibility of malaria vectors in four Districts of western Kenya.**

Ochomo E[1], Bayoh NM, Kamau L, Atieli F, Vulule J, Ouma C, Ombok M, Njagi K, Soti D, Mathenge E, Muthami L, Kinyari T, Subramaniam K, Kleinschmidt I, Donnelly MJ, Mbogo C.

Parasit Vectors. 2014 Jul 4;7:310. doi: 10.1186/1756-3305-7-310.

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**ABSTRACT**

BACKGROUND: Increasing pyrethroid resistance in malaria vectors has been reported in western Kenya where long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the mainstays of vector control. To ensure the sustainability of insecticide-based malaria vector control, monitoring programs need to be implemented. This study was designed to investigate the extent and distribution of pyrethroid resistance in 4 Districts of western Kenya (Nyando, Rachuonyo, Bondo and Teso). All four Districts have received LLINs while Nyando and Rachuonyo Districts have had IRS campaigns for 3-5 years using pyrethroids. This study is part of a programme aimed at determining the impact of insecticide resistance on malaria epidemiology.

METHODS: Three day old adult mosquitoes from larval samples collected in the field, were used for bioassays using the WHO tube bioassay, and mortality recorded 24 hours post exposure. Resistance level was assigned based on the 2013 WHO guidelines where populations with <90% mortality were considered resistant. Once exposed, samples were identified to species using PCR.

RESULTS: An. arabiensis comprised at least 94% of all An. gambiae s.l. in Bondo, Rachuonyo and Nyando. Teso was a marked contrast case with 77% of all samples being An. gambiae s.s. Mortality to insecticides varied widely between clusters even in one District with mortality to deltamethrin ranging from 45-100%, while to permethrin the range was 30-100%. Mortality to deltamethrin in Teso District was < 90% in 4 of 6 clusters tested in An arabiensis and <90% in An. gambiae s.s in 5 of 6 clusters tested. To permethrin, mortality ranged between 5.9-95%, with <90% mortality in 9 of 13 and 8 of 13 in An. arabiensis and An. gambiae s.s. respectively. Cluster specific mortality of An. arabiensis between permethin and deltamethrin were not correlated (Z = 2.9505, P = 0.2483).

CONCLUSION: High levels of pyrethroid resistance were observed in western Kenya. This resistance does not seem to be associated with either species or location. Insecticide resistance can vary within small geographical areas and such heterogeneity may make it possible to evaluate the impact of resistance on malaria and mosquito parameters within similar eco-epidemiological zones.

PMCID: PMC4094666 **Free PMC Article**
PMID: 24996418 [PubMed - indexed for MEDLINE]

17. **Genetic basis of pyrethroid resistance in a population of Anopheles arabiensis, the primary malaria vector in Lower Moshi, north-eastern Tanzania.**

Matowo J[1], Jones CM, Kabula B, Ranson H, Steen K, Mosha F, Rowland M, Weetman D.

Parasit Vectors. 2014 Jun 19;7:274. doi: 10.1186/1756-3305-7-274.

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**ABSTRACT**

BACKGROUND: Pyrethroid resistance has been slower to emerge in Anopheles arabiensis than in An. gambiae s.s and An. funestus and, consequently, studies are only just beginning to unravel the genes involved. Permethrin resistance in An. arabiensis in Lower Moshi, Tanzania has been linked to elevated levels of both P450 monooxygenases and β-esterases. We have conducted a gene expression study to identify specific genes linked with metabolic resistance in the Lower Moshi An. arabiensis population.

METHODS: Microarray experiments employing an An. gambiae whole genome expression chip were performed on An. arabiensis, using interwoven loop designs. Permethrin-exposed survivors were compared to three separate unexposed mosquitoes from the same or a nearby population. A subsection of detoxification genes were chosen for subsequent quantitative real-time PCR (qRT-PCR).

RESULTS: Microarray analysis revealed significant over expression of 87 probes and under expression of 85 probes (in pairwise comparisons between permethrin survivors and unexposed sympatric and allopatric samples from Dar es Salaam (controls). For qRT-PCR we targeted over expressed ABC transporter genes (ABC '2060'), a glutathione-S-transferase, P450s and esterases. Design of efficient, specific primers was successful for ABC '2060'and two P450s (CYP6P3, CYP6M2). For the CYP4G16 gene, we used the primers that were previously used in a microarray study of An. arabiensis from Zanzibar islands. Over expression of CYP4G16 and ABC '2060' was detected though with contrasting patterns in pairwise comparisons between survivors and controls. CYP4G16 was only up regulated in survivors, whereas ABC '2060' was similar in survivors and controls but over expressed in Lower Moshi samples compared to the Dar es Salaam samples. Increased transcription of CYP4G16 and ABC '2060' are linked directly and indirectly respectively, with permethrin resistance in Lower Moshi An. arabiensis.

CONCLUSIONS: Increased transcription of a P450 (CYP4G16) and an ABC transporter (ABC 2060) are linked directly and indirectly respectively, with permethrin resistance in Lower Moshi An. arabiensis. Our study provides replication of CYP4G16 as a candidate gene for pyrethroid resistance in An. arabiensis, although its role may not be in detoxification, and requires further investigation.

PMCID: PMC4082164 **Free PMC Article**
PMID: 24946780 [PubMed - indexed for MEDLINE]

18. **Negative cross resistance mediated by co-treated bed nets: a potential means of restoring pyrethroid-susceptibility to malaria vectors.**

White MT[1], Lwetoijera D[2], Marshall J[1], Caron-Lormier G[3], Bohan DA[4], Denholm I[5], Devine GJ[6].

PLoS One. 2014 May 1;9(5):e95640. doi: 10.1371/journal.pone.0095640. eCollection 2014.

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[5]University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom.
[6]QIMR Berghofer Medical Research Institute, Brisbane, Australia.

**ABSTRACT**

Insecticide-treated nets and indoor residual spray programs for malaria control are entirely dependent on pyrethroid insecticides. The ubiquitous exposure of Anopheles mosquitoes to this chemistry has selected for resistance in a number of populations. This threatens the sustainability of our most effective interventions but no operationally practicable way of resolving the problem currently exists. One innovative solution involves the co-application of a powerful chemosterilant (pyriproxyfen or PPF) to bed nets that are usually treated only with pyrethroids. Resistant mosquitoes that are unaffected by the pyrethroid component of a PPF/pyrethroid co-treatment remain vulnerable to PPF. There is a differential impact of PPF on pyrethroid-resistant and susceptible mosquitoes that is modulated by the mosquito's behavioural response at co-treated surfaces. This imposes a specific fitness cost on pyrethroid-resistant phenotypes and can reverse selection. The concept is demonstrated using a mathematical model.

PMCID: PMC4006834 **Free PMC Article**
PMID: 24788951 [PubMed - indexed for MEDLINE]

19. **Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis.**

Killeen GF[1], Chitnis N.

Malar J. 2014 Mar 14;13:97. doi: 10.1186/1475-2875-13-97.

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**ABSTRACT**

BACKGROUND: The ability of mosquitoes to evade fatal exposure to insecticidal nets and sprays represents the primary obstacle to eliminating malaria. However, it remains unclear which behaviours are most important for buffering mosquito and parasite populations against vector control.

METHODS: Simulated life histories were used to compare the impact of alternative feeding behaviour strategies upon overall lifetime feeding success, and upon temporal distributions of successful feeds and biting rates experienced by unprotected humans, in the presence and absence of insecticidal nets. Strictly nocturnal preferred feeding times were contrasted with 1) a wider preference window extending to dawn and dusk, and 2) crepuscular preferences wherein foraging is suppressed when humans sleep and can use nets but is maximal immediately before and after. Simulations with diversion and mortality parameters typical of endophagic, endophilic African vectors, such as Anopheles gambiae and Anopheles funestus, were compared with those for endophagic but exophilic species, such as Anopheles arabiensis, that also enter houses but leave earlier before lethal exposure to insecticide-treated surfaces occurs.

RESULTS: Insecticidal nets were predicted to redistribute successful feeding events to dawn and dusk where these were included in the profile of innately preferred feeding times. However, predicted distributions of biting unprotected humans were unaffected because extended host-seeking activity was redistributed to innately preferred feeding times. Recently observed alterations of biting activity distributions therefore reflect processes not captured in this model, such as evolutionary selection of heritably modified feeding time preferences or phenotypically plastic expression of feeding time preference caused by associative learning. Surprisingly, endophagy combined with exophily, among mosquitoes that enter houses but then feed and/or rest briefly before rapidly exiting, consistently attenuated predicted insecticide impact more than any feeding time preference trait.

CONCLUSIONS: Regardless of underlying cause, recent redistributions of host-biting activity to dawn and dusk necessitate new outdoor control strategies. However, persistently indoor-feeding vectors, that evade intradomiciliary insecticide exposure, are at least equally important. Fortunately, recent evaluations of occupied houses or odour-baited stations, with baffled entrances that retain An. arabiensis within insecticide-treated structures, illustrate how endophagic but exophilic vectors may be more effectively tackled using existing insecticides.

PMCID: PMC3995604 **Free PMC Article**
PMID: 24629066 [PubMed - indexed for MEDLINE]

20. **Contrasting Plasmodium infection rates and insecticide susceptibility profiles between the sympatric sibling species Anopheles parensis and Anopheles funestus s.s: a potential challenge for malaria vector control in Uganda.**

Mulamba C, Irving H, Riveron JM, Mukwaya LG, Birungi J, Wondji CS[1].

Parasit Vectors. 2014 Feb 17;7:71. doi: 10.1186/1756-3305-7-71.

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**ABSTRACT**

BACKGROUND: Although the An. funestus group conceals one of the major malaria vectors in Africa, little is known about the dynamics of members of this group across the continent. Here, we investigated the species composition, infection rate and susceptibility to insecticides of this species group in Uganda.

METHODS: Indoor resting blood-fed Anopheles adult female mosquitoes were collected from 3 districts in Uganda. Mosquitoes morphologically belonging to the An. funestus group were identified to species by PCR. The sporozoite infection rates were determined by TaqMan and a nested PCR. Susceptibility to major insecticides was assessed using WHO bioassays. The potential role of four candidate resistance genes was assessed using qRT-PCR.

RESULTS: An. funestus s.s. and An. parensis, were the only members of the An. funestus group identified. Both species were sympatric in Masindi (North-West), whereas only An. parensis was present in Mityana (Central) and Ntungamo (South-West). The Plasmodium falciparum infection detected in An. parensis (4.2%) by TaqMan could not be confirmed by nested PCR, whereas the 5.3% infection in An. funestus s.s. was confirmed. An. parensis was susceptible to most insecticides, however, a moderate resistance was observed against deltamethrin and DDT. In the sympatric population of Masindi, resistance was observed to pyrethroids (permethrin and deltamethrin) and DDT, but all the resistant mosquitoes belonged to An. funestus s.s. No significant over-expression was observed for the four P450 candidate genes CYP6M7, CYP9K1, CYP6P9 and CYP6AA4 between deltamethrin resistant and control An. parensis. However, when compared with the susceptible FANG An. funestus s.s strain, the CYP9K1 is significantly over-expressed in An. parensis (15-fold change; $P < 0.001$), suggesting it could play a role in the deltamethrin resistance.

CONCLUSION: The contrasting infection rates and insecticide susceptibility profiles of both species highlights the importance of accurate species identification for successful vector control programs.

PMCID: PMC3937429 **Free PMC Article**
PMID: 24533773 [PubMed - indexed for MEDLINE]

21. **Synergist bioassays: A simple method for initial metabolic resistance investigation of field Anopheles gambiae s.l. populations.**

Chouaïbou M[1], Zivanovic GB[2], Knox TB[3], Jamet HP[2], Bonfoh B[4].

Acta Trop. 2014 Feb;130:108-11. doi: 10.1016/j.actatropica.2013.10.020. Epub 2013 Nov 2.

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**ABSTRACT**

Metabolic resistance and the potential role of permeability-glycoprotein (P-gp) efflux pumps were investigated in a pyrethroid-resistant wild Anopheles gambiae s.l. Tiassalé population, using WHO susceptibility assays with deltamethrin (0.05%), with and without pre-exposure to synergists. The synergists used included an inhibitor of P-glycoprotein efflux pumps (verapamil), an inhibitor of esterases (EN 16-5), and an inhibitor of P450s and esterases (piperonyl butoxide). Pre-exposure to verapamil followed by deltamethrin led to a slight but non-significant (P=0.59) increase in mortality relative to exposure to deltamethrin alone (64.5% versus 69.2%). Similarly, pre-exposure to EN 16-5 yielded a non-significant increase in mortality (to 76.6%; P=0.85) but a significant increase in the knock down rate (from 48.3% to 78.7%; P<0.01). Pre-exposure with PBO caused a significant increase in mortality (to 93.1%; P<0.001) and knockdown rate (100%; P<0.001), which related to a 2.9 fold decrease in the resistance level. The results provide evidence that metabolic resistance mechanisms are present within the assessed mosquito population. The decrease in time to knock down of this population with deltamethrin following exposure to EN16-5 and PBO is of particular relevance to vector control, where quick knock down is a highly desired characteristic. The suspected resistance mechanisms present in this population merit further investigation through biochemical and molecular analyses for full resistance profile characterization. Bioassays with synergists can provide a quick and easy basis for initial characterization of resistant mosquito populations, without the need of preserved specimens, expensive equipment and substrates or specialized expertise.

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PMCID: PMC4786622 **Free PMC Article**
PMID: 24191946 [PubMed - indexed for MEDLINE]

22.

## Insecticide resistance mechanisms associated with different environments in the malaria vector Anopheles gambiae: a case study in Tanzania.

Nkya TE, Akhouayri I, Poupardin R, Batengana B, Mosha F, Magesa S, Kisinza W, David JP[1].

Malar J. 2014 Jan 25;13:28. doi: 10.1186/1475-2875-13-28.

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**ABSTRACT**

BACKGROUND: Resistance of mosquitoes to insecticides is a growing concern in Africa. Since only a few insecticides are used for public health and limited development of new molecules is expected in the next decade, maintaining the efficacy of control programmes mostly relies on resistance management strategies. Developing such strategies requires a deep understanding of factors influencing resistance together with characterizing the mechanisms involved. Among factors likely to influence insecticide resistance in mosquitoes, agriculture and urbanization have been implicated but rarely studied in detail. The present study aimed at comparing insecticide resistance levels and associated mechanisms across multiple Anopheles gambiae sensu lato populations from different environments.

METHODS: Nine populations were sampled in three areas of Tanzania showing contrasting agriculture activity, urbanization and usage of insecticides for vector control. Insecticide resistance levels were measured in larvae and adults through bioassays with deltamethrin, DDT and bendiocarb. The distribution of An. gambiae sub-species and pyrethroid target-site mutations (kdr) were investigated using molecular assays. A microarray approach was used for identifying transcription level variations associated to different environments and insecticide resistance.

RESULTS: Elevated resistance levels to deltamethrin and DDT were identified in agriculture and urban areas as compared to the susceptible strain Kisumu. A significant correlation was found between adult deltamethrin resistance and agriculture activity. The subspecies Anopheles arabiensis was predominant with only few An. gambiae sensu stricto identified in the urban area of Dar es Salaam. The L1014S kdr mutation was detected at elevated frequency in An gambiae s.s. in the urban area but remains sporadic in An. arabiensis specimens. Microarrays identified 416 transcripts differentially expressed in any area versus the susceptible reference strain and supported the impact of agriculture on resistance mechanisms with multiple genes encoding pesticide targets, detoxification enzymes and proteins linked to neurotransmitter activity affected. In contrast, resistance mechanisms found in the urban area appeared more specific and more related to the use of insecticides for vector control.

CONCLUSIONS: Overall, this study confirmed the role of the environment in shaping insecticide resistance in mosquitoes with a major impact of agriculture activities. Results are discussed in relation to resistance mechanisms and the optimization of resistance management strategies.

PMCID: PMC3913622 **Free PMC Article**
PMID: 24460952 [PubMed - indexed for MEDLINE]

23. **Indoor application of attractive toxic sugar bait (ATSB) in combination with mosquito nets for control of pyrethroid-resistant mosquitoes.**

Stewart ZP[1], Oxborough RM[2], Tungu PK[3], Kirby MJ[4], Rowland MW[5], Irish SR[5].

PLoS One. 2013 Dec 19;8(12):e84168. doi: 10.1371/journal.pone.0084168. eCollection 2013.

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[3]Pan-African Malaria Vector Research Consortium (PAMVERC), Moshi, Tanzania ; Amani Medical Research Centre, National Institute for Medical Research, Muheza, Tanzania.
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[5]Faculty of Infectious Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom ; Pan-African Malaria Vector Research Consortium (PAMVERC), Moshi, Tanzania.

**ABSTRACT**

BACKGROUND: Attractive toxic sugar bait (ATSB) sprayed onto vegetation has been successful in controlling Anopheles mosquitoes outdoors. Indoor application of ATSB has yet to be explored. The purpose of this study was to determine whether ATSB stations positioned indoors have the potential to kill host-seeking mosquitoes and constitute a new approach to control of mosquito-borne diseases.

METHODS: Insecticides were mixed with dyed sugar solution and tested as toxic baits against Anopheles arabiensis, An. Gambiae s.s. and Culex quinquefasciatus in feeding bioassay tests to identify suitable attractant-insecticide combinations. The most promising ATSB candidates were then trialed in experimental huts in Moshi, Tanzania. ATSB stations were hung in huts next to untreated mosquito nets occupied by human volunteers. The proportions of mosquitoes killed in huts with ATSB treatments

relative to huts with non-insecticide control treatments huts were recorded, noting evidence of dye in mosquito abdomens.

RESULTS: In feeding bioassays, chlorfenapyr 0.5% v/v, boric acid 2% w/v, and tolfenpyrad 1% v/v, mixed in a guava juice-based bait, each killed more than 90% of pyrethroid-susceptible An. Gambiae s.s. and pyrethroid-resistant An. arabiensis and Cx. quinquefasciatus. In the hut trial, mortality rates of the three ATSB treatments ranged from 41-48% against An. arabiensis and 36-43% against Cx. quinquefasciatus and all were significantly greater than the control mortalities: 18% for An. arabiensis, 7% for Cx. quinquefasciatus ($p<0.05$). Mortality rates with ATSB were comparable to those with long lasting insecticidal nets previously tested against the same species in this area.

CONCLUSIONS: Indoor ATSB shows promise as a supplement to mosquito nets for controlling mosquitoes. Indoor ATSB constitute a novel application method for insecticide classes that act as stomach poisons and have not hitherto been exploited for mosquito control. Combined with LLIN, indoor use of ATSB has the potential to serve as a strategy for managing insecticide resistance.

PMCID: PMC3868566 **Free PMC Article**
PMID: 24367638 [PubMed - indexed for MEDLINE]

24. **The dynamics of pyrethroid resistance in Anopheles arabiensis from Zanzibar and an assessment of the underlying genetic basis.**

Jones CM, Haji KA, Khatib BO, Bagi J, Mcha J, Devine GJ, Daley M, Kabula B, Ali AS, Majambere S, Ranson H[1].

Parasit Vectors. 2013 Dec 6;6:343. doi: 10.1186/1756-3305-6-343.

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**ABSTRACT**

BACKGROUND: The emergence of pyrethroid resistance in the malaria vector, Anopheles arabiensis, threatens to undermine the considerable gains made towards eliminating malaria on Zanzibar. Previously, resistance was restricted to the island of Pemba while mosquitoes from Unguja, the larger of the two islands of Zanzibar, were susceptible. Here, we characterised the mechanism(s) responsible for resistance on Zanzibar using a combination of gene expression and target-site mutation assays.

METHODS: WHO resistance bioassays were conducted using 1-5d old adult Anopheles gambiae s.l. collected between 2011 and 2013 across the archipelago. Synergist assays with the P450 inhibitor piperonyl-butoxide were performed in 2013. Members of the An. gambiae complex were PCR-identified and screened for target-site mutations (kdr and Ace-1). Gene expression in pyrethroid resistant An. arabiensis from Pemba was analysed using whole-genome microarrays.

RESULTS: Pyrethroid resistance is now present across the entire Zanzibar archipelago. Survival to the pyrethroid lambda-cyhalothrin in bioassays conducted in 2013 was 23.5-54.3% on Unguja and 32.9-81.7% on Pemba. We present evidence that resistance is mediated, in part at least, by elevated P450 monoxygenases. Whole-genome microarray scans showed that the most enriched gene terms in resistant An. arabiensis from Pemba were associated with P450 activity and synergist assays with PBO completely restored susceptibility to pyrethroids in both islands. CYP4G16 was the most consistently over-expressed gene in resistant mosquitoes compared with two susceptible strains from Unguja and Dar es Salaam. Expression of this P450 is enriched in the abdomen and it is thought to play a role in hydrocarbon synthesis. Microarray and qPCR detected several additional genes putatively involved in this pathway enriched in the Pemba pyrethroid resistant population and we hypothesise that resistance may be, in part, related to alterations in the structure of the mosquito cuticle. None of the kdr target-site mutations, associated with pyrethroid/DDT resistance in An. gambiae elsewhere in Africa, were found on the islands.

CONCLUSION: The consequences of this resistance phenotype are discussed in relation to future vector control strategies on Zanzibar to support the ongoing malaria elimination efforts on the islands.

PMCID: PMC3895773 **Free PMC Article**
PMID: 24313005 [PubMed - indexed for MEDLINE]

25. **The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance.**

Ochomo EO[1], Bayoh NM, Walker ED, Abongo BO, Ombok MO, Ouma C, Githeko AK, Vulule J, Yan G, Gimnig JE.

Malar J. 2013 Oct 24;12:368. doi: 10.1186/1475-2875-12-368.

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**ABSTRACT**

BACKGROUND: Long-lasting insecticide-treated mosquito nets (LLINs) are a primary malaria prevention strategy in sub-Saharan Africa. However, emergence of insecticide resistance threatens the effectiveness of LLINs.

METHODS: Cross-sectional surveys of LLINs were conducted in houses of seven and four villages in Gem and Bungoma Districts in western Kenya, respectively. Condition (number and area of holes in the nets), number and species of mosquitoes resting inside them, and insecticidal activity of nets were quantified. Mosquitoes collected inside nets were allowed to lay eggs and progeny tested for susceptibility to deltamethrin and permethrin, pyrethoids commonly deployed in LLINs in western Kenya.

RESULTS: In Gem, 83.3% of nets were less than three years old and 32.4% had at least one hole of any size; while in Bungoma, 92% were less than three years old and 48% had at least one hole. No anopheline and five Culex spp. mosquitoes were found resting inside nets in Gem regardless of the number and size of holes, while 552 Anopheles gambiae s.l., five Anopheles funestus s.l. and 137 Culex spp. were in nets in Bungoma. The number of mosquitoes resting inside nets increased with hole areas >50 cm in Bungoma. In WHO resistance assays, f1 offspring of samples collected in nets in Bungoma were 94 and 65% resistant to deltamethrin and permethrin, respectively. Nets from Bungoma retained strong activity against a susceptible laboratory strain, but not against f1 offspring of field-collected An. gambiae s.s. All An. gambiae s.s. samples collected in nets were homozygous for the kdr genotype L1014S.

CONCLUSIONS: In areas with pyrethroid resistant vectors, LLINs with modest hole areas permit mosquito entry and feeding, providing little protection against the vectors. LLIN formulations develop large holes within three years of use, diminishing their presupposed lifetime effectiveness.

PMCID: PMC4016513 **Free PMC Article**
PMID: 24156715 [PubMed - indexed for MEDLINE]

26. **Insecticide resistance monitoring of field-collected Anopheles gambiae s.l. populations from Jinja, eastern Uganda, identifies high levels of pyrethroid resistance.**

Mawejje HD[1], Wilding CS, Rippon EJ, Hughes A, Weetman D, Donnelly MJ.

Med Vet Entomol. 2013 Sep;27(3):276-83. doi: 10.1111/j.1365-2915.2012.01055.x. Epub 2012 Oct 10.

[1]Infectious Diseases Research Collaboration, Kampala, Uganda Vector Group, Liverpool School of Tropical Medicine, Liverpool, U.K.

**ABSTRACT**

Insecticide resistance in the malaria vector Anopheles gambiae s.l. (Diptera: Culicidae) threatens insecticide-based control efforts, necessitating regular monitoring. We assessed resistance in field-collected An. gambiae s.l. from Jinja, Uganda using World Health Organization (WHO) bioassays. Only An. gambiae s.s. and An. arabiensis (≈70%) were present. Female An. gambiae exhibited extremely high pyrethroid resistance (permethrin LT50 > 2 h; deltamethrin LT50 > 5 h). Female An. arabiensis were resistant to permethrin and exhibited reduced susceptibility to deltamethrin. However, while An. gambiae were DDT resistant, An. arabiensis were fully susceptible. Both species were fully susceptible to bendiocarb and fenitrothion. Kdr 1014S has increased rapidly in the Jinja population of An. gambiae s.s. and now approaches fixation (≈95%), consistent with insecticide-mediated selection, but is currently at a low frequency in An. arabiensis (0.07%). Kdr 1014F was also at a low frequency in An. gambiae. These frequencies preclude adequately-powered tests for an association with phenotypic resistance. PBO synergist bioassays resulted in near complete recovery of pyrethroid susceptibility suggesting involvement of CYP450s in resistance. A small number (0.22%) of An. gambiae s.s. ×An. arabiensis hybrids were found, suggesting the possibility of introgression of resistance alleles between

species. The high levels of pyrethroid resistance encountered in Jinja threaten to reduce the efficacy of vector control programmes which rely on pyrethroid-impregnated bednets or indoor spraying of pyrethroids.

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PMCID: PMC3543752 **Free PMC Article**
PMID: 23046446 [PubMed - indexed for MEDLINE]

27. **Pyrethroid resistance in Anopheles gambiae s.s. and Anopheles arabiensis in western Kenya: phenotypic, metabolic and target site characterizations of three populations.**

Ochomo E[1], Bayoh MN, Brogdon WG, Gimnig JE, Ouma C, Vulule JM, Walker ED.

Med Vet Entomol. 2013 Jun;27(2):156-64. doi: 10.1111/j.1365-2915.2012.01039.x. Epub 2012 Aug 2.

[1]Department of Biomedical Science and Technology, School of Public Health and Community Development, Maseno University, Maseno, Kenya. ericochomo@yahoo.com

**ABSTRACT**

Field and laboratory investigations revealed phenotypic, target site and metabolic resistance to permethrin in an Anopheles gambiae s.s. (Diptera: Culicidae) population in Bungoma District, a region in western Kenya in which malaria is endemic and rates of ownership of insecticide-treated bednets are high. The sensitivity of individual An. gambiae s.l. females as indicated in assays using World Health Organization (WHO) test kits demonstrated reduced mortality in response to permethrin, deltamethrin and bendiocarb. Estimated time to knock-down of 50% (KDT50) of the test population in Centers for Disease Control (CDC) bottle bioassays was significantly lengthened for the three insecticides compared with that in a susceptible control strain. Anopheles arabiensis from all three sites showed higher mortality to all three insecticides in the WHO susceptibility assays compared with the CDC bottle assays, in which they showed less sensitivity and longer KDT50 than the reference strain for permethrin and deltamethrin. Microplate assays revealed elevated activity of β-esterases and oxidases, but not glutathione-S-transferase, in An. gambiae s.s. survivors exposed to permethrin in bottle bioassays compared with knocked down and unexposed individuals. No An. arabiensis showed elevated enzyme activity. The 1014S kdr allele was fixed in the Bungoma An. gambiae s.s. population and absent from An. arabiensis, whereas the 1014F kdr allele was absent from all samples of both species. Insecticide resistance could compromise vector control in Bungoma and could spread to other areas as coverage with longlasting insecticide-treated bednets increases.

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PMCID: PMC4744475 **Free PMC Article**
PMID: 22861380 [PubMed - indexed for MEDLINE]

28. **High level of resistance in the mosquito Anopheles gambiae to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania.**

Protopopoff N[1], Matowo J, Malima R, Kavishe R, Kaaya R, Wright A, West PA, Kleinschmidt I, Kisinza W, Mosha FW, Rowland M.

Malar J. 2013 May 2;12:149. doi: 10.1186/1475-2875-12-149.

[1]Department of Disease Control, London School of Hygiene and Tropical Medicine, Keppel Street, London, UK. natacha.protopopoff@lshtm.ac.uk

**ABSTRACT**

BACKGROUND: To control malaria in Tanzania, two primary vector control interventions are being scaled up: long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). The main threat to effective malaria control is the selection of insecticide resistance. While resistance to pyrethroids, the primary insecticide used for LLINs and IRS, has been reported among mosquito vectors in only a few sites in Tanzania, neighbouring East African countries are recording increasing levels of resistance. To monitor the rapidly evolving situation, the resistance status of the malaria vector Anopheles gambiae s.l to different insecticides and the prevalence of the kdr resistance allele involved in pyrethroid resistance were investigated in north-western Tanzania, an area that has been subject to several rounds of pyrethroid IRS since 2006.

METHODS: Household collections of anopheline mosquitoes were exposed to diagnostic dosages of pyrethroid, DDT, and bendiocarb using WHO resistance test kits. The relative proportions of An. gambiae s.s and Anopheles arabiensis were also investigated among mosquitoes sampled using indoor CDC light traps. Anophelines were identified to species and the kdr mutation was detected using real time PCR TaqMan assays.

RESULTS: From the light trap collections 80% of An. gambiae s.l were identified as An. gambiae s.s and 20% as An. arabiensis. There was cross-resistance between pyrethroids and DDT with mortality no higher than 40% reported in any of the resistance tests. The kdr-eastern variant was present in homozygous form in 97% of An. gambiae s.s but was absent in An. arabiensis. Anopheles gambiae s.s showed reduced susceptibility to the carbamate insecticide, bendiocarb, the proportion surviving WHO tests ranging from 0% to 30% depending on season and location.

CONCLUSION: Anopheles gambiae s.s has developed phenotypic resistance to pyrethroids and DDT and kdr frequency has almost reached fixation. Unlike in coastal Tanzania, where the ratio of An. gambiae s.s to An. arabiensis has decreased in response to vector control, An. gambiae s.s persists at high frequency in north-western Tanzania, probably due to selection of pyrethroid resistance, and this trend is likely to arise in other areas as resistance spreads or is subject to local selection from IRS or LLINs.

PMCID: PMC3655935 **Free PMC Article**
PMID: 23638757 [PubMed - indexed for MEDLINE]

29. **Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of Anopheles gambiae s.s. from different malaria transmission zones in Uganda.**

Okia M[1], Ndyomugyenyi R, Kirunda J, Byaruhanga A, Adibaku S, Lwamafa DK, Kironde F.

Parasit Vectors. 2013 May 2;6:130. doi: 10.1186/1756-3305-6-130.

[1]National Malaria Control Programme, Ministry of Health, P.O. Box 7272, Kampala, Uganda.

**ABSTRACT**

BACKGROUND: There are major concerns over sustaining the efficacy of current malaria vector control interventions given the rapid spread of resistance, particularly to pyrethroids. This study assessed the bioefficacy of five WHO-recommended long-lasting insecticidal nets (LLINs) against pyrethroid-resistant Anopheles gambiae field populations from Uganda.

METHODS: Adult An. gambiae from Lira, Tororo, Wakiso and Kanungu districts were exposed to permethrin (0.75%) or deltamethrin (0.05%) in standard WHO susceptibility tests. Cone bioassays were used to measure the bioefficacy of four mono-treated LLINs (Olyset®, Interceptor®, Netprotect® and PermaNet® 2.0) and one combination LLIN (PermaNet® 3.0) against the four mosquito populations. Wireball assays were similarly conducted to determine knockdown rates. Species composition and kdr mutation frequency were determined for a sample of mosquitoes from each population. Chemical assays confirmed that test nets fell within target dose ranges.

RESULTS: Anopheles gambiae s.s. predominated at all four sites (86-99% of Anopheles spp.) with moderate kdr L1014S allelic frequency (0.34-0.37). Confirmed or possible resistance to both permethrin and deltamethrin was identified for all four test populations. Reduced susceptibility to standard LLINs was observed for all four populations, with mortality rates as low as 45.8% even though the nets were unused. The combination LLIN PermaNet®3.0 showed the highest overall bioefficacy against all four An. gambiae s.l. populations (98.5-100% mortality). Wireball assays provided a more sensitive indicator of comparative bioefficacy, and PermaNet 3.0 was again associated with the highest bioefficacy against all four populations (76.5-91.7% mortality after 30 mins).

CONCLUSIONS: The bioefficacy of mono-treated LLINs against pyrethroid-resistant field populations of An. gambiae varied by LLIN type and mosquito population, indicating that certain LLINs may be more suitable than others at particular sites. In contrast, the combination LLIN PermaNet 3.0 performed optimally against the four An. gambiae populations tested. The observed reduced susceptibility of malaria vectors to mono-treated LLINs is of particular concern, especially considering all nets were unused. With ongoing scale-up of insecticidal tools in the advent of increasing resistance, it is essential

that those interventions with proven enhanced efficacy are given preference particularly in areas with high resistance.

PMCID: PMC3656772 **Free PMC Article**
PMID: 23634798 [PubMed - indexed for MEDLINE]

30. **Reconsideration of Anopheles rivulorum as a vector of Plasmodium falciparum in western Kenya: some evidence from biting time, blood preference, sporozoite positive rate, and pyrethroid resistance.**

Kawada H[1], Dida GO, Sonye G, Njenga SM, Mwandawiro C, Minakawa N.

Parasit Vectors. 2012 Oct 10;5:230. doi: 10.1186/1756-3305-5-230.

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**ABSTRACT**

BACKGROUND: Anopheles gambiae, An. arabiensis, and An. funestus are widespread malaria vectors in Africa. Anopheles rivulorum is the next most widespread species in the An. funestus group. The role of An. rivulorum as a malaria vector has not been fully studied, although it has been found to be a minor or opportunistic transmitter of Plasmodium falciparum.

METHODS: Mosquitoes were collected indoors over a 12-hour period using a light source attached to a rotating bottle collector in order to determine peak activity times and to provide DNA for meal source identification. Gravid female mosquitoes were collected indoors via an aspirator to generate F1 progeny for testing insecticidal susceptibility. Blood meal sources were identified using a multiplexed PCR assay for human and bovine cytochrome-B, and by matching sequences generated with primers targeting vertebrate and mammalian cytochrome-B segments to the Genbank database.

RESULTS: Anopheles rivulorum fed on human blood in the early evening between 18:00 and 20:00, when insecticide-treated bed nets are not in use, and the presence of Plasmodium falciparum sporozoites in 0.70% of the An. rivulorum individuals tested was demonstrated. Susceptibility to permethrin, deltamethrin, and DDT is higher in An. rivulorum (84.8%, 91.4%, and 100%, respectively) than in An. funestus s.s. (36.8%, 36.4%, and 70%, respectively), whereas mortality rates for propoxur and fenitrothion were 100% for both species. Resistance to pyrethroids was very high in An. funestus s.s. and the potential of the development of high resistance was suspected in An. rivulorum.

CONCLUSION: Given the tendency for An. rivulorum to be active early in the evening, the presence of P. falciparum in the species, and the potential for the development of pyrethroid resistance, we strongly

advocate reconsideration of the latent ability of this species as an epidemiologically important malaria vector.

PMCID: PMC3485129 **Free PMC Article**
PMID: 23050856 [PubMed - indexed for MEDLINE]

31. **Susceptibility status of malaria vectors to insecticides commonly used for malaria control in Tanzania.**

Kabula B[1], Tungu P, Matowo J, Kitau J, Mweya C, Emidi B, Masue D, Sindato C, Malima R, Minja J, Msangi S, Njau R, Mosha F, Magesa S, Kisinza W.

Trop Med Int Health. 2012 Jun;17(6):742-50. doi: 10.1111/j.1365-3156.2012.02986.x. Epub 2012 Apr 23.

[1]National Institute for Medical Research, Amani Research Centre, Muheza, Tanzania. bika72@yahoo.com

**ABSTRACT**

OBJECTIVE: The aim of the study was to monitor the insecticide susceptibility status of malaria vectors in 12 sentinel districts of Tanzania.

METHODS: WHO standard methods were used to detect knock-down and mortality in the wild female Anopheles mosquitoes collected in sentinel districts. The WHO diagnostic doses of 0.05% deltamethrin, 0.05% lambdacyhalothrin, 0.75% permethrin and 4% DDT were used.

RESULTS: The major malaria vectors in Tanzania, Anopheles gambiae s.l., were susceptible (mortality rate of 98-100%) to permethrin, deltamethrin, lambdacyhalothrin and DDT in most of the surveyed sites. However, some sites recorded marginal susceptibility (mortality rate of 80-97%); Ilala showed resistance to DDT (mortality rate of 65% [95% CI, 54-74]), and Moshi showed resistance to lambdacyhalothrin (mortality rate of 73% [95% CI, 69-76]) and permethrin (mortality rate of 77% [95% CI, 73-80]).

CONCLUSIONS: The sustained susceptibility of malaria vectors to pyrethroid in Tanzania is encouraging for successful malaria control with Insecticide-treated nets and IRS. However, the emergency of focal points with insecticide resistance is alarming. Continued monitoring is essential to ensure early containment of resistance, particularly in areas that recorded resistance or marginal susceptibility and those with heavy agricultural and public health use of insecticides.

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**Free Article**
PMID: 22519840 [PubMed - indexed for MEDLINE]

32. **Insecticide resistance in Culex quinquefasciatus from Zanzibar: implications for vector control programmes.**

Jones CM[1], Machin C, Mohammed K, Majambere S, Ali AS, Khatib BO, McHa J, Ranson H, Kelly-Hope LA.

Parasit Vectors. 2012 Apr 21;5:78. doi: 10.1186/1756-3305-5-78.

[1]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

**ABSTRACT**

BACKGROUND: Zanzibar has a long history of lymphatic filariasis (LF) caused by the filarial parasite Wuchereria bancrofti, and transmitted by the mosquito Culex quinquefasciatus Say. The LF Programme in Zanzibar has successfully implemented mass drug administration (MDA) to interrupt transmission, and is now in the elimination phase. Monitoring infections in mosquitoes, and assessing the potential role of interventions such as vector control, is important in case the disease re-emerges as a public health problem. Here, we examine Culex mosquito species from the two main islands to detect W. bancrofti infection and to determine levels of susceptibility to the insecticides used for vector control.

METHODS: Culex mosquitoes collected during routine catches in Vitongoji, Pemba Island, and Makadara, Unguja Island were tested for W. bancrofti infection using PCR. Insecticide bioassays on Culex mosquitoes were performed to determine susceptibility to permethrin, deltamethrin, lambda-cyhalothrin, DDT and bendiocarb. Additional synergism assays with piperonyl butoxide (PBO) were used for lambda-cyhalothrin. Pyrosequencing was used to determine the kdr genotype and sequencing of the mitochondrial cytochrome oxidase I (mtCOI) subunit performed to identify ambiguous Culex species.

RESULTS: None of the wild-caught Culex mosquitoes analysed were found to be positive for W. bancrofti. High frequencies of resistance to all insecticides were found in Wete, Pemba Island, whereas Culex from the nearby site of Tibirinzi (Pemba) and in Kilimani, Unguja Island remained relatively susceptible. Species identification confirmed that mosquitoes from Wete were Culex quinquefasciatus. The majority of the Culex collected from Tibirinzi and all from Kilimani could not be identified to species by molecular assays. Two alternative kdr alleles, both resulting in a L1014F substitution were detected in Cx. quinquefasciatus from Wete with no homozygote susceptible detected. Metabolic resistance to pyrethroids was also implicated by PBO synergism assays.

CONCLUSIONS: Results from the xenomonitoring are encouraging for the LF programme in Zanzibar. However, the high levels of pyrethroid resistance found in the principle LF vector in Pemba Island will need to be taken into consideration if vector control is to be implemented as part of the elimination programme.

PMCID: PMC3349604 **Free PMC Article**
PMID: 22520274 [PubMed - indexed for MEDLINE]

33. **Comparative transcriptome analyses of deltamethrin-resistant and -susceptible Anopheles gambiae mosquitoes from Kenya by RNA-Seq.**

Bonizzoni M[1], Afrane Y, Dunn WA, Atieli FK, Zhou G, Zhong D, Li J, Githeko A, Yan G.

PLoS One. 2012;7(9):e44607. doi: 10.1371/journal.pone.0044607. Epub 2012 Sep 7.

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**ABSTRACT**

Malaria causes more than 300 million clinical cases and 665,000 deaths each year, and the majority of the mortality and morbidity occurs in sub-Saharan Africa. Due to the lack of effective vaccines and wide-spread resistance to antimalarial drugs, mosquito control is the primary method of malaria prevention and control. Currently, malaria vector control relies on the use of insecticides, primarily pyrethroids. The extensive use of insecticides has imposed strong selection pressures for resistance in the mosquito populations. Consequently, resistance to pyrethroids in Anopheles gambiae, the main malaria vector in sub-Saharan Africa, has become a major obstacle for malaria control. A key element of resistance management is the identification of resistance mechanisms and subsequent development of reliable resistance monitoring tools. Field-derived An. gambiae from Western Kenya were phenotyped as deltamethrin-resistant or -susceptible by the standard WHO tube test, and their expression profile compared by RNA-seq. Based on the current annotation of the An. gambiae genome, a total of 1,093 transcripts were detected as significantly differentially accumulated between deltamethrin-resistant and -susceptible mosquitoes. These transcripts are distributed over the entire genome, with a large number mapping in QTLs previously linked to pyrethorid resistance, and correspond to heat-shock proteins, metabolic and transport functions, signal transduction activities, cytoskeleton and others. The detected differences in transcript accumulation levels between resistant and susceptible mosquitoes reflect transcripts directly or indirectly correlated with pyrethroid resistance. RNA-seq data also were used to perform a de-novo Cufflinks assembly of the An. gambiae genome.

PMCID: PMC3436877 **Free PMC Article**
PMID: 22970263 [PubMed - indexed for MEDLINE]

34. **Malaria entomological profile in Tanzania from 1950 to 2010: a review of mosquito distribution, vectorial capacity and insecticide resistance.**

Kabula B[1], Derua YA, Tungui P, Massue DJ, Sambu E, Stanley G, Mosha FW, Kisinza WN.

Tanzan J Health Res. 2011 Dec;13(5 Suppl 1):319-31.

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**ABSTRACT**

In Sub Saharan Africa where most of the malaria cases and deaths occur, members of the Anopheles gambiae species complex and Anophelesfunestus species group are the important malaria vectors. Control efforts against these vectors in Tanzania like in most other Sub Saharan countries have failed to achieve the set objectives of eliminating transmission due to scarcity of information about the enormous diversity of Anopheles mosquito species and their susceptibility status to insecticides used for malaria vector control. Understanding the diversity and insecticide susceptibility status of these vectors and other factors relating to their importance as vectors (such as malaria transmission dynamics, vector biology, ecology, behaviour and population genetics) is crucial to developing a better and sound intervention strategies that will reduce man-vector contact and also manage the emergency of insecticide resistance early and hence.a success in malaria control. The objective of this review was therefore to obtain the information from published and unpublished documents on spatial distribution and composition of malaria vectors, key features of their behaviour, transmission indices and susceptibility status to insecticides in Tanzania. All data available were collated into a database. Details recorded for each data source were the locality, latitude/longitude, time/period of study, species, abundance, sampling/collection methods, species identification methods, insecticide resistance status, including evidence of the kdr allele, and Plasmodium falciparum sporozoite rate. This collation resulted in a total of 368 publications, encompassing 806,273 Anopheles mosquitoes from 157 georeferenced locations being collected and identified across Tanzania from 1950s to 2010. Overall, the vector species most often reported included An. gambiae complex (66.8%), An. funestus complex (21.8%), An. gambiae s.s. (2.1%) and An. arabiensis (9%). A variety of sampling/ collection and species identification methods were used with an increase in molecular techniques in recent decades. Only 32.2% and 8.4% of the data sets reported on sporozoite analysis and entomological inoculation rate (EIR), respectively which highlights the paucity of such important information in the country. Studies demonstrated efficacy of all four major classes of insecticides against malaria vectors in Tanzania with focal points showing phenotypic resistance. About 95% of malaria entomological data was obtained from northeastern Tanzania. This shows the disproportionate nature of the available information with the western part of the country having none. Therefore it is important for the country to establish entomological surveillance system with state of the art to capture all vitally important entomological indices including vector bionomics in areas of Tanzania where very few or no studies have been done. This is vital in planning and implementing evidence based malaria vector control programmes as well as in monitoring the current malaria control interventions.

PMID: 26591987 [PubMed - indexed for MEDLINE]

35. **Distribution of a knockdown resistance mutation (L1014S) in Anopheles gambiae s.s. and Anopheles arabiensis in western and southern Kenya.**

Kawada H[1], Futami K, Komagata O, Kasai S, Tomita T, Sonye G, Mwatele C, Njenga SM, Mwandawiro C, Minakawa N, Takagi M.

PLoS One. 2011;6(9):e24323. doi: 10.1371/journal.pone.0024323. Epub 2011 Sep 9.

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**ABSTRACT**

In Kenya, insecticide-treated mosquito nets (ITNs) distributed to pregnant women and children under 5 years old through various programs have resulted in a significant reduction in malaria deaths. All of the World Health Organization-recommended insecticides for mosquito nets are pyrethroids, and vector mosquito resistance to these insecticides is one of the major obstacles to an effective malaria control program. Anopheles gambiae s.s. and Anopheles arabiensis are major malaria vectors that are widely distributed in Kenya. Two point mutations in the voltage-gated sodium channel (L1014F and L1014S) are associated with knockdown resistance (kdr) to DDT and pyrethroids in An. gambiae s.s. While the same point mutations have been reported to be rare in An. arabiensis, some evidence of metabolic resistance has been reported in this species. In order to determine the distribution of the point mutation L1014S in An. gambiae s.s. and An. arabiensis in southern and western Kenya, we collected larvae and screened for the mutation by DNA sequencing. We found high allelic and homozygous frequencies of the L1014S mutation in An. gambiae s.s. The L1014S mutation was also widely distributed in An. arabiensis, although the allelic frequency was lower than in An. gambiae s.s. The same intron sequence (length: 57 base) found in both species indicated that the mutation was introgressed by hybridization. The allelic frequency of L1014S was higher in both species in western regions, demonstrating the strong selection pressure imposed by long-lasting insecticide-treated nets (LLITN)/ITN on the An. gambiae s.s. and An. arabiensis populations in those areas. The present contribution of the L1014S mutation to pyrethroid resistance in An. arabiensis may be negligible. However, the homozygous frequency could increase with continuing selection pressure due to expanded LLITN coverage in the future.

PMCID: PMC3170322 **Free PMC Article**
PMID: 21931682 [PubMed - indexed for MEDLINE]

36. **Multimodal pyrethroid resistance in malaria vectors, Anopheles gambiae s.s., Anopheles arabiensis, and Anopheles funestus s.s. in western Kenya.**

Kawada H[1], Dida GO, Ohashi K, Komagata O, Kasai S, Tomita T, Sonye G, Maekawa Y, Mwatele C, Njenga SM, Mwandawiro C, Minakawa N, Takagi M.

PLoS One. 2011;6(8):e22574. doi: 10.1371/journal.pone.0022574. Epub 2011 Aug 11.

[1]Department of Vector Ecology and Environment, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan. vergiss@nagasaki-u.ac.jp

**ABSTRACT**

Anopheles gambiae s.s., Anopheles arabiensis, and Anopheles funestus s.s. are the most important species for malaria transmission. Pyrethroid resistance of these vector mosquitoes is one of the main obstacles against effective vector control. The objective of the present study was to monitor the pyrethroid susceptibility in the 3 major malaria vectors in a highly malaria endemic area in western Kenya and to elucidate the mechanisms of pyrethroid resistance in these species. Gembe East and West, Mbita Division, and 4 main western islands in the Suba district of the Nyanza province in western Kenya were used as the study area. Larval and adult collection and bioassay were conducted, as well as the detection of point mutation in the voltage-gated sodium channel (1014L) by using direct DNA sequencing. A high level of pyrethroid resistance caused by the high frequency of point mutations (L1014S) was detected in An. gambiae s.s. In contrast, P450-related pyrethroid resistance seemed to be widespread in both An. arabiensis and An. funestus s.s. Not a single L1014S mutation was detected in these 2 species. A lack of cross-resistance between DDT and permethrin was also found in An. arabiensis and An. funestus s.s., while An. gambiae s.s. was resistant to both insecticides. It is noteworthy that the above species in the same area are found to be resistant to pyrethroids by their unique resistance mechanisms. Furthermore, it is interesting that 2 different resistance mechanisms have developed in the 2 sibling species in the same area individually. The cross resistance between permethrin and DDT in An. gambiae s.s. may be attributed to the high frequency of kdr mutation, which might be selected by the frequent exposure to ITNs. Similarly, the metabolic pyrethroid resistance in An. arabiensis and An. funestus s.s. is thought to develop without strong selection by DDT.

PMCID: PMC3154902 **Free PMC Article**
PMID: 21853038 [PubMed - indexed for MEDLINE]

37. **Pyrethroid resistance in an Anopheles funestus population from Uganda.**

Morgan JC[1], Irving H, Okedi LM, Steven A, Wondji CS.

PLoS One. 2010 Jul 29;5(7):e11872. doi: 10.1371/journal.pone.0011872.

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom.

**ABSTRACT**

BACKGROUND: The susceptibility status of Anopheles funestus to insecticides remains largely unknown in most parts of Africa because of the difficulty in rearing field-caught mosquitoes of this malaria vector. Here we report the susceptibility status of the An. funestus population from Tororo district in Uganda and a preliminary characterisation of the putative resistance mechanisms involved.

METHODOLOGY/PRINCIPAL FINDINGS: A new forced egg laying technique used in this study significantly increased the numbers of field-caught females laying eggs and generated more than 4000 F1 adults. WHO bioassays indicated that An. funestus in Tororo is resistant to pyrethroids (62% mortality after 1 h exposure to 0.75% permethrin and 28% mortality to 0.05% deltamethrin). Suspected DDT resistance was also observed with 82% mortality. However this population is fully susceptible to bendiocarb (carbamate), malathion (organophosphate) and dieldrin with 100% mortality observed after exposure to each of these insecticides. Sequencing of a fragment of the sodium channel gene containing the 1014 codon conferring pyrethroid/DDT resistance in An. gambiae did not detect the L1014F kdr mutation but a correlation between haplotypes and resistance phenotype was observed indicating that mutations in other exons may be conferring the knockdown resistance in this species. Biochemical assays suggest that resistance in this population is mediated by metabolic resistance with elevated level of GSTs, P450s and pNPA compared to a susceptible strain of Anopheles gambiae. RT-PCR further confirmed the involvement of P450s with a 12-fold over-expression of CYP6P9b in the Tororo population compared to the fully susceptible laboratory colony FANG.

CONCLUSION: This study represents the first report of pyrethroid/DDT resistance in An. funestus from East Africa. With resistance already reported in southern and West Africa, this indicates that resistance in An. funestus may be more widespread than previously assumed and therefore this should be taken into account for the implementation and management of vector control programs in Africa.

PMCID: PMC2912372 **Free PMC Article**
PMID: 20686697 [PubMed - indexed for MEDLINE]

38. **Biochemical basis of permethrin resistance in Anopheles arabiensis from Lower Moshi, north-eastern Tanzania.**

Matowo J[1], Kulkarni MA, Mosha FW, Oxborough RM, Kitau JA, Tenu F, Rowland M.

Malar J. 2010 Jul 7;9:193. doi: 10.1186/1475-2875-9-193.

[1]Pan-African Malaria Vector Research Consortium www.pamverc.org. johntowo@yahoo.com

**ABSTRACT**

BACKGROUND: Development of resistance to different classes of insecticides is a potential threat to malaria control. With the increasing coverage of long-lasting insecticide-treated nets in Tanzania, the continued monitoring of resistance in vector populations is crucial. It may facilitate the development of novel strategies to prevent or minimize the spread of resistance. In this study, metabolic-based mechanisms conferring permethrin (pyrethroid) resistance were investigated in Anopheles arabiensis of Lower Moshi, Kilimanjaro region of north-eastern Tanzania.

METHODS: WHO susceptibility test kits were used to detect resistance to permethrin in An. arabiensis. The levels and mechanisms of permethrin resistance were determined using CDC bottle bioassays and microplate (biochemical) assays. In bottle bioassays, piperonyl butoxide (PBO) and s,s,s-tributyl phosphorotrithioate (DEF) were used as synergists to inhibit mixed function oxidases and non-specific esterases respectively. Biochemical assays were carried out in individual mosquitoes to detect any increase in the activity of enzymes typically involved in insecticide metabolism (mixed function oxidases, alpha- and beta-esterases).

RESULTS: Anopheles arabiensis from the study area was found to be partially resistant to permethrin, giving only 87% mortality in WHO test kits. Resistance ratios at KT50 and KT95 were 4.0 and 4.3 respectively. The permethrin resistance was partially synergized by DEF and by PBO when these were mixed with permethrin in bottle bioassays and was fully synergized when DEF and PBO were used together. The levels of oxidase and beta-esterase activity were significantly higher in An. arabiensis from Lower Moshi than in the laboratory susceptible strain. There was no difference in alpha-esterase activity between the two strains.

CONCLUSION: Elevated levels of mixed function oxidases and beta-esterases play a role in detoxification of permethrin in the resistant An. arabiensis population of Lower Moshi.

PMCID: PMC3224900 **Free PMC Article**
PMID: 20609220 [PubMed - indexed for MEDLINE]

39.

## Pyrethroid resistance in Anopheles gambiae leads to increased susceptibility to the entomopathogenic fungi Metarhizium anisopliae and Beauveria bassiana.

Howard AF[1], Koenraadt CJ, Farenhorst M, Knols BG, Takken W.

Malar J. 2010 Jun 16;9:168. doi: 10.1186/1475-2875-9-168.

[1]Laboratory of Entomology, Wageningen University and Research Centre, PO Box 8031, 6700 EH Wageningen, The Netherlands. afv.howard@gmail.com

**ABSTRACT**

BACKGROUND: Entomopathogenic fungi are being investigated as a new mosquito control tool because insecticide resistance is preventing successful mosquito control in many countries, and new methods are required that can target insecticide-resistant malaria vectors. Although laboratory studies have previously examined the effects of entomopathogenic fungi against adult mosquitoes, most application methods used cannot be readily deployed in the field. Because the fungi are biological organisms it is important to test potential field application methods that will not adversely affect them. The two objectives of this study were to investigate any differences in fungal susceptibility between an insecticide-resistant and insecticide-susceptible strain of Anopheles gambiae sensu stricto, and to test a potential field application method with respect to the viability and virulence of two fungal species

METHODS: Pieces of white polyester netting were dipped in Metarhizium anisopliae ICIPE-30 or Beauveria bassiana IMI391510 mineral oil suspensions. These were kept at 27 +/- 1 degrees C, 80 +/- 10% RH and the viability of the fungal conidia was recorded at different time points. Tube bioassays were used to infect insecticide-resistant (VKPER) and insecticide-susceptible (SKK) strains of An. gambiae s.s., and survival analysis was used to determine effects of mosquito strain, fungus species or time since fungal treatment of the net.

RESULTS: The resistant VKPER strain was significantly more susceptible to fungal infection than the insecticide-susceptible SKK strain. Furthermore, B. bassiana was significantly more virulent than M. anisopliae for both mosquito strains, although this may be linked to the different viabilities of these fungal species. The viability of both fungal species decreased significantly one day after application onto polyester netting when compared to the viability of conidia remaining in suspension.

CONCLUSIONS: The insecticide-resistant mosquito strain was susceptible to both species of fungus indicating that entomopathogenic fungi can be used in resistance management and integrated vector management programmes to target insecticide-resistant mosquitoes. Although fungal viability significantly decreased when applied to the netting, the effectiveness of the fungal treatment at killing mosquitoes did not significantly deteriorate. Field trials over a longer trial period need to be carried out

to verify whether polyester netting is a good candidate for operational use, and to see if wild insecticide-resistant mosquitoes are as susceptible to fungal infection as the VKPER strain.

PMCID: PMC2898789 **Free PMC Article**
PMID: 20553597 [PubMed - indexed for MEDLINE]

40. **Insecticide resistance and its association with target-site mutations in natural populations of Anopheles gambiae from eastern Uganda.**

Ramphul U[1], Boase T, Bass C, Okedi LM, Donnelly MJ, Müller P.

Trans R Soc Trop Med Hyg. 2009 Nov;103(11):1121-6. doi: 10.1016/j.trstmh.2009.02.014. Epub 2009 Mar 19.

[1]Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

**ABSTRACT**

Insecticide resistance in Anopheles gambiae threatens the success of malaria vector control programmes in sub-Saharan Africa. In order to manage insecticide resistance successfully, it is essential to assess continuously the target mosquito population. Here, we collected baseline information on the distribution and prevalence of insecticide resistance and its association with target-site mutations in eastern Uganda. Anopheles gambiae s.l. adults were raised from wild-caught larvae sampled from two ecologically distinct breeding sites and exposed to WHO discriminating concentrations of DDT, permethrin, deltamethrin, bendiocarb and malathion. Survival rates to DDT were as high as 85.4%, alongside significant resistance levels to permethrin (38.5%), reduced susceptibility to deltamethrin, but full susceptibility to bendiocarb and malathion. Using molecular diagnostics, susceptible and resistant specimens were further tested for the presence of knockdown resistance (kdr) and acetylcholinesterase 1 resistance (ace-1(R)) alleles. While ace-1(R) and kdrL1014F ('kdr west') alleles were absent, the kdr L1014S ('kdr east') allele was present in both populations. In A. gambiae s.s., L1014S was closely associated with DDT and, to a lesser degree, with permethrin resistance. Intriguingly, the association between DDT resistance and the presence of L1014S is consistent with a co-dominant effect, with heterozygous individuals showing an intermediate phenotype.
PMID: 19303125 [PubMed - indexed for MEDLINE]

41. **A significant increase in kdr in Anopheles gambiae is associated with an intensive vector control intervention in Burundi highlands.**

Protopopoff N[1], Verhaeghen K, Van Bortel W, Roelants P, Marcotty T, Baza D, D'Alessandro U, Coosemans M.

Trop Med Int Health. 2008 Dec;13(12):1479-87. doi: 10.1111/j.1365-3156.2008.02164.x. Epub 2008 Oct 6.

[1]Department of Parasitology, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. nprotopopoff@itg.be

**ABSTRACT**

OBJECTIVES AND METHODS: In Burundi, the occurrence of the knock down resistance (kdr) mutation in Anopheles gambiae sensu lato (s.l.) was determined for six consecutive years within the framework of a vector control programme. Findings were also linked with the insecticide resistance status observed with bioassay in An. gambiae s.l. and An. funestus.

RESULTS: The proportion of An. gambiae s.l. carrying the East Leu-Ser kdr mutation was 1% before the spraying intervention in 2002; by 2007 it was 86% in sprayed valleys and 67% in untreated valleys. Multivariate analysis showed that increased risk of carrying the kdr mutation is associated with spraying interventions, location and time. In bioassays conducted between 2005 and 2007 at five sites, An. funestus was susceptible to permethrin, deltamethrin and DDT. Anopheles gambiae s.l. remained susceptible or tolerant to deltamethrin and resistant to DDT and permethrin, but only when kdr allele carriers reached 90% of the population.

CONCLUSIONS: The cross-resistance against DDT and permethrin in Karuzi suggests a possible kdr resistance mechanism. Nevertheless, the homozygous resistant genotype alone does not entirely explain the bioassay results, and other mechanisms conferring resistance cannot be ruled out. After exposure to all three insecticides, homozygote individuals for the kdr allele dominate among the surviving An. gambiae s.l. This confirms the potential selection pressure of pyrethroids on kdr mutation. However, the high occurrence of the kdr mutation, even at sites far from the sprayed areas, suggests a selection pressure other than that exerted by the vector control programme.

**Free Article**
PMID: 18983277 [PubMed - indexed for MEDLINE]

42. **Distribution of knock-down resistance mutations in Anopheles gambiae molecular forms in west and west-central Africa.**

Santolamazza F[1], Calzetta M, Etang J, Barrese E, Dia I, Caccone A, Donnelly MJ, Petrarca V, Simard F, Pinto J, della Torre A.

Malar J. 2008 Apr 29;7:74. doi: 10.1186/1475-2875-7-74.

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**ABSTRACT**

BACKGROUND: Knock-down resistance (kdr) to DDT and pyrethroids in the major Afrotropical vector species, Anopheles gambiae sensu stricto, is associated with two alternative point mutations at amino acid position 1014 of the voltage-gated sodium channel gene, resulting in either a leucine-phenylalanine (L1014F), or a leucine-serine (L1014S) substitution. In An. gambiae S-form populations, the former mutation appears to be widespread in west Africa and has been recently reported from Uganda, while the latter, originally recorded in Kenya, has been recently found in Gabon, Cameroon and Equatorial Guinea. In M-form populations surveyed to date, only the L1014F mutation has been found, although less widespread and at lower frequencies than in sympatric S-form populations.

METHODS: Anopheles gambiae M- and S-form specimens from 19 sites from 11 west and west-central African countries were identified to molecular form and genotyped at the kdr locus either by Hot Oligonucleotide Ligation Assay (HOLA) or allele-specific PCR (AS-PCR).

RESULTS: The kdr genotype was determined for about 1,000 An. gambiae specimens. The L1014F allele was found at frequencies ranging from 6% to 100% in all S-form samples (N = 628), with the exception of two samples from Angola, where it was absent, and coexisted with the L1014S allele in samples from Cameroon, Gabon and north-western Angola. The L1014F allele was present in M-form samples (N = 354) from Benin, Nigeria, and Cameroon, where both M- and S-forms were sympatric.

CONCLUSION: The results represent the most comprehensive effort to analyse the overall distribution of the L1014F and L1014S mutations in An. gambiae molecular forms, and will serve as baseline data for resistance monitoring. The overall picture shows that the emergence and spread of kdr alleles in An. gambiae is a dynamic process and that there is marked intra- and inter-form heterogeneity in resistance allele frequencies. Further studies are needed to determine: i) the importance of selection pressure exerted by both agricultural and public health use of pyrethroid insecticides, ii) the phenotypic effects, particularly when the two mutations co-occur; and iii) the epidemiological importance of kdr for both pyrethroid- and DDT-based malaria control operations, particularly if/when the two insecticides are to be used in concert.

PMCID: PMC2405802 **Free PMC Article**
PMID: 18445265 [PubMed - indexed for MEDLINE]

43. **Status of insecticide susceptibility in Anopheles gambiae sensu lato and Anopheles funestus mosquitoes from western Kenya.**

Kamau L[1], Agai D, Matoke D, Wachira L, Gikandi G, Vulule JM.

J Insect Sci. 2008;8:11. doi: 10.1673/031.008.1101.

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**ABSTRACT**

The status of resistance was investigated in Anopheles gambiae sensu lato and An. funestus (Diptera: Culicidae) mosquitoes from western Kenya to four classes of insecticides approved by World Health Organization for indoor residual spraying. The prevalence of the knockdown-resistance (kdr) mutation associated with resistance to pyrethroids and DDT was determined in An. gambiae s.l.. Standard World Health Organization diagnostic bioassay kits for DDT (an organochlorine), fenitrothion (an organophosphate), bendiocarb (a carbamate), and the pyrethoirds, lambdacyhalothrin and permethrin, were used. Knockdown every 10 min and mortality 24 h after exposure were noted. Controls not treated with insecticides and with the susceptible An. gambiae KISUMU strain were included in the bioassays. The presence of the kdr gene was determined using a standard diagnostic polymerase chain reaction assay. Over 98% mortality was observed for tests with all insecticides for both An. gambiae s.l. and An. funestus. Knockdown rates were not significantly different between An. gambiae s.l. and the KISUMU strain control. 50% and 95% knockdown times were either slightly lower than those for the KISUMU strain or higher by factors of less than 1.6. The mean frequency of the East African kdr mutation was 24.7% in An. gambiae sensu strictu. Based on conventional criteria where susceptibility is defined by mortality rates >98% 24 h after exposure, no evidence for resistance was found, implying that vector control measures employing any of the insecticides tested would be unhampered by resistance. The observed frequencies of the kdr mutation do not appear to compromise the effectiveness of the insecticides. The need for continuous monitoring of the status of insecticide resistance and of the impact of any observed resistance on the efficacy of vector control programs employing insecticides is apparent.

PMCID: PMC3061582 **Free PMC Article**
PMID: 20345290 [PubMed - indexed for MEDLINE]

44. **Occurrence of the leucine-to-phenylalanine knockdown resistance (kdr) mutation in Anopheles arabiensis populations in Tanzania, detected by a simplified high-throughput SSOP-ELISA method.**

Kulkarni MA[1], Rowland M, Alifrangis M, Mosha FW, Matowo J, Malima R, Peter J, Kweka E, Lyimo I, Magesa S, Salanti A, Rau ME, Drakeley C.

Malar J. 2006 Jul 5;5:56.

[1]Department of Natural Resource Sciences, McGill University, Macdonald Campus, Ste Anne de Bellevue, Quebec H9X 3V9, Canada. manisha.kulkarni@elf.mcgill.ca

**ABSTRACT**

BACKGROUND: Molecular markers of insecticide resistance can provide sensitive indicators of resistance development in malaria vector populations. Monitoring of insecticide resistance in vector populations is an important component of current malaria control programmes. Knockdown resistance (kdr) confers resistance to the pyrethroid class of insecticides with cross-resistance to DDT through single nucleotide polymorphisms (SNPs) in the voltage-gated sodium channel gene.

METHODS: To enable detection of kdr mutations at low frequency a method was developed that uses polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA)-based technology, allowing rapid, reliable and cost-effective testing of large numbers of individual mosquitoes. This was used to assay mosquitoes from sites in lower Moshi, Tanzania.

RESULTS: Sequence-specific oligonucleotide probes (SSOP) were used for simultaneous detection of both East and West African kdr mutations with high specificity and sensitivity. Application of the SSOP-ELISA method to 1,620 field-collected Anopheles arabiensis from Tanzania identified the West African leucine-phenylalanine kdr mutation in two heterozygous individuals, indicating the potential for resistance development that requires close monitoring.

CONCLUSION: The presence of the West African kdr mutation at low frequency in this East African population of An. arabiensis has implications for the spread of the kdr gene across the African continent.

PMCID: PMC1526444 **Free PMC Article**
PMID: 16820067 [PubMed - indexed for MEDLINE]

45. **Status of insecticide susceptibility in Anopheles arabiensis from Mwea rice irrigation scheme, Central Kenya.**

Kamau L[1], Vulule JM.

Malar J. 2006 Jun 6;5:46.

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**ABSTRACT**

BACKGROUND: Control of the Anopheline mosquito vectors of malaria by use of insecticides has been shown to impact on both morbidity and mortality due to this disease. Evidence of insecticide resistance in different settings necessitates surveillance studies to allow prompt detection of resistance should it arise and thus enable its management. Possible resistance by Anopheles arabiensis mosquitoes from Mwea rice irrigation scheme in Central Kenya to insecticides in the four classes of insecticides approved by WHO for indoor residual spraying was investigated.

METHODS: Susceptibility to DDT (an organochlorine), fenitrothion (an organophosphate), bendiocarb (a carbamate), lambdacyhalothrin and permethrin (both pyrethroids) was tested using standard WHO diagnostic bioassay kits. Bioassays were performed on non-blood fed mosquitoes one- to three-day old. Knockdown was recorded every 10 min and mortality 24 h post-exposure was noted.

RESULTS: Mortality 24 h post-exposure was 100% for all insecticides except for lambdacyhalothrin, which averaged 99.46%. Knockdown rates at 10 min intervals were not significantly different between the Mwea population and the susceptible KISUMU strain of Anopheles gambiae sensu stricto control. The KDT50 and KDT95 values for the Mwea population were either lower than those for the control or higher by factors of no more than 2 for most comparisons and compared well with those of An. gambiae sensu lato categorized as susceptible in other studies.

CONCLUSION: These results suggest that the Mwea population of An. arabiensis is susceptible to all the insecticides tested. This implies that vector control measures employing any of these insecticides would not be hampered by resistance.

PMCID: PMC1501029 **Free PMC Article**
PMID: 16756645 [PubMed - indexed for MEDLINE]

46. **Co-occurrence of East and West African kdr mutations suggests high levels of resistance to pyrethroid insecticides in Anopheles gambiae from Libreville, Gabon.**

Pinto J[1], Lynd A, Elissa N, Donnelly MJ, Costa C, Gentile G, Caccone A, do Rosário VE.

Med Vet Entomol. 2006 Mar;20(1):27-32.

[1]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal. jpinto@ihmt.unl.pt

**ABSTRACT**

Point mutations in the voltage-gated sodium channel gene involved in knockdown resistance to DDT and pyrethroid insecticides have been described in several insect species. In the malaria vector Anopheles gambiae Giles sensu stricto (Diptera: Culicidae) two mutations have been identified. The first, consisting of a leucine-phenylalanine substitution at amino acid position 1014, is widespread in West Africa. The second, a leucine-serine substitution at the same position, has to date only been detected in western Kenya. Analysis of the kdr polymorphism in a sample of 106 An. gambiae s.s. of the rDNA S-form/Type I collected in Libreville (Gabon) surprisingly revealed the presence of both East and West African kdr mutations with frequencies of 63% and 37%, respectively. No wild-type alleles were detected and there was an excess of heterozygous genotypes (P = 0.04). In addition, an inconsistency was found during the kdr genotyping procedures by polymerase chain reaction, which could have lead to an underestimation of resistance alleles. The implications of these findings are discussed.
PMID: 16608487 [PubMed - indexed for MEDLINE]

47. **Effects of a novel pesticide resistance management strategy on tick control in a smallholding exotic-breed dairy herd in Kenya.**

Kamidi RE[1], Kamidi MK.
Trop Anim Health Prod. 2005 Aug;37(6):469-78.

[1]International Livestock Research Institute, Nairobi, Kenya. r.kamidi@cgiar.org

**ABSTRACT**

Effects of a novel pesticide resistance management strategy on tick control are evaluated in this study. The study is based on a temporal analysis of tick management practices on a smallholding in western Kenya. Results are reported of an innovation to tackle individual resistance in a pair of alternative pesticides using relay application. Incidence of tick-borne diseases at the farm were reduced from 79.6% per annum to 4.5% and no cases were observed in the last two years of the study. Negative cross-resistance is believed to be the mechanism in play for this effective tick control practice. Tick-borne disease control and management costs were halved in comparison to application of a single

ineffective pesticide at the same treatment frequency. The acaricide relay strategy is suitable for smallholdings and is expected to significantly extend the useful lifespan of the pesticide pair.

PMID: 16248218 [PubMed - indexed for MEDLINE]

48. **Dynamics of the pyrethroid knockdown resistance allele in western Kenyan populations of Anopheles gambiae in response to insecticide-treated bed net trials.**

Stump AD[1], Atieli FK, Vulule JM, Besansky NJ.

Am J Trop Med Hyg. 2004 Jun;70(6):591-6.

[1]Center for Tropical Disease Research and Training, Department of Biologic Sciences, University of Notre Dame, Notre Dame, Indiana 46556, USA.

**ABSTRACT**

Permethrin and DDT resistance in Anopheles gambiae s.s. associated with a leucine-serine knockdown resistance (kdr) mutation in the voltage-gated sodium channel gene was discovered recently in western Kenya where a large scale permethrin-impregnated bed net (ITN) program has been implemented. Collections of An. gambiae s.l. were made from intervention and control villages prior to and after onset of the program. The kdr genotypes were determined using allele-specific polymerase chain reaction diagnostic tests. In An. gambiae s.s., the frequency of the kdr mutation prior to ITN introduction was approximately 3-4% in western Kenya and zero in samples from the coast. After ITN introduction, the kdr mutation increased in ITN and neighboring villages from approximately 4% to approximately 8%, but remained unchanged in villages at least 20 km distant and was not detected in coastal Kenya. The identical leucine-serine mutation was found in a single An. arabiensis individual among 658 tested. The leucine-phenylalanine kdr mutation common in west African An. gambiae populations was not detected in An. gambiae s.l. from Kenya. Implications for the population structure and control of An. gambiae are discussed.

**Free Article**

PMID: 15210997 [PubMed - indexed for MEDLINE]

49. **Pyrethroid resistance in tropical bedbugs, Cimex hemipterus, associated with use of treated bednets.**

Myamba J[1], Maxwell CA, Asidi A, Curtis CF.

Med Vet Entomol. 2002 Dec;16(4):448-51.

[1]Ubwari Field Station of Tanzanian National Institute of Medical Research, Muheza, Tanga, Tanzania.

**ABSTRACT**

When Tanzanian villages were provided with pyrethroid-treated bednets, bedbugs (Cimicidae) disappeared; however, after about 6 years they have re-appeared in these villages. Using a newly devised test-kit, susceptibility tests of bedbugs Cimex hemipterus (Fabricius) from five of these villages showed that there is resistance to permethrin and alphacypermethrin in bedbugs from each of the villages, in contrast to those from five villages without treated nets. Circumstantial evidence indicates that bedbug resistance to pyrethroid insecticides may evolve more readily in villages with incomplete coverage rates of treated bednets, allowing bedbug infestations to become re-established. Bedbugs have not returned to a village where nearly all the beds have been provided with pyrethroid-treated bednets for 14 years.
PMID: 12510899 [PubMed - indexed for MEDLINE]

50. **Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids.**

Ranson H[1], Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH.

Insect Mol Biol. 2000 Oct;9(5):491-7.

[1]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, USA. RansonHA@cardiff.ac.uk

**ABSTRACT**

A field trial of permethrin-impregnated bednets and curtains was initiated in Western Kenya in 1990, and a strain of Anopheles gambiae showing reduced susceptibility to permethrin was colonized from this site in 1992. A leucine-phenylalanine substitution at position 1014 of the voltage-gated sodium channel is associated with resistance to permethrin and DDT in many insect species, including Anopheles gambiae from West Africa. We cloned and sequenced a partial sodium channel cDNA from the Kenyan permethrin-resistant strain and we identified an alternative substitution (leucine to serine) at the same position, which is linked to the inheritance of permethrin resistance in the F(2) progeny of genetic crosses between susceptible and resistant individuals. The diagnostic polymerase chain reaction (PCR) developed by Martinez-Torres et al. [(1998) Insect Mol Biol 7: 179-184] to detect kdr alleles in field populations of An. gambiae will not detect the Kenyan allele and hence reliance on this assay may lead to an underestimate of the prevalence of pyrethroid resistance in this species. We adapted the diagnostic PCR to detect the leucine-serine mutation and with this diagnostic we were able to demonstrate that this kdr allele was present in individuals collected from the Kenyan trial site in 1986, prior to the introduction of pyrethroid-impregnated bednets. The An. gambiae sodium channel was physically mapped to chromosome 2L, division 20C. This position corresponds to the location of a major quantitative trait locus determining resistance to permethrin in the Kenyan strain of An. gambiae.
PMID: 11029667 [PubMed - indexed for MEDLINE]

51. **Modifications of pyrethroid effects associated with kdr mutation in Anopheles gambiae.**

Chandre F[1], Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, Guillet P.

Med Vet Entomol. 2000 Mar;14(1):81-8.

[1]Laboratoire de Lutte contre les Insectes Nuisibles, Institute for Research & Development (IRD formerly ORSTOM), Montpellier, France. chandre@ipr.ird.ci

**ABSTRACT**

Effects of knockdown resistance (kdr) were investigated in three pyrethroid-resistant (RR) strains of the Afrotropical mosquito Anopheles gambiae Giles (Diptera: Culicidae): Kou from Burkina Faso, Tola and Yao from Côte d'Ivoire; compared with a standard susceptible (SS) strain from Kisumu, Kenya. The kdr factor was incompletely recessive, conferring 43-fold resistance ratio at LD50 level and 29-fold at LD95 level, as determined by topical application tests with Kou strain. When adult mosquitoes were exposed to 0.25% permethrin-impregnated papers, the 50% and 95% knockdown times (KdT) were 23 and 42 min for SS females, compared with 40 and 62 min for RS (F1 Kou x Kisumu) females. On 1% permethrin the KdT50 and KdT95 were 11 and 21 min for SS compared with 18 and 33 min for RS females. Following 1 h exposure to permethrin (0.25% or 1%), no significant knockdown of Kou RR females occurred within 24 h. Permethrin irritancy to An. gambiae was assessed by comparing 'time to first take-off' (TO) for females. The standard TO50 and TO95 values for Kisumu SS on untreated paper were 58 and 1044 s, respectively, vs. 3.7 and 16.5 s on 1% permethrin. For Kou RR females the comparable values were 27.3 s for TO50 and 294 s for TO95, with intermediate RS values of 10.1 s for TO50 and 71.9 s for TO95. Thus, TO values for RS were 2.7-4.4 times more than for SS, and those for RR were 7-18 times longer than for SS. Experiments with pyrethroid-impregnated nets were designed to induce hungry female mosquitoes to pass through holes cut in the netting. Laboratory 'tunnel tests' used a bait guinea-pig to attract mosquitoes through circular holes (5 x 1 cm) in a net screen. With untreated netting, 75-83% of laboratory-reared females passed through the holes overnight, 63-69% blood-fed successfully and 9-17% died, with no significant differences between SS and RR genotypes. When the netting was treated with permethrin 250mg ai/m2 the proportions that passed through the holes overnight were only 10% of SS vs. 40-46% of RR (Tola & Kou); mortality rates were 100% of SS compared with 59-82% of RR; bloodmeals were obtained by 9% of Kou RR and 17% of Tola RR, but none of the Kisumu SS females. When the net was treated with deltamethrin 25 mg ai/m2 the proportions of An. gambiae that went through the holes and blood-fed successfully were 3.9% of Kisumu SS and 3.5% of Yaokoffikro field population (94% R). Mortality rates were 97% of Kisumu SS vs. 47% of Yaokoffikro R. Evidently this deltamethrin treatment was sufficient to kill nearly all SS and half of the Yaokoffikro R An. gambiae population despite its high kdr frequency. Experimental huts at Yaokoffikro were used for overnight evaluation of bednets against An. gambiae females. The huts were sealed to prevent egress of mosquitoes released at 20.00 hours and collected at 05.00 hours. Each net was perforated with 225 square holes (2 x 2 cm). A man slept under the net as bait. With untreated nets, only 4-6% of mosquitoes died overnight and bloodmeals were taken by 17% of SS vs. 29% of Yaokoffikro R ($P<0.05$). Nets treated with permethrin 500 mg/m2 caused mortality rates of 95% Kisumu SS and 45% Yao R ($P<0.001$) and blood-feeding rates were reduced to 1.3% of SS vs. 8.1% of Yao R ($P<0.05$). Nets treated with deltamethrin 25 mg/m2 caused mortality rates of 91% Kisumu SS and 54%

Yao R (P<0.001) and reduced blood-feeding rates to zero for SS vs. 2.5% for Yao R (P>0.05). (ABSTRACT TRUNCATED)
PMID: 10759316 [PubMed - indexed for MEDLINE]

52. **Some emerging issues on the malaria problem in Kenya.**

Oloo AJ[1], Vulule JM, Koech DK.

East Afr Med J. 1996 Jan;73(1):50-3.

[1]Vector Biology and Control Research Centre, Kenya Medical Research Institute, Kisumu, Kenya.

**ABSTRACT**

Malaria in Kenya has been characterised by marked variability in its epidemiology, partly reflecting the obstacles and progress made in the control strategies. The impact of anti-vector activities in the 1970s and before have been observed for variable lengths of time afterwards. Malaria has re-emerged in areas previously with little or no transmission. The recovery of infective Anopheles gambiae vector in higher altitudes affirms the potential for transmission in areas where epidemics have been known to occur. Morbidity and mortality patterns in the otherwise endemic lowlands have become increasingly severe, an observation which would be attributed to the increasing inefficacy of chloroquine. Efforts to promote personal protection suffer substantial setbacks in sustainability inspite of apparent acceptability. There are indications that the mosquito vector susceptibility to permethrin and other insecticides will now require continual monitoring in order to detect development of significant resistance. In this communication, we review some emergent issues in malaria transmission in Kenya and the potential for control as adduced from historical and contemporary perspectives.
PMID: 8625864 [PubMed - indexed for MEDLINE]

53. **Long-term use of permethrin-impregnated nets does not increase Anopheles gambiae permethrin tolerance.**

Vulule JM[1], Beach RF, Atieli FK, Mount DL, Roberts JM, Mwangi RW.

Med Vet Entomol. 1996 Jan;10(1):71-9.

[1]Vector Biology and Control Research Centre (Kenya Medical Research Institute), Kisumu, Kenya.

**ABSTRACT**

Previous use of permethrin-impregnated bednets (mosquito nets) and curtains in four Kenyan villages for one year, 1990-91, raised the permethrin LT50 of Anopheles gambiae to 2.4-fold above its baseline value, designated permethrin tolerance (PT), as measured by exposure to 0.25% permethrin-impregnated papers in W.H.O. test-kits. During 1992-93, with ongoing use of permethrin-impregnated

nets and curtains, PT regressed slightly compared with the contemporary susceptibility level of An.gambiae from non-intervention villages, to 1.8-fold in 1992 and only 1.6-fold in 1993. Thus the selection pressure of impregnated nets for PT in An.gambiae appears to be minimal in our study villages, although the impact of permethrin was demonstrated by a significantly lower parous-rate of An.gambiae females in the intervention (63-66%) than in non-intervention (79%) villages, and by reduced malaria transmission (reported elsewhere). In a selected stock of An.gambiae from the study area, PT did not affect the susceptibility to deltamethrin, fenitrothion, propoxur or DDT. Bioassays described herein provide easy procedures for field-monitoring of mosquito susceptibility/tolerance/resistance to insecticides used for net impregnation in operational programmes.
PMID: 8834745 [PubMed - indexed for MEDLINE]

54. **Detection of pyrethroid resistance in Anopheles mosquitos.**

Magesa SM[1], Aina O, Curtis CF.

Bull World Health Organ. 1994;72(5):737-40.

[1]National Institute for Medical Research, Amani Medical Research Centre, Tanga, United Republic of Tanzania.

**ABSTRACT**

Although pyrethroid insecticides are a promising means of controlling Anopheles malaria vectors, there is a need to monitor for resistance. It has been proposed that the results of the WHO-recommended testing method, involving exposure to impregnated paper for 1 hour, might be misleading because of knockdown during this period, and that exposure to a higher dose of pyrethroid for 2 minutes might be preferable. However, comparative tests with a susceptible and a permethrin-resistant strain of A. stephensi showed that exposure for 1 hour was at least as sensitive in detecting resistance as was the short exposure method.

PMCID: PMC2486547 **Free PMC Article**
PMID: 7955022 [PubMed - indexed for MEDLINE]

55. **Resistance to malathion in a strain of body lice from Burundi.**

Cole MM, Clark PH, Washington F, Ellerbe W, VanNatta DL.

J Econ Entomol. 1973 Feb;66(1):118-9.

**Free Article**
PMID: 4690250 [PubMed - indexed for MEDLINE]

56. **First report of resistance of human body lice to malathion.**

Miller RN, Wisseman CL, Sweeney GW, Verschueren A, Fabrikant IB.

Trans R Soc Trop Med Hyg. 1972;66(2):372-5.

PMID: 5048872 [PubMed - indexed for MEDLINE]

57. **Insecticide-resistance in bed-bugs and flies in Zanzibar.**

GRATZ NG.
PMCID: PMC2555887 **Free PMC Article**
PMID: 13707974 [PubMed - indexed for MEDLINE]

# MALARIA DRUG RESISTANCE

## 70 Citations

(sorted newest to oldest)

# MALARIA DRUG RESISTANCE

1. **Most outdoor malaria transmission by behaviourally-resistant Anopheles arabiensis is mediated by mosquitoes that have previously been inside houses.**

Killeen GF[1,2], Govella NJ[3], Lwetoijera DW[3], Okumu FO[3,4].

Malar J. 2016 Apr 19;15:225. doi: 10.1186/s12936-016-1280-z.

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[4]School of Public Health, University of the Witwatersrand, Johannesburg, Republic of South Africa.

**ABSTRACT**

BACKGROUND: Anopheles arabiensis is stereotypical of diverse vectors that mediate residual malaria transmission globally, because it can feed outdoors upon humans or cattle, or enter but then rapidly exit houses without fatal exposure to insecticidal nets or sprays.

METHODS: Life histories of a well-characterized An. arabiensis population were simulated with a simple but process-explicit deterministic model and relevance to other vectors examined through sensitivity analysis.

RESULTS: Where most humans use bed nets, two thirds of An. arabiensis blood feeds and half of malaria transmission events were estimated to occur outdoors. However, it was also estimated that most successful feeds and almost all (>98 %) transmission events are preceded by unsuccessful attempts to attack humans indoors. The estimated proportion of vector blood meals ultimately obtained from humans indoors is dramatically attenuated by availability of alternative hosts, or partial ability to attack humans outdoors. However, the estimated proportion of mosquitoes old enough to transmit malaria, and which have previously entered a house at least once, is far less sensitive to both variables. For vectors with similarly modest preference for cattle over humans and similar ability to evade fatal indoor insecticide exposure once indoors, >80 % of predicted feeding events by mosquitoes old enough to transmit malaria are preceded by at least one house entry event, so long as ≥40 % of attempts to attack humans occur indoors and humans outnumber cattle ≥4-fold.

CONCLUSIONS: While the exact numerical results predicted by such a simple deterministic model should be considered only approximate and illustrative, the derived conclusions are remarkably insensitive to substantive deviations from the input parameter values measured for this particular An. arabiensis

population. This life-history analysis, therefore, identifies a clear, broadly-important opportunity for more effective suppression of residual malaria transmission by An. arabiensis in Africa and other important vectors of residual transmission across the tropics. Improved control of predominantly outdoor residual transmission by An. arabiensis, and other modestly zoophagic vectors like Anopheles darlingi, which frequently enter but then rapidly exit from houses, may be readily achieved by improving existing technology for killing mosquitoes indoors.

PMCID: PMC4837512 **Free PMC Article**
PMID: 27093890 [PubMed - indexed for MEDLINE]

2. **Assessment of the Worldwide Antimalarial Resistance Network Standardized Procedure for In Vitro Malaria Drug Sensitivity Testing Using SYBR Green Assay for Field Samples with Various Initial Parasitemia Levels.**

Cheruiyot AC[1], Auschwitz JM[2], Lee PJ[2], Yeda RA[1], Okello CO[1], Leed SE[2], Talwar M[2], Murthy T[2], Gaona HW[2], Hickman MR[2], Akala HM[1], Kamau E[1], Johnson JD[3].

Antimicrob Agents Chemother. 2016 Mar 25;60(4):2417-24. doi: 10.1128/AAC.00527-15. Print 2016 Apr.

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[2]Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA.
[3]Department of Emerging Infectious Diseases-Global Emerging Infections Surveillance and Response System (DEID-GEIS) Program, U.S. Army Medical Research Directorate-Kenya (USAMRD-K), Kenya Medical Research Institute (KEMRI)-Walter Reed Project, Kisumu, Kenya jacob.d.johnson.mil@mail.mil.

**ABSTRACT**

The malaria SYBR green assay, which is used to profilein vitrodrug susceptibility ofPlasmodium falciparum, is a reliable drug screening and surveillance tool. Malaria field surveillance efforts provide isolates with various low levels of parasitemia. To be advantageous, malaria drug sensitivity assays should perform reproducibly among various starting parasitemia levels rather than at one fixed initial value. We examined the SYBR green assay standardized procedure developed by the Worldwide Antimalarial Resistance Network (WWARN) for its sensitivity and ability to accurately determine the drug concentration that inhibits parasite growth by 50% (IC50) in samples with a range of initial parasitemia levels. The initial sensitivity determination of the WWARN procedure yielded a detection limit of 0.019% parasitemia.P. falciparumlaboratory strains and field isolates with various levels of initial parasitemia were then subjected to a range of doses of common antimalarials. The IC50s were comparable for laboratory strains with between 0.0375% and 0.6% parasitemia and for field isolates with between 0.075% and 0.6% parasitemia for all drugs tested. Furthermore, assay quality (Z') analysis

indicated that the WWARN procedure displays high robustness, allowing for drug testing of malaria field samples within the derived range of initial parasitemia. The use of the WWARN procedure should allow for the inclusion of more malaria field samples in malaria drug sensitivity screens that would have otherwise been excluded due to low initial parasitemia levels.

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PMCID: PMC4808143 **Free PMC Article**
PMID: 26856829 [PubMed - indexed for MEDLINE]

3. **Within-host competition and drug resistance in the human malaria parasite Plasmodium falciparum.**

Bushman M[1], Morton L[2], Duah N[3], Quashie N[4], Abuaku B[3], Koram KA[3], Dimbu PR[5], Plucinski M[6], Gutman J[2], Lyaruu P[7], Kachur SP[2], de Roode JC[8], Udhayakumar V[2].

Proc Biol Sci. 2016 Mar 16;283(1826):20153038. doi: 10.1098/rspb.2015.3038.

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[5]National Malaria Control Program, Luanda, Angola.
[6]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA Epidemic Intelligence Service, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA.
[7]Ifakara Health Institute, Dar es Salaam, Tanzania.
[8]Department of Biology, Emory University, Atlanta, GA 30322, USA.

**ABSTRACT**

Infections with the malaria parasite Plasmodium falciparum typically comprise multiple strains, especially in high-transmission areas where infectious mosquito bites occur frequently. However, little is known about the dynamics of mixed-strain infections, particularly whether strains sharing a host compete or grow independently. Competition between drug-sensitive and drug-resistant strains, if it occurs, could be a crucial determinant of the spread of resistance. We analysed 1341 P. falciparum infections in children from Angola, Ghana and Tanzania and found compelling evidence for competition

in mixed-strain infections: overall parasite density did not increase with additional strains, and densities of individual chloroquine-sensitive (CQS) and chloroquine-resistant (CQR) strains were reduced in the presence of competitors. We also found that CQR strains exhibited low densities compared with CQS strains (in the absence of chloroquine), which may underlie observed declines of chloroquine resistance in many countries following retirement of chloroquine as a first-line therapy. Our observations support a key role for within-host competition in the evolution of drug-resistant malaria. Malaria control and resistance-management efforts in high-transmission regions may be significantly aided or hindered by the effects of competition in mixed-strain infections. Consideration of within-host dynamics may spur development of novel strategies to minimize resistance while maximizing the benefits of control measures.

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PMCID: PMC4810865 [Available on 2017-03-16]
PMID: 26984625 [PubMed - indexed for MEDLINE]

4. **Impact of Sulfadoxine-Pyrimethamine Resistance on Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing Infections and Preventing Low Birth Weight.**

Desai M[1], Gutman J[2], Taylor SM[3], Wiegand RE[2], Khairallah C[4], Kayentao K[5], Ouma P[6], Coulibaly SO[7], Kalilani L[8], Mace KE[2], Arinaitwe E[9], Mathanga DP[8], Doumbo O[10], Otieno K[6], Edgar D[7], Chaluluka E[8], Kamuliwo M[11], Ades V[12], Skarbinski J[2], Shi YP[2], Magnussen P[13], Meshnick S[14], Ter Kuile FO[4].

Clin Infect Dis. 2016 Feb 1;62(3):323-33. doi: 10.1093/cid/civ881. Epub 2015 Oct 20.

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[1]Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, Georgia Malaria Branch, Center for Global Health Research, Kenya Medical Research Institute, Kisumu.
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[14]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill.

**ABSTRACT**

BACKGROUND: Owing to increasing sulfadoxine-pyrimethamine (SP) resistance in sub-Saharan Africa, monitoring the effectiveness of intermittent preventive therapy in pregnancy (IPTp) with SP is crucial.

METHODS: Between 2009 and 2013, both the efficacy of IPTp-SP at clearing existing peripheral malaria infections and the effectiveness of IPTp-SP at reducing low birth weight (LBW) were assessed among human immunodeficiency virus-uninfected participants in 8 sites in 6 countries. Sites were classified as high, medium, or low resistance after measuring parasite mutations conferring SP resistance. An individual-level prospective pooled analysis was conducted.

RESULTS: Among 1222 parasitemic pregnant women, overall polymerase chain reaction-uncorrected and -corrected failure rates by day 42 were 21.3% and 10.0%, respectively (39.7% and 21.1% in high-resistance areas; 4.9% and 1.1% in low-resistance areas). Median time to recurrence decreased with increasing prevalence of Pfdhps-K540E. Among 6099 women at delivery, IPTp-SP was associated with a 22% reduction in the risk of LBW (prevalence ratio [PR], 0.78; 95% confidence interval [CI],.69-.88; P <.001). This association was not modified by insecticide-treated net use or gravidity, and remained significant in areas with high SP resistance (PR, 0.81; 95% CI,.67-.97; P =.02).

CONCLUSIONS: The efficacy of SP to clear peripheral parasites and prevent new infections during pregnancy is compromised in areas with >90% prevalence of Pfdhps-K540E. Nevertheless, in these high-resistance areas, IPTp-SP use remains associated with increases in birth weight and maternal hemoglobin. The effectiveness of IPTp in eastern and southern Africa is threatened by further increases in SP resistance and reinforces the need to evaluate alternative drugs and strategies for the control of malaria in pregnancy.

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PMCID: PMC4762476 [Available on 2017-02-01]
PMID: 26486699 [PubMed - indexed for MEDLINE]

5. **Lack of effect of intermittent preventive treatment for malaria in pregnancy and intense drug resistance in western Uganda.**

Braun V[1], Rempis E[2], Schnack A[3], Decker S[4], Rubaihayo J[5], Tumwesigye NM[6], Theuring S[7], Harms G[8], Busingye P[9], Mockenhaupt FP[10].

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**ABSTRACT**

BACKGROUND: Intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is widely implemented in sub-Saharan Africa for the prevention of malaria in pregnancy and adverse birth outcomes. However, in areas of intense SP resistance, the efficacy of IPTp may be compromised.

METHODS: A cross-sectional study among 915 delivering women (728 analysable live singleton deliveries) was conducted in Fort Portal, western Uganda, to assess associations of reported IPTp use, Plasmodium falciparum infection, maternal anaemia, low birth weight, and preterm delivery, and to estimate the degree of SP resistance as reflected by pfdhfr/pfdhps mutations.

RESULTS: Plasmodium falciparum infection was detected by PCR in 8.9 % and by microscopy of placental blood samples in 4.0 %. Infection was significantly associated with stillbirth, early neonatal death, anaemia, low birth weight, and pre-term delivery. Eighty percent of the women had taken at least one dose of IPTp, and more than half had taken two doses. As compared to women without

chemoprophylaxis against malaria, IPTp had no significant influence on the presence of P. falciparum infection (13.8 vs. 9.6 %, P = 0.31). Nor was it associated with reductions in anaemia, low birth weight or preterm delivery. P. falciparum with intense SP resistance (pfdhfr/pfdhps quintuple or sextuple mutations) were observed in 93 % (pfdhps 581G, 36 %), and the additional high resistance allele pfhdr 164L in 36 %.

CONCLUSIONS: In Fort Portal, Uganda, reported use of IPTp with SP does not provide an observable benefit. The molecular markers of P. falciparum indicate high grade SP resistance reaching the threshold set by WHO for the discontinuation of IPTp with SP. Alternative approaches for the prevention of malaria in pregnancy are urgently needed.

PMCID: PMC4583758 **Free PMC Article**
PMID: 26410081 [PubMed - indexed for MEDLINE]

6. **Prevalence of Plasmodium falciparum resistance markers to sulfadoxine-pyrimethamine among pregnant women receiving intermittent preventive treatment for malaria in Uganda.**

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Antimicrob Agents Chemother. 2015 Sep;59(9):5475-82. doi: 10.1128/AAC.00507-15. Epub 2015 Jun 29.

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**ABSTRACT**

The aim of this study was to assess the prevalence of mutations in Plasmodium falciparum dihydrofolate reductase (Pfdhfr) and dihydropteroate synthase (Pfdhps) genes among pregnant women using sulfadoxine-pyrimethamine (SP) as an intermittent preventive treatment (IPTp). A molecular epidemiological study of P. falciparum parasite resistance markers to SP was conducted from August 2010 to February 2012 in Mukono district in central Uganda. DNA was extracted from 413 P. falciparum-positive samples. Real-time PCR, followed by melting curve analysis, was used to characterize point mutations in the Pfdhfr and Pfdhps genes that are associated with SP resistance. The prevalence of the single-nucleotide mutations in Pfdhfr at codons 51I, 59R, and 108N and in Pfdhps at codons 437G and

540E was high (>98%), reaching 100% fixation after one dose of SP, while the prevalence of 581G was 3.3% at baseline, reaching 12.5% after one dose of SP. At baseline, the prevalence of Pfdhfr and Pfdhps quintuple mutations was 89%, whereas the sextuple mutations (including 581G) were not prevalent (3.9%), reaching 16.7% after one dose of SP. However, the numbers of infections at follow-up visits were small, and hence there was insufficient statistical power to test whether there was a true rise in the prevalence of this allele. The overall high frequency of Pfdhfr and Pfdhps quintuple mutations throughout pregnancy excluded further analyses of possible associations between certain haplotypes and the risk of lower birth weight and anemia. However, women infected with P. falciparum had 1.3-g/dl-lower hemoglobin levels (P = 0.001) and delivered babies with a 400-g-lower birth weight (P = 0.001) compared to nonparasitemic women. Despite this, 44 women who were P. falciparum positive at baseline became negative after one or two doses of SP (i.e., 50.5%), implying that SP-IPTp still has some efficacy. P. falciparum resistance markers to SP are high in this population, whereas P. falciparum infection was associated with poor birth outcomes.

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PMCID: PMC4538518 **Free PMC Article**
PMID: 26124154 [PubMed - indexed for MEDLINE]

7. **Design of a study to determine the impact of insecticide resistance on malaria vector control: a multi-country investigation.**

Kleinschmidt I[1,2], Mnzava AP[3], Kafy HT[4], Mbogo C[5], Bashir AI[6,7], Bigoga J[8], Adechoubou A[9], Raghavendra K[10], Knox TB[11], Malik EM[12], Nkuni ZJ[13], Bayoh N[14], Ochomo E[15], Fondjo E[16], Kouambeng C[17], Awono-Ambene HP[18], Etang J[19,20], Akogbeto M[21], Bhatt R[22], Swain DK[23], Kinyari T[24], Njagi K[25], Muthami L[26], Subramaniam K[27], Bradley J[28], West P[29], Massougbodji A[30], Okê-Sopoh M[31], Hounto A[32], Elmardi K[33], Valecha N[34], Kamau L[35], Mathenge E[36], Donnelly MJ[37,38].

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**ABSTRACT**

BACKGROUND: Progress in reducing the malaria disease burden through the substantial scale up of insecticide-based vector control in recent years could be reversed by the widespread emergence of insecticide resistance. The impact of insecticide resistance on the protective effectiveness of insecticide-treated nets (ITN) and indoor residual spraying (IRS) is not known. A multi-country study was undertaken in Sudan, Kenya, India, Cameroon and Benin to quantify the potential loss of epidemiological effectiveness of ITNs and IRS due to decreased susceptibility of malaria vectors to insecticides. The design of the study is described in this paper.

METHODS: Malaria disease incidence rates by active case detection in cohorts of children, and indicators of insecticide resistance in local vectors were monitored in each of approximately 300 separate locations (clusters) with high coverage of malaria vector control over multiple malaria seasons. Phenotypic and genotypic resistance was assessed annually. In two countries, Sudan and India, clusters were randomly assigned to receive universal coverage of ITNs only, or universal coverage of ITNs combined with high coverage of IRS. Association between malaria incidence and insecticide resistance, and protective effectiveness of vector control methods and insecticide resistance were estimated, respectively.

RESULTS: Cohorts have been set up in all five countries, and phenotypic resistance data have been collected in all clusters. In Sudan, Kenya, Cameroon and Benin data collection is due to be completed in 2015. In India data collection will be completed in 2016.

DISCUSSION: The paper discusses challenges faced in the design and execution of the study, the analysis plan, the strengths and weaknesses, and the possible alternatives to the chosen study design.

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PMID: 26194648 [PubMed - indexed for MEDLINE]

8. **Assessment of molecular markers for anti-malarial drug resistance after the introduction and scale-up of malaria control interventions in western Kenya.**

Shah M[1,2], Omosun Y[3,4], Lal A[5,6], Odero C[7], Gatei W[8], Otieno K[9], Gimnig JE[10], ter Kuile F[11], Hawley WA[12,13], Nahlen B[14], Kariuki S[15], Walker E[16], Slutsker L[17], Hamel M[18,19], Shi YP[20].

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**ABSTRACT**

BACKGROUND: Although it is well known that drug pressure selects for drug-resistant parasites, the role of transmission reduction by insecticide-treated bed nets (ITNs) on drug resistance remains unclear. In this study, the drug resistance profile of current and previous first-line anti-malarials in Kenya was assessed within the context of drug policy change and scale-up of ITNs. National first-line treatment changed from chloroquine (CQ) to sulphadoxine-pyrimethamine (SP) in 1998 and to artemether-lumefantrine (AL) in 2004. ITN use was scaled-up in the Asembo, Gem and Karemo areas of western Kenya in 1997, 1999 and 2006, respectively.

METHODS: Smear-positive samples (N = 253) collected from a 2007 cross-sectional survey among children in Asembo, Gem and Karemo were genotyped for mutations in pfcrt and pfmdr1 (CQ), dhfr and dhps (SP), and at pfmdr-N86 and the gene copy number in pfmdr1 (lumefantrine). Results were compared among the three geographic areas in 2007 and to retrospective molecular data from children in Asembo in 2001.

RESULTS: In 2007, 69 and 85% of samples harboured the pfmdr1-86Y mutation and dhfr/dhps quintuple mutant, respectively, with no significant differences by study area. However, the prevalence of the pfcrt-76T mutation differed significantly among areas ($p < 0.02$), between 76 and 94%, with the highest prevalence in Asembo. Several 2007 samples carried mutations at dhfr-164L, dhps-436A, or dhps-613T. From 2001 to 2007, there were significant increases in the pfcrt-76T mutation from 82 to 94% ($p < 0.03$), dhfr/dhps quintuple mutant from 62 to 82% ($p < 0.03$), and an increase in the septuple CQ and SP combined mutant haplotype, K 76 Y 86 I 51 R 59 N 108 G 437 E 540, from 28 to 39%. The prevalence of the pfmdr1-86Y mutation remained unchanged. All samples were single copy for pfmdr1.

CONCLUSIONS: Molecular markers associated with lumefantrine resistance were not detected in 2007. More recent samples will be needed to detect any selective effects by AL. The prevalence of CQ and SP resistance markers increased from 2001 to 2007 in the absence of changes in transmission intensity. In 2007, only the prevalence of pfcrt-76T mutation differed among study areas of varying transmission intensity. Resistant parasites were most likely selected by sustained drug pressure from the continued use of CQ, SP, and mechanistically similar drugs, such as amodiaquine and cotrimoxazole. There was no clear evidence that differences in transmission intensity, as a result of ITN scale-up, influenced the prevalence of drug resistance molecular markers.

PMCID: PMC4331436 **Free PMC Article**
PMID: 25889220 [PubMed - indexed for MEDLINE]

9. **Alternatively spliced transcripts and novel pseudogenes of the Plasmodium falciparum resistance-associated locus pfcrt detected in East African malaria patients.**

Gadalla NB[1], Malmberg M[2], Adam I[3], Oguike MC[4], Beshir K[4], Elzaki SE[5], Mukhtar I[6], Gadalla AA[5], Warhurst DC[7], Ngasala B[8], Mårtensson A[9], El-Sayed BB[5], Gil JP[10], Sutherland CJ[11].

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**ABSTRACT**

OBJECTIVES: Polymorphisms in the lysosomal transporter encoded by the pfcrt gene directly impact on Plasmodium falciparum susceptibility to aminoquinolines. The Lys76Thr mutation is the critical change conferring chloroquine resistance in vitro and in vivo, but always occurs with additional non-synonymous changes in the pfcrt coding sequence. We sought to better describe pfcrt polymorphisms distal to codon 76.

METHODS: Small-volume samples (≤ 500 μL) of parasite-infected blood collected directly from malaria patients presenting for treatment in Sudan and Tanzania were immediately preserved for RNA

extraction. The pfcrt locus was amplified from cDNA preparations by nested PCR and sequenced directly to derive full-length mRNA sequences.

RESULTS: In one of two sites in Sudan, two patients were found with an unorthodox spliced form of pfcrt mRNA in which two exons were skipped, but it was not possible to test for the presence of the putative protein products of these aberrant transcripts. Genomic DNA sequencing from dried blood spots collected in parallel confirmed the presence of spliced pfcrt pseudogenes in a minority of parasite isolates. Full-length cDNA from conventionally spliced mRNA molecules in all study sites demonstrated the existence of a variety of pfcrt haplotypes in East Africa, and thus provides evidence of intragenic recombination.

CONCLUSIONS: The presence of pseudogenes, although unlikely to have any direct public health impact, may confound results obtained from simple genotyping methods that consider only codon 76 and the adjacent residues of pfcrt.

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PMID: 25253286 [PubMed - indexed for MEDLINE]

10. **Investigating mosquito net durability for malaria control in Tanzania - attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): study protocol.**

Lorenz LM, Overgaard HJ[1], Massue DJ, Mageni ZD, Bradley J, Moore JD, Mandike R, Kramer K, Kisinza W, Moore SJ.

BMC Public Health. 2014 Dec 13;14:1266. doi: 10.1186/1471-2458-14-1266.

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**ABSTRACT**

BACKGROUND: Long-Lasting Insecticidal Nets (LLINs) are one of the major malaria vector control tools, with most countries adopting free or subsidised universal coverage campaigns of populations at-risk from malaria. It is essential to understand LLIN durability so that public health policy makers can select the most cost effective nets that last for the longest time, and estimate the optimal timing of repeated distribution campaigns. However, there is limited knowledge from few countries of the durability of LLINs under user conditions.

METHODS/DESIGN: This study investigates LLIN durability in eight districts of Tanzania, selected for their demographic, geographic and ecological representativeness of the country as a whole. We use a two-stage approach: First, LLINs from recent national net campaigns will be evaluated retrospectively

in 3,420 households. Those households will receive one of three leading LLIN products at random (Olyset®, PermaNet®2.0 or Netprotect®) and will be followed up for three years in a prospective study to compare their performance under user conditions. LLIN durability will be evaluated by measuring Attrition (the rate at which nets are discarded by households), Bioefficacy (the insecticidal efficacy of the nets measured by knock-down and mortality of mosquitoes), Chemical content (g/kg of insecticide available in net fibres) and physical Degradation (size and location of holes). In addition, we will extend the current national mosquito insecticide Resistance monitoring program to additional districts and use these data sets to provide GIS maps for use in health surveillance and decision making by the National Malaria Control Program (NMCP).

DISCUSSION: The data will be of importance to policy makers and vector control specialists both in Tanzania and the SSA region to inform best practice for the maintenance of high and cost-effective coverage and to maximise current health gains in malaria control.

PMCID: PMC4301422 **Free PMC Article**
PMID: 25495268 [PubMed - indexed for MEDLINE]

11. **Preventive effect of permethrin-impregnated long-lasting insecticidal nets on the blood feeding of three major pyrethroid-resistant malaria vectors in western Kenya.**

Kawada H[1], Ohashi K, Dida GO, Sonye G, Njenga SM, Mwandawiro C, Minakawa N.

Parasit Vectors. 2014 Aug 20;7:383. doi: 10.1186/1756-3305-7-383.

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**ABSTRACT**

BACKGROUND: Since the World Health Organization (WHO) adopted the use of long-lasting insecticidal nets (LLINs) as a principal strategy for effective malaria prevention and control, pyrethroids have been the only class of insecticides used for LLINs. The dramatic success of insecticide-treated nets (ITNs) and LLINs in African countries, however, has been threatened by the rapid development of pyrethroid resistance in vector mosquitoes. ITNs and LLINs are still used as effective self-protection measures, but there have been few studies on the effectiveness of ITNs and LLINs in areas where vector mosquitoes are pyrethroid-resistant.

METHODS: To investigate the behavioral pattern of mosquitoes in the houses where LLINs were used, indoor mosquito trappings of Anopheles gambiae s.s., An. arabiensis, and An. funestus s.s. were performed with Centers for Disease Control and Prevention (CDC) miniature light trap equipped with a collection bottle rotator at 2-hour intervals between 4:00 pm and 8:00 am. The trapped female mosquitoes were identified and classified as unfed, blood fed, and gravid. The abdominal contents of fed female mosquitoes were used for DNA extractions to identify the blood source.

RESULTS: A large proportion of human blood feeding of An. arabiensis and An. funestus s.s. (but not An. gambiae s.s.) took place during the time people were active outside LLINs. However, during the hours when people were beneath LLINs, these provided protective efficacy as indicated by reduced human blood feeding rates.

CONCLUSION: LLINs provided effective protection against pyrethroid-resistant malaria vector populations during bedtime hours. However, protection of LLINs was insufficient during the hours when people were active outside of the bed nets. Such limitation of LLINs will need to be intensively addressed in African countries in the near future.

PMCID: PMC4150967 **Free PMC Article**
PMID: 25141947 [PubMed - indexed for MEDLINE]

12. **Temporal changes in prevalence of molecular markers mediating antimalarial drug resistance in a high malaria transmission setting in Uganda.**

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**ABSTRACT**

Standard therapy for malaria in Uganda changed from chloroquine to chloroquine + sulfadoxine-pyrimethamine in 2000, and artemether-lumefantrine in 2004, although implementation of each change was slow. Plasmodium falciparum genetic polymorphisms are associated with alterations in drug sensitivity. We followed the prevalence of drug resistance-mediating P. falciparum polymorphisms in 982 samples from Tororo, a region of high transmission intensity, collected from three successive treatment trials conducted during 2003-2012, excluding samples with known recent prior treatment. Considering transporter mutations, prevalence of the mutant pfcrt 76T, pfmdr1 86Y, and pfmdr1 1246Y alleles decreased over time. Considering antifolate mutations, the prevalence of pfdhfr 51I, 59R, and 108N, and pfdhps 437G and 540E were consistently high; pfdhfr 164L and pfdhps 581G were uncommon, but most prevalent during 2008-2010. Our data suggest sequential selective pressures as

different treatments were implemented, and they highlight the importance of genetic surveillance as treatment policies change over time.

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PMCID: PMC4080569 **Free PMC Article**
PMID: 24799371 [PubMed - indexed for MEDLINE]

13. **Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis.**

Killeen GF[1], Chitnis N.

Malar J. 2014 Mar 14;13:97. doi: 10.1186/1475-2875-13-97.

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**ABSTRACT**

BACKGROUND: The ability of mosquitoes to evade fatal exposure to insecticidal nets and sprays represents the primary obstacle to eliminating malaria. However, it remains unclear which behaviours are most important for buffering mosquito and parasite populations against vector control.

METHODS: Simulated life histories were used to compare the impact of alternative feeding behaviour strategies upon overall lifetime feeding success, and upon temporal distributions of successful feeds and biting rates experienced by unprotected humans, in the presence and absence of insecticidal nets. Strictly nocturnal preferred feeding times were contrasted with 1) a wider preference window extending to dawn and dusk, and 2) crepuscular preferences wherein foraging is suppressed when humans sleep and can use nets but is maximal immediately before and after. Simulations with diversion and mortality parameters typical of endophagic, endophilic African vectors, such as Anopheles gambiae and Anopheles funestus, were compared with those for endophagic but exophilic species, such as Anopheles arabiensis, that also enter houses but leave earlier before lethal exposure to insecticide-treated surfaces occurs.

RESULTS: Insecticidal nets were predicted to redistribute successful feeding events to dawn and dusk where these were included in the profile of innately preferred feeding times. However, predicted distributions of biting unprotected humans were unaffected because extended host-seeking activity was redistributed to innately preferred feeding times. Recently observed alterations of biting activity distributions therefore reflect processes not captured in this model, such as evolutionary selection of heritably modified feeding time preferences or phenotypically plastic expression of feeding time preference caused by associative learning. Surprisingly, endophagy combined with exophily, among

mosquitoes that enter houses but then feed and/or rest briefly before rapidly exiting, consistently attenuated predicted insecticide impact more than any feeding time preference trait.

CONCLUSIONS: Regardless of underlying cause, recent redistributions of host-biting activity to dawn and dusk necessitate new outdoor control strategies. However, persistently indoor-feeding vectors, that evade intradomiciliary insecticide exposure, are at least equally important. Fortunately, recent evaluations of occupied houses or odour-baited stations, with baffled entrances that retain An. arabiensis within insecticide-treated structures, illustrate how endophagic but exophilic vectors may be more effectively tackled using existing insecticides.

PMCID: PMC3995604 **Free PMC Article**
PMID: 24629066 [PubMed - indexed for MEDLINE]

14. **Pooled deep sequencing of Plasmodium falciparum isolates: an efficient and scalable tool to quantify prevailing malaria drug-resistance genotypes.**

Taylor SM[1], Parobek CM, Aragam N, Ngasala BE, Mårtensson A, Meshnick SR, Juliano JJ.

J Infect Dis. 2013 Dec 15;208(12):1998-2006. doi: 10.1093/infdis/jit392. Epub 2013 Aug 1.

[1]Department of Epidemiology, Gillings School of Global Public Health.

**ABSTRACT**

Molecular surveillance for drug-resistant malaria parasites requires reliable, timely, and scalable methods. These data may be efficiently produced by genotyping parasite populations using second-generation sequencing (SGS). We designed and validated a SGS protocol to quantify mutant allele frequencies in the Plasmodium falciparum genes dhfr and dhps in mixed isolates. We applied this new protocol to field isolates from children and compared it to standard genotyping using Sanger sequencing. The SGS protocol accurately quantified dhfr and dhps allele frequencies in a mixture of parasite strains. Using SGS of DNA that was extracted and then pooled from individual isolates, we estimated mutant allele frequencies that were closely correlated to those estimated by Sanger sequencing (correlations, >0.98). The SGS protocol obviated most molecular steps in conventional methods and is cost saving for parasite populations >50. This SGS genotyping method efficiently and reproducibly estimates parasite allele frequencies within populations of P. falciparum for molecular epidemiologic studies.

PMCID: PMC3836461 **Free PMC Article**
PMID: 23908494 [PubMed - indexed for MEDLINE]

15. **On the effects of malaria treatment on parasite drug resistance--probability modelling of genotyped malaria infections.**

Kum CK, Thorburn D, Ghilagaber G, Gil P, Björkman A.

Int J Biostat. 2013 Oct 12;9(1). pii: /j/ijb.2013.9.issue-1/ijb-2012-0016/ijb-2012-0016.xml. doi: 10.1515/ijb-2012-0016.

**ABSTRACT**

We compare the frequency of resistant genes of malaria parasites before treatment and at first malaria incidence after treatment. The data come from a clinical trial at two health facilities in Tanzania and concerns single nucleotide polymorphisms (SNPs) at three positions believed to be related to resistance to malaria treatment. A problem is that mixed infections are common, which both obscures the underlying frequency of alleles at each locus as well as the associations between loci in samples where alleles are mixed. We use combinatorics and quite involved probability methods to handle multiple infections and multiple haplotypes. The infection with the different haplotypes seemed to be independent of each other. We showed that at two of the three studied SNPs, the proportion of resistant genes had increased after treatment with sulfadoxine-pyrimethamine alone but when treated in combination with artesunate, no effect was noticed. First recurrences of malaria associated more with sulfadoxine-pyrimethamine alone as treatment than when in combination with artesunate. We also found that the recruited children had two different ongoing malaria infections where the parasites had different gene types.

PMID: 24127546 [PubMed - indexed for MEDLINE]

16. **Impact of retreatment with an artemisinin-based combination on malaria incidence and its potential selection of resistant strains: study protocol for a randomized controlled clinical trial.**

Muhindo Mavoko H[1], Nabasumba C, Tinto H, D'Alessandro U, Grobusch MP, Lutumba P, Van Geertruyden JP.

Trials. 2013 Sep 23;14:307. doi: 10.1186/1745-6215-14-307.

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**ABSTRACT**

BACKGROUND: Artemisinin-based combination therapy is currently recommended by the World Health Organization as first-line treatment of uncomplicated malaria. Recommendations were adapted in 2010 regarding rescue treatment in case of treatment failure. Instead of quinine monotherapy, it should be combined with an antibiotic with antimalarial properties; alternatively, another artemisinin-based combination therapy may be used. However, for informing these policy changes, no clear evidence is yet available. The need to provide the policy makers with hard data on the appropriate

rescue therapy is obvious. We hypothesize that the efficacy of the same artemisinin-based combination therapy used as rescue treatment is as efficacious as quinine + clindamycin or an alternative artemisinin-based combination therapy, without the risk of selecting drug resistant strains.

DESIGN: We embed a randomized, open label, three-arm clinical trial in a longitudinal cohort design following up children with uncomplicated malaria until they are malaria parasite free for 4 weeks. The study is conducted in both the Democratic Republic of Congo and Uganda and performed in three steps. In the first step, the pre-randomized controlled trial (RCT) phase, children aged 12 to 59 months with uncomplicated malaria are treated with the recommended first-line drug and constitute a cohort that is passively followed up for 42 days. If the patients experience an uncomplicated malaria episode between days 14 and 42 of follow-up, they are randomized either to quinine + clindamycin, or an alternative artemisinin-based combination therapy, or the same first-line artemisinin-based combination therapy to be followed up for 28 additional days. If between days 14 and 28 the patients experience a recurrent parasitemia, they are retreated with the recommended first-line regimen and actively followed up for another 28 additional days (step three; post-RCT phase). The same methodology is followed for each subsequent failure. In any case, all patients without an infection at day 28 are classified as treatment successes and reach a study endpoint. The RCT phase allows the comparison of the safety and efficacy of three rescue treatments. The prolonged follow-up of all children until they are 28 days parasite-free allows us to assess epidemiological-, host- and parasite-related predictors for repeated malaria infection.

TRIAL REGISTRATION: NCT01374581 and PACTR201203000351114.

PMCID: PMC3849445 **Free PMC Article**
PMID: 24059911 [PubMed - indexed for MEDLINE]

17. **Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of Anopheles gambiae s.s. from different malaria transmission zones in Uganda.**

Okia M[1], Ndyomugyenyi R, Kirunda J, Byaruhanga A, Adibaku S, Lwamafa DK, Kironde F.

Parasit Vectors. 2013 May 2;6:130. doi: 10.1186/1756-3305-6-130.

[1]National Malaria Control Programme, Ministry of Health, P.O. Box 7272, Kampala, Uganda.

**ABSTRACT**

BACKGROUND: There are major concerns over sustaining the efficacy of current malaria vector control interventions given the rapid spread of resistance, particularly to pyrethroids. This study assessed the bioefficacy of five WHO-recommended long-lasting insecticidal nets (LLINs) against pyrethroid-resistant Anopheles gambiae field populations from Uganda.

METHODS: Adult An. gambiae from Lira, Tororo, Wakiso and Kanungu districts were exposed to permethrin (0.75%) or deltamethrin (0.05%) in standard WHO susceptibility tests. Cone bioassays were

used to measure the bioefficacy of four mono-treated LLINs (Olyset®, Interceptor®, Netprotect® and PermaNet® 2.0) and one combination LLIN (PermaNet® 3.0) against the four mosquito populations. Wireball assays were similarly conducted to determine knockdown rates. Species composition and kdr mutation frequency were determined for a sample of mosquitoes from each population. Chemical assays confirmed that test nets fell within target dose ranges.

RESULTS: Anopheles gambiae s.s. predominated at all four sites (86-99% of Anopheles spp.) with moderate kdr L1014S allelic frequency (0.34-0.37). Confirmed or possible resistance to both permethrin and deltamethrin was identified for all four test populations. Reduced susceptibility to standard LLINs was observed for all four populations, with mortality rates as low as 45.8% even though the nets were unused. The combination LLIN PermaNet®3.0 showed the highest overall bioefficacy against all four An. gambiae s.l. populations (98.5-100% mortality). Wireball assays provided a more sensitive indicator of comparative bioefficacy, and PermaNet 3.0 was again associated with the highest bioefficacy against all four populations (76.5-91.7% mortality after 30 mins).

CONCLUSIONS: The bioefficacy of mono-treated LLINs against pyrethroid-resistant field populations of An. gambiae varied by LLIN type and mosquito population, indicating that certain LLINs may be more suitable than others at particular sites. In contrast, the combination LLIN PermaNet 3.0 performed optimally against the four An. gambiae populations tested. The observed reduced susceptibility of malaria vectors to mono-treated LLINs is of particular concern, especially considering all nets were unused. With ongoing scale-up of insecticidal tools in the advent of increasing resistance, it is essential that those interventions with proven enhanced efficacy are given preference particularly in areas with high resistance.

PMCID: PMC3656772 **Free PMC Article**
PMID: 23634798 [PubMed - indexed for MEDLINE]

18. **Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations.**

Govella NJ[1], Chaki PP, Killeen GF.

Malar J. 2013 Apr 11;12:124. doi: 10.1186/1475-2875-12-124.

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**ABSTRACT**

BACKGROUND: The most potent malaria vectors rely heavily upon human blood so they are vulnerable to attack with insecticide-treated nets (ITNs) and indoor residual spraying (IRS) within houses. Mosquito taxa that can avoid feeding or resting indoors, or by obtaining blood from animals, mediate a growing proportion of the dwindling transmission that persists as ITNs and IRS are scaled up.

PRESENTATION OF THE HYPOTHESIS: Increasing frequency of behavioural evasion traits within persisting residual vector systems usually reflect the successful suppression of the most potent and vulnerable vector taxa by IRS or ITNs, rather than their failure. Many of the commonly observed changes in mosquito behavioural patterns following intervention scale-up may well be explained by modified taxonomic composition and expression of phenotypically plastic behavioural preferences, rather than altered innate preferences of individuals or populations.

TESTING THE HYPOTHESIS: Detailed review of the contemporary evidence base does not yet provide any clear-cut example of true behavioural resistance and is, therefore, consistent with the hypothesis presented.

IMPLICATIONS OF THE HYPOTHESIS: Caution should be exercised before over-interpreting most existing reports of increased frequency of behavioural traits which enable mosquitoes to evade fatal contact with insecticides: this may simply be the result of suppressing the most behaviourally vulnerable of the vector taxa that constituted the original transmission system. Mosquito taxa which have always exhibited such evasive traits may be more accurately described as behaviourally resilient, rather than resistant. Ongoing national or regional entomological monitoring surveys of physiological susceptibility to insecticides should be supplemented with biologically and epidemiologically meaningfully estimates of malaria vector population dynamics and the behavioural phenotypes that determine intervention impact, in order to design, select, evaluate and optimize the implementation of vector control measures.

PMCID: PMC3637503 **Free PMC Article**
PMID: 23577656 [PubMed - indexed for MEDLINE]

19. **Challenges for malaria elimination in Zanzibar: pyrethroid resistance in malaria vectors and poor performance of long-lasting insecticide nets.**

Haji KA, Khatib BO, Smith S, Ali AS, Devine GJ, Coetzee M, Majambere S.

Parasit Vectors. 2013 Mar 28;6:82. doi: 10.1186/1756-3305-6-82.

**ABSTRACT**

BACKGROUND: Long-lasting insecticide treated nets (LLINs) and indoor residual house spraying (IRS) are the main interventions for the control of malaria vectors in Zanzibar. The aim of the present study was to assess the susceptibility status of malaria vectors against the insecticides used for LLINs and IRS and to determine the durability and efficacy of LLINs on the island.

METHODS: Mosquitoes were sampled from Pemba and Unguja islands in 2010-2011 for use in WHO susceptibility tests. One hundred and fifty LLINs were collected from households on Unguja, their physical state was recorded and then tested for efficacy as well as total insecticide content.

RESULTS: Species identification revealed that over 90% of the Anopheles gambiae complex was An. arabiensis with a small number of An. gambiae s.s. and An. merus being present. Susceptibility tests showed that An. arabiensis on Pemba was resistant to the pyrethroids used for LLINs and IRS. Mosquitoes from Unguja Island, however, were fully susceptible to all pyrethroids tested. A physical examination of 150 LLINs showed that two thirds were damaged after only three years in use. All used nets had a significantly lower ($p < 0.001$) mean permethrin concentration of 791.6 mg/m2 compared with 944.2 mg/m2 for new ones. Their efficacy decreased significantly against both susceptible An. gambiae s.s. colony mosquitoes and wild-type mosquitoes from Pemba after just six washes ($p < 0.001$).

CONCLUSION: The sustainability of the gains achieved in malaria control in Zanzibar is seriously threatened by the resistance of malaria vectors to pyrethroids and the short-lived efficacy of LLINs. This study has revealed that even in relatively well-resourced and logistically manageable places like Zanzibar, malaria elimination is going to be difficult to achieve with the current control measures.

PMCID: PMC3639098 **Free PMC Article**
PMID: 23537463 [PubMed - indexed for MEDLINE]

20. **Efficacy of artemether-lumefantrine in treatment of malaria among under-fives and prevalence of drug resistance markers in Igombe-Mwanza, north-western Tanzania.**

Kamugisha E[1], Jing S, Minde M, Kataraihya J, Kongola G, Kironde F, Swedberg G.

Malar J. 2012 Feb 27;11:58. doi: 10.1186/1475-2875-11-58.

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erasmuskamugisha@yahoo.com

**ABSTRACT**

BACKGROUND: Drug resistance to anti-malarials is a major public health problem worldwide. This study aimed at establishing the efficacy of artemether-lumefantrine (ACT) in Igombe-Mwanza, north-western Tanzania after a few years of ACT use, and establish the prevalence of mutations in key targets for artemisinin, chloroquine and sulphadoxine/pyrimetamine (SP) drugs.

METHODS: A prospective single cohort study was conducted at Igombe health centre using artemether-lumefantrine combination therapy between February 2010 and March 2011. The follow-up period was 28 days and outcome measures were according to WHO guidelines. Blood was collected on Whatman filter paper for DNA analysis. DNA extraction was done using TRIS-EDTA method, and mutations in Pfcrt, Pfmdr1, Pfdhfr, Pfdhps and Pfatp6 were detected using PCR-RFLP methods established previously.

RESULTS: A total of 103 patients completed the 28 days follow-up. The mean haemoglobin was 8.9 g/dl (range 5.0 to 14.5 g/dl) and mean parasite density was 5,608 parasites/µl. Average parasite clearance time was 34.7 hours and all patients cleared the parasites by day 3. There was no early treatment

failure in this study. Late clinical failure was seen in three (2.9%) patients and late parasitological failure (LPF) was seen in two (1.9%). PCR-corrected LPF was 1% and adequate clinical and parasitological response was 96%. The majority of parasites have wild type alleles on pfcrt 76 and pfmdr1 86 positions being 87.8% and 93.7% respectively. Mutant parasites predominated at pfdhfr gene at the main three positions 108, 51 and 59 with prevalence of 94.8%, 75.3% and 82.5% respectively. Post-treatment parasites had more wild types of pfdhps at position 437 and 540 than pre-treatment parasites. No mutation was seen in pfatp6 769 in re-infecting or recrudescing parasites.

CONCLUSION: The efficacy of artemether-lumefantrine for treatment of uncomplicated malaria is still high in the study area although the rate of re-infection is higher than previously reported. Parasite clearance after 48 hours was lower compared to previous studies. The prevalence of wild type allele pfcrt 76 K and pfmdr1 86 N was high in the study area while markers for SP resistance is still high. Artemether-lumefantrine may be selecting for wild type alleles on both positions (437 and 540) of pfdhps.

PMCID: PMC3305412 **Free PMC Article**
PMID: 22369089 [PubMed - indexed for MEDLINE]

21. **Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance.**

Harrington WE[1], Mutabingwa TK, Kabyemela E, Fried M, Duffy PE.

Clin Infect Dis. 2011 Aug 1;53(3):224-30. doi: 10.1093/cid/cir376.

[1]Malaria Program, Seattle Biomedical Research Institute, Seattle, Washington, USA.

**Comment in:**

- The use of intermittent preventive treatment with sulfadoxine-pyrimethamine for preventing malaria in pregnant women. [Clin Infect Dis. 2011]

**ABSTRACT**

BACKGROUND: Millions of African women receive sulfadoxine-pyrimethamine (SP) as intermittent preventive treatment during pregnancy (IPTp) to avoid poor outcomes that result from malaria. However, parasites resistant to SP are widespread in parts of Africa, and IPTp may perversely exacerbate placental infections that contain SP-resistant parasites.

METHODS: The study used a cross-sectional design. We determined IPTp use in a delivery cohort of 880 pregnant women in Muheza, Tanzania, by report and by plasma sulfa measurements, and we examined its effects on maternal and fetal delivery outcomes.

RESULTS: In the overall cohort, IPTp was not associated with decreased odds of placental malaria or with increased mean maternal hemoglobin or mean birth weight. Unexpectedly, IPTp was associated with decreased cord hemoglobin level and increased risk of fetal anemia, which may be related to in utero SP exposure.

CONCLUSIONS: IPTp does not improve overall pregnancy outcomes in Muheza, Tanzania, where SP-resistant parasites predominate and may increase the odds of fetal anemia. As parasite resistance increases in a community, the overall effect of IPTp may transition from net benefit to neutral or net harm.

PMCID: PMC3202321 **Free PMC Article**
PMID: 21765070 [PubMed - indexed for MEDLINE]

22. **Detecting adenosine triphosphatase 6 (pfATP6) point mutations that may be associated with Plasmodium falciparum resistance to artemisinin: prevalence at baseline, before policy change in Uganda.**

Kamugisha E[1], Sendagire H[2], Kaddumukasa M[3], Enweji N[4], Gheysari F[4], Swedberg G[4], Kironde F[2].

Tanzan J Health Res. 2011 Jan;13(1):40-7.

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[2]Department of Microbiology, Makerere University-Kampala, Uganda.
[3]Department of Medicine, Makerere University, Kampala, Uganda.
[4]Department of Medical Biochemistry and Microbiology, Uppsala University, Sweden.

**ABSTRACT**

The artemisinin based combination therapy (ACT) of artemether and lumefantrine (Co-artem) has recently replaced chloroquine and fansidar as the first line treatment policy drug in Uganda. It is necessary to develop practical procedures to monitor the likely emergence and spread of artemisinin resistant P. falciparum strains. We have analyzed the genotypes of PfATP6 in parasites from 300 stored filter paper samples from malaria patients who were diagnosed and treated in the years 1999 to 2004 at three field sites in Uganda. This is a period just prior to introduction of Co-artem. In order to develop a simple molecular procedure for mutation detection, regions of PfATP6 encoding protein domains important in artemisinin binding was amplified by nested PCR. Three DNA products, which together contain most of the coding region of amino acids located within the putative active site of pfATP6 were readily amplified. The amplified DNA was digested by restriction enzymes and the fragments sized by agarose gel electrophoresis. For the important codons 260, 263 and 769, methods using engineered restriction sites were employed. We did not find mutations at codons for the key residues Lys 260, Leu263, Gln266, Ser769 and Asn1039. Nucleotide sequencing of pfATPase6 gene DNA from at least 15

clinical isolates confirmed the above findings and suggested that mutations at these amino acid residues have not emerged in our study sites.
PMID: 24409646 [PubMed - indexed for MEDLINE]

23. **Protecting the malaria drug arsenal: halting the rise and spread of amodiaquine resistance by monitoring the PfCRT SVMNT type.**

Sa JM[1], Twu O.

Malar J. 2010 Dec 23;9:374. doi: 10.1186/1475-2875-9-374.

[1]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 12735 Twinbrook Parkway, Room 3E-10C, Rockville, MD 20852, USA. jsa@niaid.nih.gov

**ABSTRACT**

The loss of chloroquine due to selection and spread of drug resistant Plasmodium falciparum parasites has greatly impacted malaria control, especially in highly endemic areas of Africa. Since chloroquine removal a decade ago, the guidelines to treat falciparum malaria suggest combination therapies, preferentially with an artemisinin derivative. One of the recommended partner drugs is amodiaquine, a pro-drug that relies on its active metabolite monodesethylamodiaquine, and is still effective in areas of Africa, but not in regions of South America. Genetic studies on P. falciparum parasites have shown that different pfcrt mutant haplotypes are linked to distinct levels of chloroquine and amodiaquine responses. The pfcrt haplotype SVMNT (termed after the amino acids from codon positions 72-76) is stably present in several areas where amodiaquine was introduced and widely used. Parasites with this haplotype are highly resistant to monodesethylamodiaquine and also resistant to chloroquine. The presence of this haplotype in Africa was found for the first time in 2004 in Tanzania and a role for amodiaquine in the selection of this haplotype was suggested. This commentary discusses the finding of a second site in Africa with high incidence of this haplotype. The >50% SVMNT haplotype prevalence in Angola represents a threat to the rise and spread of amodiaquine resistance. It is paramount to monitor pfcrt haplotypes in every country currently using amodiaquine and to re-evaluate current combination therapies in areas where SVMNT type parasites are prevalent.

PMCID: PMC3020158 **Free PMC Article**
PMID: 21182787 [PubMed - indexed for MEDLINE]

24. **Drug coverage in treatment of malaria and the consequences for resistance evolution--evidence from the use of sulphadoxine/pyrimethamine.**

Malisa AL[1], Pearce RJ, Abdulla S, Mshinda H, Kachur PS, Bloland P, Roper C.

Malar J. 2010 Jul 5;9:190. doi: 10.1186/1475-2875-9-190.

[1]Department of Biological Sciences, Faculty of Science, Sokoine University of Agriculture, SUA, PO Box 3038, Morogoro, Tanzania.

**ABSTRACT**

BACKGROUND: It is argued that, the efficacy of anti-malarials could be prolonged through policy-mediated reductions in drug pressure, but gathering evidence of the relationship between policy, treatment practice, drug pressure and the evolution of resistance in the field is challenging. Mathematical models indicate that drug coverage is the primary determinant of drug pressure and the driving force behind the evolution of drug resistance. These models show that where the basis of resistance is multigenic, the effects of selection can be moderated by high recombination rates, which disrupt the associations between co-selected resistance genes.

METHODS: To test these predictions, dhfr and dhps frequency changes were measured during 2000-2001 while SP was the second-line treatment and contrasted these with changes during 2001-2002 when SP was used for first-line therapy. Annual cross sectional community surveys carried out before, during and after the policy switch in 2001 were used to collect samples. Genetic analysis of SP resistance genes was carried out on 4,950 Plasmodium falciparum infections and the selection pressure under the two policies compared.

RESULTS: The influence of policy on the parasite reservoir was profound. The frequency of dhfr and dhps resistance alleles did not change significantly while SP was the recommended second-line treatment, but highly significant changes occurred during the subsequent year after the switch to first line SP. The frequency of the triple mutant dhfr (N51I,C59R,S108N) allele (conferring pyrimethamine resistance) increased by 37% - 63% and the frequency of the double A437G, K540E mutant dhps allele (conferring sulphadoxine resistance) increased 200%-300%. A strong association between these unlinked alleles also emerged, confirming that they are co-selected by SP.

CONCLUSION: The national policy change brought about a shift in treatment practice and the resulting increase in coverage had a substantial impact on drug pressure. The selection applied by first-line use is strong enough to overcome recombination pressure and create significant linkage disequilibrium between the unlinked genetic determinants of pyrimethamine and sulphadoxine resistance, showing that recombination is no barrier to the emergence of resistance to combination treatments when they are used as the first-line malaria therapy.

PMCID: PMC2908640 **Free PMC Article**
PMID: 20602754 [PubMed - indexed for MEDLINE]

25. **Effect of trimethoprim-sulphamethoxazole on the risk of malaria in HIV-infected Ugandan children living in an area of widespread antifolate resistance.**

Gasasira AF[1], Kamya MR, Ochong EO, Vora N, Achan J, Charlebois E, Ruel T, Kateera F, Meya DN, Havlir D, Rosenthal PJ, Dorsey G.

Malar J. 2010 Jun 23;9:177. doi: 10.1186/1475-2875-9-177.

[1]School of Medicine, Makerere University Kampala, Uganda. agasasira@gmail.com

**ABSTRACT**

BACKGROUND: Daily trimethoprim-sulfamethoxazole (TS) protects against malaria, but efficacy may be diminished as anti-folate resistance increases. This study assessed the incidence of falciparum malaria and the prevalence of resistance-conferring Plasmodium falciparum mutations in HIV-infected children receiving daily TS and HIV-uninfected children not taking TS.

MATERIALS AND METHODS: Subjects were 292 HIV-infected and 517 uninfected children from two cohort studies in Kampala, Uganda observed from August 2006 to December 2008. Daily TS was given to HIV-infected, but not HIV-uninfected children and all participants were provided an insecticide-treated bed net. Standardized protocols were used to measure the incidence of malaria and identify markers of antifolate resistance.

RESULTS: Sixty-five episodes of falciparum malaria occurred in HIV-infected and 491 episodes in uninfected children during the observation period. TS was associated with a protective efficacy of 80% (0.10 vs. 0.45 episodes per person year, $p < 0.001$), and efficacy did not vary over three consecutive 9.5 month periods (81%, 74%, 80% respectively, $p = 0.506$). The prevalences of dhfr 51I, 108N, and 59R and dhps 437G and 540E mutations were each over 90% among parasites infecting both HIV-infected and uninfected children. Prevalence of the dhfr 164L mutation, which is associated with high-level resistance, was significantly higher in parasites from HIV-infected compared to uninfected children (8% vs. 1%, $p = 0.001$). Sequencing of the dhfr and dhps genes identified only one additional polymorphism, dhps 581G, in 2 of 30 samples from HIV-infected and 0 of 54 samples from uninfected children.

CONCLUSION: Despite high prevalence of known anti-folate resistance-mediating mutations, TS prophylaxis was highly effective against malaria, but was associated with presence of dhfr 164L mutation.

PMCID: PMC2903607 **Free PMC Article**
PMID: 20573194 [PubMed - indexed for MEDLINE]

26. **The effect of point mutations in dihydrofolate reductase genes and multidrug resistance gene 1-86 on treatment of falciparum malaria in Sudan.**

Yusuf RU[1], Omar SA, Ngure RM.

J Infect Dev Ctries. 2010 Mar 8;4(2):61-9.

[1]Kenya Medical Research Institute, Centre for Biotechnology Research and Development, (KEMRI) Nairobi, Kenya. rudu@kemri.org

**ABSTRACT**

BACKGROUND: One of the major problems to the treatment of malaria is the emergence and spread of parasite resistant to antimalarial drugs. Due to increased chloroquine (CQ) resistance, the antifolate combinations are becoming important in the chemotherapy of falciparum malaria. However, resistance to antifolate exists and they are still effective in the above combinations. This study aimed at determining the prevalence of antimalarial drug resistance markers in P. falciparum isolates, involving the detection of mutations at the mdr 1- 86 which associates with amodiaquine resistance, and dhfr mutations associated with SP resistances.

METHODS: The dot-blot/ probe hybridization, which is more sensitive and specific; it detects parasitaemia of less than 100 parasites/microl of blood, and can identify a minority parasite genotype down to 1% in a mixture, was adopted to determine multi-drug resistance (mdr1-86) to show the correlation of Amodiaquine (AQ) resistance and PCR/ RFLP adopted to determine dihydrofolate reductase (dhfr) baseline resistance to Sulphadoxine- Pyrimethamine (SP) resistance in Nubian region of southern Sudan. A randomized open label trial of Artesunate (AS) + SP and AS+ SP was carried out in children less than 5 years. Molecular analysis of filter paper preserved blood samples collected was carried out to provide a baseline estimate of allele prevalences.

RESULTS: Baseline of the allele prevalence of the mdr1 86 locus in the AS+ AQ was successful for 80 isolates: 71(8.11%) carried parasites harbouring the mdr1-86 Tyr resistance allele, while 7 (89.19%) carried mdr1-86 Asn sensitivity allele and 2 (2.7%) were of mixed infection, having both resistance and wild type allele. Overall, the prevalence of the dhfr point mutation, codon 51, 59 and 108: 82.5% (132/160) carried mutations at dhfr (N51I, C59R or S108N), but triple mutants were rare (3.1%) in the AS + SP arm.

CONCLUSION: The research provides the evidence that mutations present in dhfr and mdr1 86 has a significant effect on the type of treatment following SP and AQ chemotherapy. SP resistance may spread rapidly, and AS + AQ is likely to be a better option, provided AQ use is restricted to the combination. The significance of the study shows that definitely combination of drugs improves SP therapy at the study site.

**Free Article**

PMID: 20212335 [PubMed - indexed for MEDLINE]

27. **Molecular correlates of high-level antifolate resistance in Rwandan children with Plasmodium falciparum malaria.**

Karema C[1], Imwong M, Fanello CI, Stepniewska K, Uwimana A, Nakeesathit S, Dondorp A, Day NP, White NJ.

Antimicrob Agents Chemother. 2010 Jan;54(1):477-83. doi: 10.1128/AAC.00498-09. Epub 2009 Oct 19.

[1]National Malaria Control Program, Kigali, Rwanda.

**ABSTRACT**

Antifolate drugs have an important role in the treatment of malaria. Polymorphisms in the genes encoding the dihydrofolate reductase and dihydropteroate synthetase enzymes cause resistance to the antifol and sulfa drugs, respectively. Rwanda has the highest levels of antimalarial drug resistance in Africa. We correlated the efficacy of chlorproguanil-dapsone plus artesunate (CPG-DDS+A) and amodiaquine plus sulfadoxine-pyrimethamine (AQ+SP) in children with uncomplicated malaria caused by Plasmodium falciparum parasites with pfdhfr and pfdhps mutations, which are known to confer reduced drug susceptibility, in two areas of Rwanda. In the eastern province, where the cure rates were low, over 75% of isolates had three or more pfdhfr mutations and two or three pfdhps mutations and 11% had the pfdhfr 164-Leu polymorphism. In the western province, where the cure rates were significantly higher ($P < 0.001$), the prevalence of multiple resistance mutations was lower and the pfdhfr I164L polymorphism was not found. The risk of treatment failure following the administration of AQ+SP more than doubled for each additional pfdhfr resistance mutation (odds ratio [OR] = 2.4; 95% confidence interval [CI] = 1.01 to 5.55; $P = 0.048$) and each pfdhps mutation (OR = 2.1; 95% CI = 1.21 to 3.54; $P = 0.008$). The risk of failure following CPG-DDS+A treatment was 2.2 times higher (95% CI = 1.34 to 3.7) for each additional pfdhfr mutation, whereas there was no association with mutations in the pfdhps gene ($P = 0.13$). The pfdhfr 164-Leu polymorphism is prevalent in eastern Rwanda. Antimalarial treatments with currently available antifol-sulfa combinations are no longer effective in Rwanda because of high-level resistance.

PMCID: PMC2798539 **Free PMC Article**
PMID: 19841150 [PubMed - indexed for MEDLINE]

28. **Residual antimalarials in malaria patients from Tanzania--implications on drug efficacy assessment and spread of parasite resistance.**

Hodel EM[1], Kabanywanyi AM, Malila A, Zanolari B, Mercier T, Beck HP, Buclin T, Olliaro P, Decosterd LA, Genton B.

PLoS One. 2009 Dec 14;4(12):e8184. doi: 10.1371/journal.pone.0008184.

[1]Swiss Tropical Institute, Basel, Switzerland.

**ABSTRACT**

BACKGROUND: Repeated antimalarial treatment for febrile episodes and self-treatment are common in malaria-endemic areas. The intake of antimalarials prior to participating in an in vivo study may alter treatment outcome and affect the interpretation of both efficacy and safety outcomes. We report the findings from baseline plasma sampling of malaria patients prior to inclusion into an in vivo study in Tanzania and discuss the implications of residual concentrations of antimalarials in this setting.

METHODS AND FINDINGS: In an in vivo study conducted in a rural area of Tanzania in 2008, baseline plasma samples from patients reporting no antimalarial intake within the last 28 days were screened for the presence of 14 antimalarials (parent drugs or metabolites) using liquid chromatography-tandem mass spectrometry. Among the 148 patients enrolled, 110 (74.3%) had at least one antimalarial in their plasma: 80 (54.1%) had lumefantrine above the lower limit of calibration (LLC = 4 ng/mL), 7 (4.7%) desbutyl-lumefantrine (4 ng/mL), 77 (52.0%) sulfadoxine (0.5 ng/mL), 15 (10.1%) pyrimethamine (0.5 ng/mL), 16 (10.8%) quinine (2.5 ng/mL) and none chloroquine (2.5 ng/mL).

CONCLUSIONS: The proportion of patients with detectable antimalarial drug levels prior to enrollment into the study is worrying. Indeed artemether-lumefantrine was supposed to be available only at government health facilities. Although sulfadoxine-pyrimethamine is only recommended for intermittent preventive treatment in pregnancy (IPTp), it was still widely used in public and private health facilities and sold in drug shops. Self-reporting of previous drug intake is unreliable and thus screening for the presence of antimalarial drug levels should be considered in future in vivo studies to allow for accurate assessment of treatment outcome. Furthermore, persisting sub-therapeutic drug levels of antimalarials in a population could promote the spread of drug resistance. The knowledge on drug pressure in a given population is important to monitor standard treatment policy implementation.

PMCID: PMC2788605 **Free PMC Article**
PMID: 20011529 [PubMed - indexed for MEDLINE]

29. **Evolution of a malaria resistance gene in wild primates.**

Tung J[1], Primus A, Bouley AJ, Severson TF, Alberts SC, Wray GA.

Nature. 2009 Jul 16;460(7253):388-91. doi: 10.1038/nature08149. Epub 2009 Jun 24.

[1]Department of Biology, Duke University, North Carolina 27708, USA. jt5@duke.edu

**ABSTRACT**

The ecology, behaviour and genetics of our closest living relatives, the nonhuman primates, should help us to understand the evolution of our own lineage. Although a large amount of data has been amassed on primate ecology and behaviour, much less is known about the functional and evolutionary genetic aspects of primate biology, especially in wild primates. As a result, even in well-studied populations in which nongenetic factors that influence adaptively important characteristics have been identified, we have almost no understanding of the underlying genetic basis for such traits. Here, we report on the functional consequences of genetic variation at the malaria-related FY (DARC) gene in a well-studied population of yellow baboons (Papio cynocephalus) living in Amboseli National Park in Kenya. FY codes for a chemokine receptor normally expressed on the erythrocyte surface that is the known entry point for the malarial parasite Plasmodium vivax. We identified variation in the cis-regulatory region of the baboon FY gene that was associated with phenotypic variation in susceptibility to Hepatocystis, a malaria-like pathogen that is common in baboons. Genetic variation in this region also influenced gene expression in vivo in wild individuals, a result we confirmed using in vitro reporter gene assays. The patterns of genetic variation in and around this locus were also suggestive of non-neutral evolution, raising the possibility that the evolution of the FY cis-regulatory region in baboons has exhibited both mechanistic and selective parallels with the homologous region in humans. Together, our results represent the first reported association and functional characterization linking genetic variation and a complex trait in a natural population of nonhuman primates.

PMID: 19553936 [PubMed - indexed for MEDLINE]

30. **Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment.**

Harrington WE[1], Mutabingwa TK, Muehlenbachs A, Sorensen B, Bolla MC, Fried M, Duffy PE.

Proc Natl Acad Sci U S A. 2009 Jun 2;106(22):9027-32. doi: 10.1073/pnas.0901415106. Epub 2009 May 18.

[1]Seattle Biomedical Research Institute, 307 Westlake Avenue N, Seattle, WA 98109, USA.

**ABSTRACT**

Intermittent preventive treatment in pregnancy (IPTp) is used to prevent Plasmodium falciparum malaria. However, parasites resistant to the IPTp drug sulfadoxine-pyrimethamine (SP) have emerged

worldwide, and infections with mixed resistant and susceptible parasites are exacerbated by pyrimethamine in mice. In a prospective delivery cohort in Muheza, Tanzania, we examined the effects of SP IPTp on parasite resistance alleles, parasite diversity, level of parasitemia, and inflammation in the placenta. IPTp use was associated with an increased fraction of parasites carrying the resistance allele at DHPS codon 581, an increase in the level of parasitemia, and more intense placental inflammation. The lowest mean level of parasite diversity and highest mean level of parasitemia occurred in women after recent IPTp use. These findings support a model of parasite release and facilitation, whereby the most highly resistant parasites out-compete less fit parasite populations and overgrow under drug pressure. Use of partially effective anti-malarial agents for IPTp may exacerbate malaria infections in the setting of widespread drug resistance.

PMCID: PMC2690058 **Free PMC Article**
PMID: 19451638 [PubMed - indexed for MEDLINE]

31. **Natural selection of FLT1 alleles and their association with malaria resistance in utero.**

Muehlenbachs A[1], Fried M, Lachowitzer J, Mutabingwa TK, Duffy PE.

Proc Natl Acad Sci U S A. 2008 Sep 23;105(38):14488-91. doi: 10.1073/pnas.0803657105. Epub 2008 Sep 8.

[1]Mother-Offspring Malaria Studies Project, Seattle Biomedical Research Institute, Seattle, WA 98109, USA.

**Comment in:**

- Flt1, pregnancy, and malaria: evolution of a complex interaction. [Proc Natl Acad Sci U S A. 2008]

**ABSTRACT**

Placental malaria (PM) caused by Plasmodium falciparum contributes significantly to infant mortality in sub-Saharan Africa and is associated with pregnancy loss. We hypothesized that fetal genes that modify PM would be associated with fetal fitness. During PM, placental trophoblasts produce soluble fms-like tyrosine kinase 1 (sFlt1), also known as soluble VEGF receptor 1, an angiogenesis inhibitor associated with preeclampsia. Here we present a study examining the genotype of the fms-related tyrosine kinase 1 (FLT1) 3' UTR in Tanzanian mother-infant pairs. First-time mothers suffer the most PM, and newborn FLT1 genotype distribution differed by birth order, with newborns of first-time mothers outside of Hardy-Weinberg equilibrium (HWE) during peak PM season. Among first-time but not other mothers, maternal FLT1 genotype was associated with a history of prior pregnancy loss. During PM, newborn FLT1 genotype was associated with low birth weight and placental inflammatory gene expression. FLT1 genotype was also associated with Flt1 levels among study subjects and in vitro. Thus, FLT1 variants confer fetal fitness in utero and are associated with the maternal immune response during PM. This

indicates that FLT1 is under natural selection in a malaria endemic area and that human exposure to malaria can influence the evolutionary genetics of the maternal-fetal relationship.

PMCID: PMC2567167 **Free PMC Article**
PMID: 18779584 [PubMed - indexed for MEDLINE]

32. **Molecular epidemiology of drug-resistant malaria in western Kenya highlands.**

Zhong D[1], Afrane Y, Githeko A, Cui L, Menge DM, Yan G.

BMC Infect Dis. 2008 Jul 31;8:105. doi: 10.1186/1471-2334-8-105.

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**ABSTRACT**

BACKGROUND: Since the late 1980s a series of malaria epidemics has occurred in western Kenya highlands. Among the possible factors that may contribute to the highland malaria epidemics, parasite resistance to antimalarials has not been well investigated.

METHODS: Using parasites from highland and lowland areas of western Kenya, we examined key mutations associated with Plasmodium falciparum resistance to sulfadoxine - pyrimethamine and chloroquine, including dihydrofolate reductase (pfdhfr) and dihydropteroate synthetase (pfdhps), chloroquine resistance transporter gene (pfcrt), and multi-drug resistance gene 1 (pfmdr1).

RESULTS: We found that >70% of samples harbored 76T pfcrt mutations and over 80% of samples harbored quintuple mutations (51I/59R/108N pfdhfr and 437G/540E pfdhps) in both highland and lowland samples. Further, we did not detect significant difference in the frequencies of these mutations between symptomatic and asymptomatic malaria volunteers, and between highland and lowland samples.

CONCLUSION: These findings suggest that drug resistance of malaria parasites in the highlands could be contributed by the mutations and their high frequencies as found in the lowland. The results are discussed in terms of the role of drug resistance as a driving force for malaria outbreaks in the highlands.

PMCID: PMC2533336 **Free PMC Article**
PMID: 18671871 [PubMed - indexed for MEDLINE]

33. **Comparison of different artemisinin-based combinations for the treatment of Plasmodium falciparum malaria in children in Kigali, Rwanda, an area of resistance to sulfadoxine-pyrimethamine: artesunate plus sulfadoxine/pyrimethamine versus artesunate plus sulfamethoxypyrazine/pyrimethamine.**

Rulisa S[1], Gatarayiha JP, Kabarisa T, Ndayisaba G.

Am J Trop Med Hyg. 2007 Oct;77(4):612-6.

[1]Central University Hospital of Kigali, Kigali, Rwanda. stevenruse@yahoo.com

**ABSTRACT**

In view of the changing policy towards artemisinin-based combination therapies (ACTs), the efficacy, tolerance, and degree of re-infection of two ACTs were investigated: artesunate plus sulfadoxine/pyrimethamine (As + SP) and AS plus sulfamethoxypyrazine/pyrimethamine (As + SMP). One hundred three children were assigned to receive As + SP and 109 to receive As + SMP. In spite of the high incidence of resistance to SP, As + SP showed satisfactory results consistent with recent recommendations for ACTs (adequate clinical and parasitologic response on day 28 [ACPR] > or = 90%), but results with As + SMP fulfilled the most stringent criteria (ACPR > or = 95%). The absence of side effects and the low price of these drugs make them it worth to reconsider national therapies in favor of either of these two drug combinations.

**Free Article**

PMID: 17978058 [PubMed - indexed for MEDLINE]

34. **Combining evidence of natural selection with association analysis increases power to detect malaria-resistance variants.**

Ayodo G[1], Price AL, Keinan A, Ajwang A, Otieno MF, Orago AS, Patterson N, Reich D.

Am J Hum Genet. 2007 Aug;81(2):234-42. Epub 2007 Jun 15.

[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

**ABSTRACT**

Statistical power to detect disease variants can be increased by weighting candidates by their evidence of natural selection. To demonstrate that this theoretical idea works in practice, we performed an association study of 10 putative resistance variants in 471 severe malaria cases and 474 controls from the Luo in Kenya. We replicated associations at HBB (P=.0008) and CD36 (P=.03) but also showed that the same variants are unusually differentiated in frequency between the Luo and Yoruba (who historically have been exposed to malaria) and the Masai and Kikuyu (who have not been exposed). This empirically demonstrates that combining association analysis with evidence of natural selection

can increase power to detect risk variants by orders of magnitude--up to P=.000018 for HBB and P=.00043 for CD36.

PMCID: PMC1950820 **Free PMC Article**
PMID: 17668374 [PubMed - indexed for MEDLINE]

35. **Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study.**

Blomberg B[1], Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, Msangi V, Tellevik MG, Holberg-Petersen M, Harthug S, Maselle SY, Langeland N.

BMC Infect Dis. 2007 May 22;7:43.

[1]Department of Medicine, Haukeland University Hospital, Bergen, Norway. bjorn.blomberg@med.uib.no

**ABSTRACT**

BACKGROUND: Bloodstream infection is a common cause of hospitalization, morbidity and death in children. The impact of antimicrobial resistance and HIV infection on outcome is not firmly established.

METHODS: We assessed the incidence of bloodstream infection and risk factors for fatal outcome in a prospective cohort study of 1828 consecutive admissions of children aged zero to seven years with signs of systemic infection. Blood was obtained for culture, malaria microscopy, HIV antibody test and, when necessary, HIV PCR. We recorded data on clinical features, underlying diseases, antimicrobial drug use and patients' outcome.

RESULTS: The incidence of laboratory-confirmed bloodstream infection was 13.9% (255/1828) of admissions, despite two thirds of the study population having received antimicrobial therapy prior to blood culture. The most frequent isolates were klebsiella, salmonellae, Escherichia coli, enterococci and Staphylococcus aureus. Furthermore, 21.6% had malaria and 16.8% HIV infection. One third (34.9%) of the children with laboratory-confirmed bloodstream infection died. The mortality rate from Gram-negative bloodstream infection (43.5%) was more than double that of malaria (20.2%) and Gram-positive bloodstream infection (16.7%). Significant risk factors for death by logistic regression modeling were inappropriate treatment due to antimicrobial resistance, HIV infection, other underlying infectious diseases, malnutrition and bloodstream infection caused by Enterobacteriaceae, other Gram-negatives and candida.

CONCLUSION: Bloodstream infection was less common than malaria, but caused more deaths. The frequent use of antimicrobials prior to blood culture may have hampered the detection of organisms susceptible to commonly used antimicrobials, including pneumococci, and thus the study probably underestimates the incidence of bloodstream infection. The finding that antimicrobial resistance, HIV-

infection and malnutrition predict fatal outcome calls for renewed efforts to curb the further emergence of resistance, improve HIV care and nutrition for children.

PMCID: PMC1891109 **Free PMC Article**
PMID: 17519011 [PubMed - indexed for MEDLINE]

36. **Modelling the impact of intermittent preventive treatment for malaria on selection pressure for drug resistance.**

Alexander N[1], Sutherland C, Roper C, Cissé B, Schellenberg D.

Malar J. 2007 Jan 22;6:9.

[1]Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK. neal.alexander@lshtm.ac.uk

**ABSTRACT**

BACKGROUND: Intermittent preventive treatment (IPT) is a promising intervention for malaria control, although there are concerns about its impact on drug resistance.

METHODS: The key model inputs are age-specific values for a) baseline anti-malarial dosing rate, b) parasite prevalence, and c) proportion of those treated with anti-malarials (outside IPT) who are infected. These are used to estimate the immediate effect of IPT on the genetic coefficient of selection (s). The scenarios modelled were year round IPT to infants in rural southern Tanzania, and three doses at monthly intervals of seasonal IPT in Senegal.

RESULTS: In the simulated Tanzanian setting, the model suggests a high selection pressure for drug resistance, but that IPTi would only increase this by a small amount (4.4%). The percent change in s is larger if parasites are more concentrated in infants, or if baseline drug dosing is less common or less specific. If children aged up to five years are included in the Tanzanian scenario then the predicted increase in s rises to 31%. The Senegalese seasonal IPT scenario, in children up to five years, results in a predicted increase in s of 16%.

CONCLUSION: There is a risk that the useful life of drugs will be shortened if IPT is implemented over a wide childhood age range. On the other hand, IPT delivered only to infants is unlikely to appreciably shorten the useful life of the drug used.

PMCID: PMC1796884 **Free PMC Article**
PMID: 17241476 [PubMed - indexed for MEDLINE]

37. **Drug resistance to sulphadoxine-pyrimethamine in Plasmodium falciparum malaria in Mlimba, Tanzania.**

Mbugi EV[1], Mutayoba BM, Malisa AL, Balthazary ST, Nyambo TB, Mshinda H.

Malar J. 2006 Oct 31;5:94.

[1]Department of Veterinary Physiology, Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Sokoine University of Agriculture (SUA), Morogoro, Tanzania. rerasto@yahoo.com

**ABSTRACT**

BACKGROUND: Sulphadoxine-pyrimethamine (SP) has been and is currently used for treatment of uncomplicated Plasmodium falciparum malaria in many African countries. Nevertheless, the response of parasites to SP treatment has shown significant variation between individuals.

METHODS: The genes for dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) were used as markers, to investigate parasite resistance to SP in 141 children aged less than 5 years. Parasite DNA was extracted by Chelex method from blood samples collected and preserved on filter papers. Subsequently, polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP) were applied to detect the SP resistance-associated point mutations on dhfr and dhps. Commonly reported point mutations at codons 51, 59, 108 and 164 in the dhfr and codons 437, 540 and 581 in the dhps domains were examined.

RESULTS: Children infected with parasites harbouring a range of single to quintuple dhfr/dhps mutations were erratically cured with SP. However, the quintuple dhfr/dhps mutant genotypes were mostly associated with treatment failures. High proportion of SP resistance-associated point mutations was detected in this study but the adequate clinical response (89.4%) observed clinically at day 14 of follow up reflects the role of semi-immunity protection and parasite clearance in the population.

CONCLUSION: In monitoring drug resistance to SP, concurrent studies on possible confounding factors pertaining to development of resistance in falciparum malaria should be considered. The SP resistance potential detected in this study, cautions on its useful therapeutic life as an interim first-line drug against malaria in Tanzania and other malaria-endemic countries.

PMCID: PMC1636063 **Free PMC Article**
PMID: 17076899 [PubMed - indexed for MEDLINE]

38. **Malaria transmission intensity and the rate of spread of chloroquine resistant Plasmodium falciparum: Why have theoretical models generated conflicting results?**

Talisuna AO[1], Erhart A, Samarasinghe S, Van Overmeir C, Speybroeck N, D'Alessandro U.

Infect Genet Evol. 2006 May;6(3):241-8. Epub 2005 Aug 22.

[1]Ministry of Health, Epidemiological Surveillance Division, PO Box 7272, Kampala, Uganda. atalisuna@yahoo.com

**ABSTRACT**

The rate at which falciparum resistant malaria spreads in different transmission settings is still a controversial subject. We have assessed the spread of mutant Plasmodium falciparum parasites in six Ugandan populations with varying prevalence of chloroquine resistance (CQR), malaria transmission intensity, multiplicity of parasite clones and prevalence of CQ use. For each population, we have determined the wild and mutant allele frequency at codons 76 and 86 of the pfcrt and pfmdr1 genes, respectively. The highest frequency (median = 16.3%, range: 0.0-70.4%) of infections with two pure mutants (no wild genotype in either gene), adjusted for clone multiplicity, was observed at the extremes of malaria transmission intensity. The wild/mutant (W/M) allele ratio (an index for tracking the progression of CQR) was less than one in all sites (median = 0.51, range: 0.09-0.98) for the pfcrt-76 gene, while it was greater than one in two of six sites (median = 0.75, range: 0.4-1.6) for the pfmdr1-86 gene, suggesting that the pfcrt-76 mutants were the predominant parasites at all sites. Furthermore, the pfmdr1-86 W/M allele ratio was consistently higher than that of the pfcrt-76. The spread of mutations linked to CQR in P. falciparum commences with the pfcrt-76 gene mutations, followed later by the pfmdr1-86 gene mutations that modulate higher CQR. Such spread occurs faster at the extremes of the transmission spectrum and could explain why mathematical models have previously generated conflicting results with respect to malaria transmission intensity and spread of CQR.

PMID: 16112915 [PubMed - indexed for MEDLINE]

39. **Complement receptor 1 polymorphisms associated with resistance to severe malaria in Kenya.**

Thathy V[1], Moulds JM, Guyah B, Otieno W, Stoute JA.

Malar J. 2005 Nov 8;4:54.

[1]The US Army Medical Research Unit, The Kenya Medical Research Institute, Nairobi, Kenya. vthathy@wrp-ksm.org

**ABSTRACT**

BACKGROUND: It has been hypothesized that the African alleles Sl2 and McCb of the Swain-Langley (Sl) and McCoy (McC) blood group antigens of the complement receptor 1 (CR1) may confer a survival advantage in the setting of Plasmodium falciparum malaria, but this has not been demonstrated.

METHODS: To test this hypothesis, children in western Kenya with severe malaria-associated anaemia or cerebral malaria were matched to symptomatic uncomplicated malaria controls by age and gender. Swain-Langley and McCoy blood group alleles were determined by restriction fragment length polymorphism and conditional logistic regression was carried out.

RESULTS: No significant association was found between the African alleles and severe malaria-associated anaemia. However, children with Sl2/2 genotype were less likely to have cerebral malaria (OR = 0.17, 95% CI 0.04 to 0.72, P = 0.02) than children with Sl1/1. In particular, individuals with Sl2/2 McC(a/b) genotype were less likely to have cerebral malaria (OR = 0.18, 95% CI 0.04 to 0.77, P = 0.02) than individuals with Sl1/1 McC(a/a).

CONCLUSION: These results support the hypothesis that the Sl2 allele and, possibly, the McCb allele evolved in the context of malaria transmission and that in certain combinations probably confer a survival advantage on these populations.

PMCID: PMC1308855 **Free PMC Article**
PMID: 16277654 [PubMed - indexed for MEDLINE]

40. **Genetic resistance to malaria in mouse models.**

Hernandez-Valladares M[1], Naessens J, Iraqi FA.

Trends Parasitol. 2005 Aug;21(8):352-5.

[1]International Livestock Research Institute, Naivasha Road, PO Box 30709, Nairobi 00100, Kenya. mhernandez@bxsoft.com

**ABSTRACT**

Murine models have proved to be excellent tools in the support of studies of the human genetic bases of malaria resistance and have enabled the mapping of 12 resistance loci, eight of them controlling parasitic levels and four controlling cerebral malaria. Further studies using this method have identified a Pklr variant that confers resistance to murine malaria, a result that shows the potential of this approach to aid the understanding of mechanisms of disease resistance. In the future, the use of murine models for genetic resistance to malaria could lead to the identification of relevant genetic factors that control this devastating disease.
PMID: 15967723 [PubMed - indexed for MEDLINE]

41. **Principal role of dihydropteroate synthase mutations in mediating resistance to sulfadoxine-pyrimethamine in single-drug and combination therapy of uncomplicated malaria in Uganda.**

Dorsey G[1], Dokomajilar C, Kiggundu M, Staedke SG, Kamya MR, Rosenthal PJ.

Am J Trop Med Hyg. 2004 Dec;71(6):758-63.

[1]Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California 94143, USA. grantd@itsa.ucsf.edu

**ABSTRACT**

Antimalarial resistance to sulfadoxine-pyrimethamine (SP) is mediated by mutations in the dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) genes. However, the relative importance of different mutations is incompletely understood and has not been studied with combination therapy. Samples from 812 patients treated for uncomplicated malaria in Kampala, Uganda were tested for the presence of mutations commonly found in Africa. The dhps Glu-540 mutation was the strongest independent predictor of treatment failure. The dhfr Arg-59 mutation was only predictive of treatment failure in the presence of the dhps Glu-540 mutation. Comparing combination regimens with SP monotherapy, the addition of chloroquine to SP did not improve efficacy, the addition of artesunate lowered the risk of treatment failure only for infections with both the dhfr Arg-59 and dhps Glu-540 mutations, and the addition of amodiaquine lowered this risk for all dhfr/dhps mutation patterns. The dhps Glu-540 mutation played a principal role and the dhfr Arg-59 mutation a secondary role in mediating resistance to SP alone and in combination.

**Free Article**

PMID: 15642967 [PubMed - indexed for MEDLINE]

42. **Therapeutic efficacy of sulfadoxine-pyrimethamine and prevalence of resistance markers in Tanzania prior to revision of malaria treatment policy: Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase mutations in monitoring in vivo resistance.**

Mugittu K[1], Ndejembi M, Malisa A, Lemnge M, Premji Z, Mwita A, Nkya W, Kataraihya J, Abdulla S, Beck HP, Mshinda H.

Am J Trop Med Hyg. 2004 Dec;71(6):696-702.

[1]Ifakara Health Research and Development Centre, Ifakara, Tanzania. Kmugittu@ifakara.mimcom.net

**ABSTRACT**

Prior to the 2001 malarial treatment policy change in Tanzania, we conducted trials to assess the efficacy of sulfadoxine-pyrimethamine (SP) and the usefulness of molecular markers in monitoring resistance. A total of 383 uncomplicated Plasmodium falciparum malaria patients (between 6 and 59 months old) were treated with SP and their responses were assessed. Mutations in the P. falciparum dihydrofolate reductase (pfdhfr) and dihydropteroate synthase (pfdhps) genes in admission day blood samples were analyzed. Results indicated that 85.6% of the patients showed an adequate clinical response, 9.7% an early treatment failure, and 4.7% a late treatment failure. The quintuple mutant genotype (pfdhfr 51 Ile, 59 Arg, and 108 Asn and pfdhps 437 Gly and 540 Glu) showed an association with treatment outcome (odds ratio = 2.1; 95% confidence interval = 0.94-4.48, P = 0.045). The prevalence of the triple pfdhfr mutant genotype (51 Ile, 59 Arg, and 108 Asn) at a site of high SP resistance (23.6%) was four times higher compared with that observed at sites of moderate SP resistance (6.8-14.4%) (P = 0.000001). The genotype failure index calculated by using this marker was invariable (1.96-2.1) at sites with moderate SP resistance, but varied (3.4) at a site of high SP resistance. In conclusion, our clinical and molecular findings suggest that SP may have a short useful therapeutic life in Tanzania; thus, its adoption as an interim first-line antimalarial drug. The findings also point to the potential of the triple pfdhfr mutant genotype as an early warning tool for increasing SP resistance. These data form the baseline SP efficacy and molecular markers profile in Tanzania prior to the policy change.

**Free Article**

PMID: 15642957 [PubMed - indexed for MEDLINE]

43. **Mapping of a new quantitative trait locus for resistance to malaria in mice by a comparative mapping approach with human Chromosome 5q31-q33.**

Hernandez-Valladares M[1], Rihet P, ole-MoiYoi OK, Iraqi FA.

Immunogenetics. 2004 May;56(2):115-7. Epub 2004 Apr 29.

[1]International Livestock Research Institute (ILRI), P.O. Box 30709, 00100, Nairobi, Kenya.

**ABSTRACT**

A number of linkage studies in human populations have identified a locus ( pfbi) on Chromosome 5q31-q33 controlling Plasmodiun falciparum blood infection levels. This region contains numerous candidate genes encoding immunological molecules such as cytokines, growth factors and growth-factor receptors. We have used an F(11) advance intercross line (AIL) population of mice infected with Plasmodium chabaudi to identify additional mouse quantitative trait loci (QTL) for control of parasitaemia on Chrs 11 and 18, which carry regions homologous to human Chr 5q31-q33. Herein, we report a novel QTL for parasitaemia control ( char8) on the mouse Chr 11, linked to marker D11Mit242, and involved in the clearance stages of the parasites from the bloodstream. Strikingly, several Th2

cytokines that are located within char8 have been identified to play a predominant role in the late stages of the infection.

PMID: 15118851 [PubMed - indexed for MEDLINE]

44. **Sulfadoxine-pyrimethamine in treatment of malaria in Western Kenya: increasing resistance and underdosing.**

Terlouw DJ[1], Nahlen BL, Courval JM, Kariuki SK, Rosenberg OS, Oloo AJ, Kolczak MS, Hawley WA, Lal AA, Kuile FO.

Antimicrob Agents Chemother. 2003 Sep;47(9):2929-32.

[1]Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA. dianne.terlouw@student.uva.nl

**ABSTRACT**

Between 1993 and 1999, we monitored the efficacy of sulfadoxine-pyrimethamine in 1175 children aged <24 months receiving 2789 treatments for falciparum malaria in western Kenya using a widely deployed age-based dose regimen: infants, 125 plus 6.25 mg (sulfadoxine plus pyrimethamine); children aged 12 to 23 months; 250 plus 12.5 mg. Cumulative treatment failure by day 7, defined as early clinical failure by day 3 or presence of parasitemia on day 7, increased from 18% in 1993 to 1994 to 22% in 1997 to 1998 (P-trend test = 0.20). Based on body weight, the median dose received was 20 plus 1.00 mg/kg, and 73% of the treatments were given at lower than the recommended target dose of 25 plus 1.25 mg/kg. Underdosing accounted for 26% of cumulative treatment failures. After the dose was increased in 1998 (median, 36 plus 1.8 mg/kg), only 4.2% of patients received less than 25 plus 1.25 mg/kg and there was no association with treatment failure. However, the proportion of cumulative treatment failure continued to increase to 27% by 1999 (P-trend test = 0.03). These results raise concern about the longevity of sulfadoxine-pyrimethamine in these settings. Underdosing may have contributed to the rate at which sulfadoxine-pyrimethamine resistance developed in this area. Treatment guidelines should ensure that adequate doses are given from the initial deployment of antimalarials onward.

PMCID: PMC182608 **Free PMC Article**

PMID: 12936996 [PubMed - indexed for MEDLINE]

45. **Genetic confirmation of atovaquone-proguanil-resistant Plasmodium falciparum malaria acquired by a nonimmune traveler to East Africa.**

Schwartz E[1], Bujanover S, Kain KC.

Clin Infect Dis. 2003 Aug 1;37(3):450-1. Epub 2003 Jul 18.

[1]Center for Geographic Medicine and Department of Medicine, Chaim Sheba Medical Center, Tel Hashomer, Israel.

**ABSTRACT**

We report a case of atovaquone-proguanil-resistant Plasmodium falciparum malaria acquired by a nonimmune traveler to Kenya. Recurrent parasitemia occurred 30 days after directly observed therapy with a combination of atovaquone and proguanil. Treatment failure was confirmed by genetic fingerprinting and sequencing. The primary isolate had wild-type sequence of cytochrome b; however, the recrudescent isolate had a single mutation at position 268 (Tyr268Ser).

**Free Article**

PMID: 12884171 [PubMed - indexed for MEDLINE]

46. **Role of the pfcrt codon 76 mutation as a molecular marker for population-based surveillance of chloroquine (CQ)-resistant Plasmodium falciparum malaria in Ugandan sentinel sites with high CQ resistance.**

Talisuna AO[1], Kyosiimire-Lugemwa J, Langi P, Mutabingwa TK, Watkins W, Van Marck E, Egwang T, D'Alessandro U.

Trans R Soc Trop Med Hyg. 2002 Sep-Oct;96(5):551-6.

[1]Ministry of Health, P.O. Box 7272, Kampala, Uganda. atalisuna@kla1.afsat.com

**ABSTRACT**

The mutant genotype at codon 76 of the pfcrt gene (T76) has been proposed as a molecular marker for surveillance of chloroquine (CQ)-resistant Plasmodium falciparum malaria but this proposal has not been validated by population-based surveys. In 1998-99, in 6 Ugandan sentinel sites, the prevalence of P. falciparum infections with the T76 genotype and the level of CQ use were measured by community surveys, and CQ resistance was determined by in-vivo tests on 6-59-month-old children with clinical malaria. The prevalence of T76 was not related to the overall clinical (early and late treatment failure: ETF + LTF; r = 0.14, P = 0.78) or parasitological (RI + RII + RIII; r = 0.17, P = 0.73) CQ resistance. However, the percentage of individuals carrying only infections with the T76 genotype (T76 alone) increased with increasing ETF (r = 0.76, P = 0.07) and type RIII parasitological failure (r = 0.69, P = 0.12). Similarly, the ratio between T76 and K76 (the wild type) prevalences (T76/K76) was strongly and positively correlated

with ETF (r = 0.85, P = 0.03) and RIII (r = 0.82, P = 0.04). Moreover, T76 alone (r = 0.90, P = 0.01) as well as T76/K76 (r = 0.90, P = 0.01) significantly increased with increasing community CQ use. T76 alone and T76/K76 can be useful markers to estimate the ETF and RIII prevalence as well as the amount of CQ use in the community.

PMID: 12474488 [PubMed - indexed for MEDLINE]

47. **Intensity of malaria transmission, antimalarial-drug use and resistance in Uganda: what is the relationship between these three factors?**

Talisuna AO[1], Langi P, Bakyaita N, Egwang T, Mutabingwa TK, Watkins W, Van Marck E, D'Alessandro U.

Trans R Soc Trop Med Hyg. 2002 May-Jun;96(3):310-7.

[1]Ministry of Health, P. O. Box 7272, Kampala, Uganda. atalisuna@kla1.afsat.com

**ABSTRACT**

We studied (in 1998 and 1999) some factors that may be linked to the spread of chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) resistance in 7 discrete communities in Uganda. Exposure to malaria infection was measured by parasitological surveys in children aged 1-9 years, drug use by community surveys and drug resistance by in-vivo tests on children aged 6-59 months with clinical malaria. CQ use was inversely related to parasite prevalence (r = -0.85, P = 0.01). CQ and SP treatment failure rates varied significantly according to parasite prevalence (P = 0.001 and 0.04 respectively). The highest CQ (42.4%, 43.8%) and SP (12.5%, 14.8%) treatment failure rates were observed in sites characterized by high parasite prevalence. Using areas with medium parasite prevalence as reference, the relative risk (RR) for CQ treatment failure was 3.2 (95% CI 1.6-6.4) in high parasite prevalence sites and 3.1 (95% CI 1.2-7.7) in low parasite prevalence sites. The RR for SP treatment failure was also higher in sites with high parasite prevalence but low in those with low parasite prevalence. According to our findings, drug resistance seems to spread faster in higher transmission areas, regardless of drug pressure. In low transmission areas, drug pressure seems to be the critical factor. A decrease in transmission coupled with rational use of drugs may delay the spread of resistance.

PMID: 12174786 [PubMed - indexed for MEDLINE]

48. **Resistance patterns of Plasmodium falciparum malaria to chloroquine in Kampala, Uganda.**

Mulindwa HC[1], Mayanja-Kizza H, Freers J.

East Afr Med J. 2002 Mar;79(3):115-9.

[1]Makerere University Hospital, Kampala, Uganda.

**ABSTRACT**

BACKGROUND: Chloroquine is a first line drug for the treatment of uncomplicated Plasmodium falciparum malaria in Uganda. Recently, there have been increasing reports of resistance of Plasmodium falciparum malaria to chloroquine, as well as an increase in malaria morbidity and mortality among adults and children.

OBJECTIVES: To assess the current effectiveness (clinical and parasitological response) of chloroquine in the treatment of uncomplicated Plasmodium falciparum malaria, and to define the magnitude of chloroquine resistant Plasmodium falciparum malaria in Kampala.

DESIGN: A descriptive cross-sectional study among adults and children.

SETTING: Mulago hospital complex (the national referral and teaching hospital in Kampala, Uganda) between September 1998 and March 1999.

RESULTS: Ninety six patients with Plasmodiumfalciparum parasitaemia of 1000 to 100,000/microl of blood were treated with oral chloroquine phosphate, and followed up for 14 days. Sixty three (65.6%) patients showed clinical improvement, 29 (30.2%) deteriorated and four (4.2%) had no change. Adequate parasitological response was seen in 71(74 %), moderate in four (4.2%) and poor in 21(21.8%) patients. Treatment failures were highest among children below five years, with eleven (57.9%) children not responding to chloroquine.

CONCLUSION: Although chloroquine was found to be effective in two thirds of all patients, the high treatment failure, especially seen in children below five years is of concern. This necessitates further countrywide studies, and possibly a need to review the use of chloroquine as single first line drug for the treatment of uncomplicated malaria in Uganda, especially in children below five years of age.
PMID: 12389954 [PubMed - indexed for MEDLINE]

49. **Plasmodium falciparum genotypes, low complexity of infection, and resistance to subsequent malaria in participants in the Asembo Bay Cohort Project.**

Branch OH[1], Takala S, Kariuki S, Nahlen BL, Kolczak M, Hawley W, Lal AA.
Infect Immun. 2001 Dec;69(12):7783-92.

[1]Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

**ABSTRACT**

To assess the relationship between the within-host diversity of malaria infections and the susceptibility of the host to subsequent infection, we genotyped 60 children's successive infections from birth through 3 years of life. MSP-1 Block2 genotypes were used to estimate the complexity of infection (COI). Malaria transmission and age were positively associated with the number of K1 and Mad20 alleles detected (COI(KM)) ($P < 0.003$). Controlling for previous parasitemia, transmission, drug treatment, parasite density, sickle cell, and age, COI(KM) was negatively correlated with resistance to parasitemia of > 500/microl ($P < 0.0001$). Parasitemias with the RO-genotype were more resistant than those without this genotype ($P < 0.0000$). The resistance in low COI(KM) infections was not genotype specific. We discuss the impact of genotype-transcending immunity to conserved antigenic determinants. We also propose a diversity-driven immunomodulation hypothesis that may explain the delayed development of natural immunity in the first few years of life and suggest that interventions that decrease the COI(KM) could facilitate the development of protective immunity.

PMCID: PMC98874 **Free PMC Article**
PMID: 11705960 [PubMed - indexed for MEDLINE]

50. **Monitoring antimalarial drug resistance within National Malaria Control Programmes: the EANMAT experience.**

East African Network for Monitoring Antimalarial Treatment (EANMAT).

Trop Med Int Health. 2001 Nov;6(11):891-8.

**ABSTRACT**

The National Malaria Control Programme (NMCP), organized within the Ministry of Health (MoH), is an essential component for the planning, execution and coordination of malaria control activities. As effective case management remains the mainstay of malaria control in almost every African country, antimalarial drug resistance is a major barrier to the implementation of effective malaria control policies. In order to function effectively, these units must have an efficient surveillance system which can provide reliable and current estimates of the severity of drug resistance. Without this information, it is impossible for the MoH to design and promote a rational antimalarial policy, but because of limited resources, especially of people and expertise, most NMCPs have been unable to initiate and manage

such a system. The need for collaborative partnerships between the MoH and the research community prompted the establishment of the East Africa Network for Monitoring Antimalarial Treatment (EANMAT). EANMAT has attempted to bring together the complimentary skills of malaria researchers and MoH staff in four east African countries. After 3 years of operation, data generated by EANMAT have been used to review and modify national malaria treatment policies in Kenya, Uganda, Rwanda and Tanzania. This new approach, which forges a closer working relationship between the research and policy communities, has effectively built capacity around the complex of surveillance, interpretation and use of evidence within a policy environment. The added-value of this approach is that the research community has learned to appreciate the constraints of policy development, and that the control community has established the need to build capacity and ownership of research evidence. Networks similar to EANMAT should be encouraged elsewhere in Africa to engender similar partnerships: to assist the development of rational treatment policies, and thus more effective malaria chemotherapy leading to significant lowering of malaria morbidity and mortality.

**Free Article**

PMID: 11703843 [PubMed - indexed for MEDLINE]

51. **Chlorproguanil-dapsone for treatment of drug-resistant falciparum malaria in Tanzania.**

Mutabingwa T[1], Nzila A, Mberu E, Nduati E, Winstanley P, Hills E, Watkins W.

Lancet. 2001 Oct 13;358(9289):1218-23.

[1]National Institute for Medical Research, Amani-Tanga, Tanzania. tkmuta@ud.co.tz

**Erratum in:**

- Lancet 2001 Nov 3;358(9292):1556.

**ABSTRACT**

BACKGROUND: Resistance to the affordable malaria treatments chloroquine and pyrimethamine-sulfadoxine is seriously impeding malaria control through treatment in east Africa. We did an open, alternate drug allocation study to assess the efficacy of chlorproguanil-dapsone in the treatment of falciparum malaria clinically resistant to pyrimethamine-sulfadoxine.

METHODS: Children younger than 5 years with non-severe falciparum malaria, attending Muheza district hospital in Tanzania, were treated with the standard regimen of pyrimethamine-sulfadoxine. Patients whose clinical symptoms resolved but who remained parasitaemic 7 days after pyrimethamine-sulfadoxine were followed up for 1 month. Clinical malaria episodes were retreated with either single dose pyrimethamine-sulfadoxine or a 3-day regimen of chlorproguanil-dapsone. Those with parasitaemia after 7 days were treated with chlorproguanil-dapsone. Parasite DNA was collected on day 7 after first treatment with pyrimethamine-sulfadoxine and we looked for point

mutations in the genes encoding dihydrofolate reductase (dhfr) and dyhydropteroate synthetase (dhps).

FINDINGS: 360 children were enrolled and treated with pyrimethamine-sulfadoxine. On day 7, 192 (55%) of 348 had cleared parasitaemia. Of the remaining 156 parasitaemic children, 140 (90%) were followed up to day 28, and 92 (66%) of 140 developed clinical malaria. These 92 patients were alternately retreated with either pyrimethamine-sulfadoxine (46) or chlorproguanil-dapsone (46). 28 (61%) of 46 children retreated with pyrimethamine-sulfadoxine were still parasitaemic at day 7, compared with three (7%) of 44 [corrected] children retreated with chlorproguanil-dapsone. Resistance to pyrimethamine-sulfadoxine increased from 45% (156/348) at the first treatment to 61% (28/46) after retreatment. 83 of 85 parasite isolates collected after the first pyrimethamine-sulfadoxine treatment, and before and after the second treatments with pyrimethamine-sulfadoxine and chlorproguanil-dapsone showed triple-mutant dhfr alleles, associated with a variety of dhps mutations.

INTERPRETATION: Most patients treated with pyrimethamine-sulfadoxine, who remain parasitaemic at day 7, develop new malaria symptoms within 1 month. Chlorproguanil-dapsone was a practicable therapy under these circumstances. Analysis of parasite dhfr and dhps before and after treatment supports the view that pyrimethamine-sulfadoxine resistance in this part of Africa is primarily due to parasites with three mutations in the dhfr domain.

PMID: 11675058 [PubMed - indexed for MEDLINE]

52. **Plasmodium falciparum in Kenya: high prevalence of drug-resistance-associated polymorphisms in hospital admissions with severe malaria in an epidemic area.**

Omar SA[1], Adagu IS, Gump DW, Ndaru NP, Warhurst DC.

Ann Trop Med Parasitol. 2001 Oct;95(7):661-9.

[1]Centre for Biotechnology Research Development, Kenya Medical Research Institute, P.O. Box 54840, Mbagathi Road, Nairobi, Kenya.

**ABSTRACT**

During an epidemic of Plasmodium falciparum malaria in Chogoria, Kenya, P. falciparum DNA was collected from 24 cases of severe malaria admitted to hospital for parenteral quinine treatment. These patients had all failed first- (chloroquine) and second-line (sulfadoxine-pyrimethamine or amodiaquine) drug treatments. Twenty-two (92%) of the 24 patients sampled carried parasites with the (Asn)86(Tyr) point mutation in the pfmdr1 gene (chromosome 5), 20 (83%) had an (Asp)1246(Tyr) mutation and 18 (82%) had both of these mutations. These alleles are both reported to be associated with chloroquine-resistance. Polymorphisms in the cg2 gene (chromosome 7) are also associated with chloroquine resistance, and 18 (75%) of the 24 parasite samples each had the cg2 and pfmdr1 polymorphisms. These 18 samples also had the mutations associated with resistance to pyrimethamine and sulfadoxine: (Asn)51(Ile), (Cys)59(Arg) and (Ser)108(Asn) of gene dhfr (chromosome 4) and (Ala)437(Gly) and (Lys)540(Glu) of dhps (chromosome 8), respectively. Genotyping of the parasites from all 24 patients

revealed extensive diversity in the sequences for the merozoite surface antigens (MSA-1 and MSA-2) and the glutamate-rich protein (GLURP) and indicated that each sample contained more than one parasite clone. Although samples from non-admitted malaria cases were not available, it appears that drug resistance may have played an important role in the development of severe malaria in this epidemic.
PMID: 11784419 [PubMed - indexed for MEDLINE]

53. **Malaria chemoprophylaxis in the age of drug resistance. II. Drugs that may be available in the future.**

Shanks GD[1], Kain KC, Keystone JS.

Clin Infect Dis. 2001 Aug 1;33(3):381-5. Epub 2001 Jul 5.

[1]US Army Medical Component of the Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand. shanksdg@thai.amedd.army.mil

**ABSTRACT**

All current regimens of malaria chemoprophylaxis have serious drawbacks as a result of either suboptimal efficacy, difficulty with medication compliance, or adverse events. Two 8-aminoquinolines may be approaching registration, with primaquine having completed its prophylactic field testing and tafenoquine having begun advanced field testing at the end of 2000. Primaquine has long been used for management of relapses of malaria, but in the past decade, it has been reexamined for use in malaria prevention in order to stop infection in the liver. In field trials performed in Indonesia and Colombia, the efficacy of primaquine for malaria prevention was approximately 90%, compared with that of placebo. Because of its short half-life, primaquine requires daily administration. For adults, the prevention regimen is 30 mg base daily (0.5 mg base/kg/day), and it can probably be discontinued soon after departure from an area where malaria is endemic. To kill parasites that already exist in the liver, terminal prophylaxis is given after exposure to relapses of malaria infection; for adults, such prophylaxis usually consists of 15 mg base (0.3 mg base/kg/day) given daily for 2 weeks. Primaquine-induced gastrointestinal disturbances can be minimized if the drug is taken with food. Neither primaquine nor tafenoquine should be given to persons with glucose-6-phosphate dehydrogenase deficiency, to avoid the development of potentially severe drug-induced hemolysis. Tafenoquine is an analogue of primaquine that is more potent than the parent drug. Field trials in Kenya, Ghana, Gabon, and Southeast Asia have demonstrated an efficacy rate of approximately 90% for tafenoquine. Its long half-life allows for infrequent dosing (currently tested at 200 mg base/week), and its effect on parasites at the liver stage may allow for drug discontinuation at the time of departure from the area of endemicity.

**Free Article**
PMID: 11438908 [PubMed - indexed for MEDLINE]

54. **Assessment of drug resistance to the malaria parasite in residents of Kampala, Uganda.**

Mutanda LN[1].

East Afr Med J. 1999 Aug;76(8):421-4.

[1]Central Public Health Laboratory, Wandegeya, Kampala, Uganda.

**ABSTRACT**

OBJECTIVE: To assess drug-resistance of the malaria parasite in elite residents of Kampala city, Uganda.

DESIGN: Recruited into the study were patients with complaints of fever, backache and headache or general malaise and body joint pains, could recall their previous treatment for the current complaints and could show laboratory reports indicating presence of malaria parasites. Blood was taken from those patients and examined for malaria parasites.

SETTING: Kampala Diagnostic and Imaging Consultants Clinic, Kampala, Uganda from 1994 to 1997.

RESULTS: Out of 268 patients, 27%, 26%, 12%, 11%, 6%, 6% and 5% strains of malaria parasites were respectively resistant to chloroquine, quinine, metakelfin, fansidar, halfan, artenam and camoquine. Double drug resistance was also observed in the patients who had taken chloroquine and quinine (21%), chloroquine and fansidar (16%) and quinine and fansidar (10%) out of 86. Some strains exhibited resistance to chloroquine, quinine and fansidar (12.6%) out of 71. R III was observed in 17 strains of malaria parasites, eight of them were for chloroquine, four for fansidar and three for quinine. Twenty-six patients had frequent recurrence of malaria lasting for over one year.

CONCLUSION: One third of Plasmodium falciparum strains by 1997 had acquired resistance to chloroquine and quinine and some were gradually acquiring multi-drug resistance, leading to frequent recurrence of malaria and use of many different types of antimalarials.

PIP: This study assessed the resistance of malaria parasites to single and multiple drugs among elite residents of Kampala City, Uganda. The study was conducted at Kampala Diagnostic and Imaging Consultants Clinic from 1994 to 1997. It enrolled patients with complaints of fever, backache and headache or general body malaise and joint pains, who could recall their previous treatment for the current complaints and could show laboratory reports indicating the presence of malaria parasites. Blood samples of the patients were collected and examined. Out of 268 patients, 27%, 26%, 12%, 11%, 6%, 6%, and 5% strains of malaria parasites were respectively resistant to chloroquine, quinine, metakelfin, fansidar, halfan, artenam, and camoquine. Among patients who had taken two drugs, double drug resistance was observed in those who had taken chloroquine and quinine (21%), chloroquine and fansidar (16%), and quinine and fansidar (10%). Moreover, 26 patients had frequent recurrence of malaria lasting for over 1 year. In conclusion, one-third of Plasmodium falciparum strains

by 1997 had acquired resistance to chloroquine and quinine and some were slowly acquiring multidrug resistance resulting in frequent recurrence of malaria and use of many different types of antimalarials.

PMID: 10520345 [PubMed - indexed for MEDLINE]

55. **Chloroquine treatment for uncomplicated childhood malaria in an area with drug resistance: early treatment failure aggravates anaemia.**

Ekvall H[1], Premji Z, Björkman A.

Trans R Soc Trop Med Hyg. 1998 Sep-Oct;92(5):556-60.

[1]Division of Infectious Diseases, Karolinska Institute, Danderyd Hospital, Sweden.

**ABSTRACT**

Childhood anaemia is a major public health problem in malaria holoendemic areas. We assessed the effects of antimalarial treatment in an area with drug-resistant falciparum malaria on haemoglobin levels in small children by applying the 1996 World Health Organization in vivo method for the evaluation of standard chloroquine treatment at the community level. In Fukayosi village, coastal Tanzania, 117 children aged 5-36 months with clinical malaria episodes were treated with chloroquine syrup (25 mg/kg). Early treatment failure (ETF) occurred in 20% and late treatment failure (LTF) in 22% of cases. Age > 1 year and malnutrition were protective factors against ETF. The evidence that chloroquine treatment could not prevent an exacerbation of anaemia was (i) the fact that the fall in haemoglobin level after 72 h was significantly greater in ETF than in children with LTF and an adequate clinical response, and (ii) the absence of any haematological improvement at follow-up in children receiving chloroquine alone, even in true treatment successes. In contrast, pyrimethamine/sulfadoxine administered to treatment failures improved the haemoglobin level significantly > 21 d after treatment started (mean difference 14 g/L, 95% confidence interval 2.1-27). We conclude that, when chloroquine treatment of childhood malaria is associated with a 20% ETF rate, the haemoglobin response is unsatisfactory and there is a need to change the recommended first-line treatment.
PMID: 9861379 [PubMed - indexed for MEDLINE]

56. **Multigenic drug resistance among inbred malaria parasites.**

Dye C[1], Williams BG.

Proc Biol Sci. 1997 Jan 22;264(1378):61-7.

[1]Vector Biology and Epidemiology Unit, London School of Hygiene and Tropical Medicine, UK. dyec@who.ch

**ABSTRACT**

Recent population genetic studies on the malaria parasite Plasmodium falciparum have confirmed that selfing is more frequent where the transmission rate is lower, with inbreeding coefficients estimated to be 0.33 and 0.92 for sites in Tanzania and Papua New Guinea (PNG), respectively. These geographical differences in Plasmodium mating patterns have been linked to the rate of spread of chloroquine resistance CQR, which, according to some measures, has been slower in Tanzania than in PNG. It has been proposed that the former observation explains the latter, although the theoretical argument linking the two is based on limited simulation studies. Taking a more analytical approach here, we first establish the relevant relationship between the coefficient of inbreeding (F, within loci) and the recombination rate r, between loci, defining an 'effective recombination rate', r = r (1-F. We then show that the emergence of multigenic drug resistance can indeed be slowed (or even quickened) by more outcrossing, but only when resistance is determined by two or more genes, none of which independently confers significant protection. The resistance genes should both be initially rare, and subject to low selection pressure. The analysis does not completely discount the hypothesis that inbreeding significantly influences the spread of CQR, but we show that it can only do so under a restrictive set of conditions, and that these conditions are not satisfied by some laboratory and field data. We discuss some of the wider implications of these results for the evolution of multigenic resistance.

PMCID: PMC1688220 **Free PMC Article**
PMID: 9061961 [PubMed - indexed for MEDLINE]

57. **Resistance to chloroquine therapy in pregnant women with malaria parasitemia.**

Rukaria-Kaumbutho RM[1], Ojwang SB, Oyieke JB.

Int J Gynaecol Obstet. 1996 Jun;53(3):235-41.

[1]Department of Obstetrics and Gynaecology, University of Nairobi, Kenya.

**ABSTRACT**

OBJECTIVES: The objective of the study was to determine the efficacy of chloroquine in pregnant women with Plasmodium falciparum parasitemia at therapeutic doses of 25 mg/kg body weight divided over 3 days.

METHODS: Three-hundred pregnant women in Kilifi Hospital at the coast of Kenya were screened for malaria parasitemia using Giemsa stained thick blood smears. In vivo and in vitro parasite sensitivity to chloroquine was determined.

RESULTS: P. falciparum infections were present in 65 (22%) of 300 pregnant women. The in vivo tests showed that 46% of all the P. falciparum infections were resistant to chloroquine predominantly at RI and RII levels. The in vitro tests showed a resistance rate of 35%.

CONCLUSIONS: A large proportion of pregnant women with malaria do not respond to chloroquine therapy and alternative drugs are required.
PMID: 8793625 [PubMed - indexed for MEDLINE]

58. **Chloroquine resistant Plasmodium falciparum in coastal Tanzania. A challenge to the continued strategy of village based chemotherapy for malaria control.**

Premji Z[1], Minjas JN, Shiff CJ.

Trop Med Parasitol. 1994 Mar;45(1):47-8.

[1]Muhimbili University College of Health Science, Dar es Salaam, Tanzania.

**ABSTRACT**

An in vivo study to assess Plasmodium falciparum sensitivity to chloroquine was conducted in two villages of the Bagamoyo District, Tanzania in December 1992. The WHO standard field test (7 days) and the extended test (28 days) were carried out on symptom free children. The presence of chloroquine resistance was confirmed with 59% of infections being found resistant. Fifty-three percent were RI, 2% were at RII and 4% at RIII levels of resistance. Dosage was 25 mg/kg chloroquine base delivered over three days.
PMID: 8066382 [PubMed - indexed for MEDLINE]

59. **[A case of mefloquine-resistant Plasmodium falciparum malaria with convulsion after antimalarial treatment].**

[Article in Japanese]
Miyashita T[1], Tokumura Y, Nishiya H, Sugiyama H, Yamaguchi M, Ohyatsu I, Aoki M, Ono Y, Kamei K, Shibuya T, et al.

Kansenshogaku Zasshi. 1994 Jan;68(1):152-6.

[1]Second Department of Medicine, Teikyo University School of Medicine.

**ABSTRACT**

We report a case of 40-year-old with chloroquine- and mefloquine-resistant Plasmodium falciparum. He had a single grand mal seizure 37 days following retreatment with quinine intravenously, which resulted in rapid clearance of fever and parasitemia, in addition to mefloquine. He had a long history of

seizures, which were well controlled by phenytoin. Because he has never had such a seizure before and computerized tomographic scanning of the brain after admission showed no abnormal findings which caused convulsions, it seemed to be an adverse reaction caused by antimalarial drugs. It is possible that a double or triple combination treatment for the emergence of multiresistant falciparum malaria might more frequently produce severe side effects, such as psychiatric reactions and convulsions. This case suggests that physicians must have a long follow-up period for chronic toxicity of antimalarial drugs, especially after using drug combinations for falciparum malaria.
PMID: 8138671 [PubMed - indexed for MEDLINE]

60. **Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa.**

Bloland PB[1], Lackritz EM, Kazembe PN, Were JB, Steketee R, Campbell CC.

J Infect Dis. 1993 Apr;167(4):932-7.

[1]Malaria Branch, Centers for Disease Control, Atlanta, GA 30333.

**ABSTRACT**

Emphasis on retaining chloroquine as the first-line therapy for Plasmodium falciparum infections in most of sub-Saharan Africa for as long as it remains effective has resulted in widespread reliance on chloroquine in areas where it can have little effect on P. falciparum parasitemia. To address this issue, clinical, parasitologic, and hematologic responses to chloroquine or pyrimethamine/sulfadoxine treatment were assessed among very young children in Malawi (n = 153) and Kenya (n = 73). The median time to resumption of clinical symptoms in chloroquine-treated children was 13.5 days in Malawi and 9.5 days in Kenya. Children treated with pyrimethamine/sulfadoxine maintained clinical improvement and had greater increases in their hemoglobin concentration during the follow-up period than did children treated with chloroquine. Treatment with chloroquine failed to produce either a durable clinical improvement or optimal hematologic recovery. Consequently, chloroquine can no longer be considered adequately effective therapy of clinical P. falciparum malaria in very young children in these areas of Africa.
PMID: 8450258 [PubMed - indexed for MEDLINE]

61. **Treatment of Plasmodium falciparum malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life.**

Watkins WM[1], Mosobo M.

Trans R Soc Trop Med Hyg. 1993 Jan-Feb;87(1):75-8.

[1]Kenya Medical Research Institute Coast Research Unit, Kilifi.

**ABSTRACT**

In an area of continuing transmission of Plasmodium falciparum on the Kenya coast, children treated with pyrimethamine-sulfadoxine experienced rapid parasite clearance, although a high proportion became reinfected within a short time. The frequency of pyrimethamine resistance in vitro in new infections was higher during the elimination phase of drug from a previous treatment. In infections which occurred at times when predicted residual drug concentrations were no longer inhibitory, incidence of pyrimethamine resistance was no different from the natural or background frequency. These results are discussed in terms of the selective pressure for resistance which is exerted by drugs with long elimination half-lives and a consideration of possible ways by which the problem might be addressed.

PMID: 8465404 [PubMed - indexed for MEDLINE]

62. **Falciparum malaria fully cleared by amodiaquine, pyrimethamine-sulfadoxine and pyrimethamine-sulfalene in areas of chloroquine resistance in Dodoma, Tanzania.**

Irare SG[1], Lemnge MM, Mhina JI.

Trop Geogr Med. 1991 Oct;43(4):352-6.

[1]National Institute for Medical Research, Amani Medical Research Centre, Tanga, Tanzania.

**ABSTRACT**

The in vivo response of Plasmodium falciparum to chloroquine, amodiaquine, pyrimethamine-sulfalene (MetakelfinR) and pyrimethamine-sulfadoxine (FansidarR) was assessed in Dodoma in 1988. Asymptomatic schoolchildren with pure P. falciparum infection were given full curative doses of one of the above antimalarials. Daily parasitological follow-ups were made for seven days. Overall successful follow-up cases were 101, 108, 95 and 97 on chloroquine, amodiaquine, MetakelfinR and FansidarR respectively. The overall resistance rate in the area was 28%. Most of the resistant cases were RII type. There was only one case of MetakelfinR resistance. Amodiaquine and FansidarR were fully effective in eliminating asexual parasitaemia from the blood in all the cases during the seven days of follow-up. The results indicate that chloroquine, a commonly used antimalarial in Tanzania, is not as effective as amodiaquine, a less used drug. Although the 'antifols' are still highly effective in Tanzania, their potency could change with continued use. These drugs should, therefore, be protected and used judiciously.

PMID: 1812599 [PubMed - indexed for MEDLINE]

63. **The resistance of falciparum malaria in Africa to 4-aminoquinolines and antifolates.**

Schapira A[1].

Scand J Infect Dis Suppl. 1990;75:1-64.

[1]Instituto Nacional de Saúde, Maputo, Mozambique.

**ABSTRACT**

Falciparum malaria cannot be eradicated from sub-Saharan Africa with present technology. The mainstay of malaria control in this situation is treatment of fever cases with chloroquine, aiming principally at reduction of mortality. The efficacy of this policy is now endangered because of the appearance and spread of chloroquine-resistance on the African continent. The present review examines laboratory and field research on the resistance of African P.falciparum to chloroquine, amodiaquine, pyrimethamine, proguanil, chlorproguanil and the combination sulfadoxine-pyrimethamine. Drug-resistance in malaria may be assessed with in vivo and in vitro technology. In vivo tests are simple, but the results are difficult to compare because of the influence of immunity. In vitro tests provide a more precise epidemiological tool, but their analysis should be undertaken with consideration of their technical limitations. For parasitological, immunological and epidemiological reasons, a one-to-one correlation between in vivo and in vivo grading of resistance is usually not found. Extended in vivo tests may be at least as sensitive as in vitro tests for detecting rare resistant parasites. On the other hand, the standardized grading of higher levels of in vivo resistance is arbitrary, and it is doubtful, whether such distinction has any clinical relevance. The 4-aminoquinolines (chloroquine and amodiaquine) presumably act by interfering with vital functions in the acid vesicles of parasites. Recent experiments indicate that resistance may be related to an increased rate of efflux of chloroquine from the parasite. It is caused by mutation, and at least three genetic levels of resistance have been identified. The blood stages of resistant plasmodia seem to have a biological advantage over sensitive ones, an observation that raises some hitherto unanswered questions. In the 1970s, a low degree of resistance to chloroquine was found in African P. falciparum in several localities. Resistance to the standard dose of chloroquine of 25 mg/kg was found in 1978 in tourists, who had sojourned in Kenya and Tanzania. Since then, chloroquine-resistance has spread centrifugally with increasing rapidity from an original focus in Northern Tanzania or Southern Kenya. The rate of increase in the proportion of resistant infections has generally been more rapid in the areas, where resistance has been introduced recently than in the original epifocus. The rate of increase is also generally more rapid in urban than in rural areas, an observation that can be ascribed to differences in drug pressure.(ABSTRACT TRUNCATED AT 400 WORDS)

PMID: 2100881 [PubMed - indexed for MEDLINE]

64. **Malaria control by antivectorial measures in a zone of chloroquine-resistant malaria: a successful programme in a rice growing area of the Rusizi valley.**

Coosemans M[1], Barutwanyo M.

Trans R Soc Trop Med Hyg. 1989;83 Suppl:97-8.

[1]Institute of Tropical Medicine Prince Léopold, Antwerp, Belgium

**ABSTRACT**

Within a large project for the socio-economic development of the rice growing area of the Rusizi valley in Burundi, a malarial control programme has been set up. This programme has several components: improvement of curative services, promotion of use of impregnated mosquito nets, and use of environmental engineering and indoor spraying with residual insecticides to control infection.
PMID: 2623758 [PubMed - indexed for MEDLINE]

65. **Serial studies on the evolution of drug resistance in malaria in an area of east Africa: findings from 1979 up to 1986.**

Draper CC[1], Hills M, Kilimali VA, Brubaker G.

J Trop Med Hyg. 1988 Oct;91(5):265-73.

[1]London School of Hygiene and Tropical Medicine, UK.

**ABSTRACT**

Observations, previously reported for 1979-82, have been continued up to 1986 on the development of drug resistance in P. falciparum in the North Mara area of Tanzania, where a chloroquine chemosuppression campaign was attempted from 1977 to 1982. The WHO micro in-vitro test for chloroquine and other drugs was used. Because of the large number of tests done, each test was characterized by the mean minimum inhibitory concentrations (MIC) of drug needed to prevent schizont development instead of counting the numbers of schizonts. The MIC for chloroquine has risen progressively each year but changes in the findings of in-vivo tests were less dramatic possibly due to the effects of immunity. Resistance to amodiaquine has followed that to chloroquine at a lower level, and in the last years the MIC for quinine has risen. Sporadic resistance to mefloquine was found and, by in-vivo test, to sulphadoxine-pyrimethamine. Possible factors in the evolution of drug resistance are discussed together with implications for the future.
PMID: 3054140 [PubMed - indexed for MEDLINE]

66. **Efficacy of multiple-dose halofantrine in treatment of chloroquine-resistant falciparum malaria in children in Kenya.**

Watkins WM[1], Oloo JA, Lury JD, Mosoba M, Kariuki D, Mjomba M, Koech DK, Gilles HM.

Lancet. 1988 Jul 30;2(8605):247-50.

[1]Biomedical Sciences Research Centre, Kenya Medical Research Institute (KEMRI), Nairobi.

**ABSTRACT**

Halofantrine hydrochloride given to 46 Kenyan children with falciparum malaria at 10 mg/kg for two doses, and to 60 other children at 8 mg/kg for three doses, resulted in rapid parasite clearance, mean parasite clearance times being 45.4 h and 54.8 h, respectively. In-vitro chemosensitivity tests showed that most infections were due to chloroquine-resistant parasites, and that parasite maturation was inhibited by considerably lower concentrations of halofantrine than of chloroquine.
PMID: 2899237 [PubMed - indexed for MEDLINE]

67. **Chloroquine treatment of falciparum malaria in an area of Kenya of intermediate chloroquine resistance.**

Brandling-Bennett AD[1], Oloo AJ, Watkins WM, Boriga DA, Kariuki DM, Collins WE.

Trans R Soc Trop Med Hyg. 1988;82(6):833-7.

[1]Clinical Research Centre, Kenya Medical Research Institute, Nairobi.

**ABSTRACT**

106 children aged 1-10 years who had pure Plasmodium falciparum infections and temperatures greater than or equal to 38 degrees C were treated with chloroquine base, 25 mg/kg body weight. 29% of the infections were sensitive in vivo, 41% recurred within 4 weeks (RI), 26% were RII resistant, and 4% were RII resistant. Rieckmann micro in vitro tests were successful in 64% of isolates obtained from these children; 63% were resistant to chloroquine. In 58 paired isolates obtained before and after treatment, the level of chloroquine sensitivity was lower in the parasites persisting or recurring after treatment. All children except 2 of the 4 with RIII resistance became afebrile an average of 1.4 d after starting treatment and their other symptoms resolved in an average of 1.8 d. By day 28, 57% of the children with RI resistance and 78% of those with RII resistance had recurrence of fever and other symptoms, compared with 19% of children with sensitive infections. No relationship was observed between the clinical or parasitological response and age, nutritional status, haematocrit, splenomegaly, presence of sickle-cell trait, or seropositivity to malaria by enzyme-linked immunosorbent assay. The

study demonstrates that, in most children with malaria in an area of intermediate chloroquine resistance, fever and other symptoms resolve at least temporarily when treated with chloroquine.

PMID: 3076997 [PubMed - indexed for MEDLINE]

68. **Double-blind study to assess the efficacy of chlorproguanil given alone or in combination with chloroquine for malaria chemoprophylaxis in an area with Plasmodium falciparum resistance to chloroquine, pyrimethamine and cycloguanil.**

Coosemans MH[1], Barutwanayo M, Onori E, Otoul C, Gryseels B, Wéry M.

Trans R Soc Trop Med Hyg. 1987;81(1):151-6.

[1]Projet de Lutte contre les Maladies transmissibles et carentielles, Bujumbura, Burundi.

**ABSTRACT**

In this study the efficacy of chlorproguanil (20 mg base weekly) was compared in schoolchildren with that of chloroquine (200 mg base weekly) and that of both drugs combined (20 mg base + 200 mg base weekly). The double blind trial was performed in the rice field area of the Ruzizi valley in Burundi, where Plasmodium falciparum is widely resistant to chloroquine, and where pyrimethamine resistance with cycloguanil cross-resistance had been demonstrated. After 17 weeks, when the trial was ended, 60% breakthroughs had been observed among the children taking chloroquine, 72% among those under chlorproguanil and 61% among those under chlorproguanil and chloroquine. In children weighing between 15 and 24 kg, the failure rate was significantly higher in those treated with chlorproguanil than in the group treated with chloroquine. No difference in efficacy was observed in children weighing 25 to 39 kg. There was no significant increase of efficacy when chlorproguanil was given in association with chloroquine. The mean titre of fluorescent antibodies was the same in each treated group on week 5 and week 15. The comparison of these data with the infection rates in non-protected children suggests that malaria could not be prevented with any of the drug regimens utilized in the study.

PMID: 3328329 [PubMed - indexed for MEDLINE]

69. **Chloroquine-resistant Plasmodium falciparum malaria in Ethiopia.**

Teklehaimanot A.
Lancet. 1986 Jul 19;2(8499):127-9.

**ABSTRACT**

Standard triple-dose therapy with chloroquine (25 mg base/kg) failed to clear asexual Plasmodium falciparum parasites from the blood of 22 of 98 patients infected in various parts of Ethiopia and evaluated in Addis Ababa, a malaria-free city. RI to RIII levels of resistance were demonstrated in those

patients. The resistant isolates were confined to areas bordering Somalia, Kenya, and Sudan. In in-vitro tests 7 of 10 (70%) isolates were chloroquine-resistant.
PMID: 2873398 [PubMed - indexed for MEDLINE]

70. **Serial studies on the evolution of chloroquine resistance in an area of East Africa receiving intermittent malaria chemosuppression.**

Draper CC, Brubaker G, Geser A, Kilimali VA, Wernsdorfer WH.

Bull World Health Organ. 1985;63(1):109-18.

**ABSTRACT**

Serial in vitro and in vivo tests for chloroquine sensitivity of Plasmodium falciparum were carried out from 1979 to 1982 in an area of E. Africa where chemosuppression with chloroquine had been attempted since 1977. Within 1(1/2) years there were signs of a decreasing drug response. Chloroquine resistance was first detected in 1981 and this increased markedly in 1982. Other contributory causes for the rise of parasite rates in children were possibly a decline in the efficiency of the drug distribution system and also immunological factors. Evidence of resistance to pyrimethamine was also found. Observations were made of the heterogeneity of the parasites' responses with emerging resistance. Implications for the future are discussed.

PMCID: PMC2536351 **Free PMC Article**
PMID: 3886184 [PubMed - indexed for MEDLINE]

# TUBERCULOSIS RESISTANCE

## 61 Citations

(sorted newest to oldest)

# TUBERCULOSIS RESISTANCE

1. **A Locus at 5q33.3 Confers Resistance to Tuberculosis in Highly Susceptible Individuals.**

Sobota RS[1], Stein CM[2], Kodaman N[3], Scheinfeldt LB[4], Maro I[5], Wieland-Alter W[6], Igo RP Jr[7], Magohe A[8], Malone LL[9], Chervenak K[9], Hall NB[7], Modongo C[10], Zetola N[10], Matee M[8], Joloba M[11], Froment A[12], Nyambo TB[13], Moore JH[14], Scott WK[15], Lahey T[6], Boom WH[16], von Reyn CF[6], Tishkoff SA[17], Sirugo G[18], Williams SM[19].

Am J Hum Genet. 2016 Mar 3;98(3):514-24. doi: 10.1016/j.ajhg.2016.01.015.

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**ABSTRACT**

Immunosuppression resulting from HIV infection increases the risk of progression to active tuberculosis (TB) both in individuals newly exposed to Mycobacterium tuberculosis (MTB) and in those with latent infections. We hypothesized that HIV-positive individuals who do not develop TB, despite living in areas where it is hyperendemic, provide a model of natural resistance. We performed a genome-wide association study of TB resistance by using 581 HIV-positive Ugandans and Tanzanians enrolled in prospective cohort studies of TB; 267 of these individuals developed active TB, and 314 did not. A common variant, rs4921437 at 5q33.3, was significantly associated with TB (odds ratio = 0.37, p = 2.11 × 10(-8)). This variant lies within a genomic region that includes IL12B and is embedded in an H3K27Ac histone mark. The locus also displays consistent patterns of linkage disequilibrium across African populations and has signals of strong selection in populations from equatorial Africa. Along with prior studies demonstrating that therapy with IL-12 (the cytokine encoded in part by IL12B, associated with longer survival following MTB infection in mice deficient in CD4 T cells), our results suggest that this pathway might be an excellent target for the development of new modalities for treating TB, especially for HIV-positive individuals. Our results also indicate that studying extreme disease resistance in the face of extensive exposure can increase the power to detect associations in complex infectious disease.

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PMCID: PMC4800052 **Free PMC Article**
PMID: 26942285 [PubMed - indexed for MEDLINE]

2. **Resistance related metabolic pathways for drug target identification in Mycobacterium tuberculosis.**

Cloete R[1], Oppon E[2], Murungi E[3,4], Schubert WD[5,6], Christoffels A[7].

BMC Bioinformatics. 2016 Feb 8;17:75. doi: 10.1186/s12859-016-0898-8.

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**ABSTRACT**

BACKGROUND: Increasing resistance to anti-tuberculosis drugs has driven the need for developing new drugs. Resources such as the tropical disease research (TDR) target database and AssessDrugTarget can help to prioritize putative drug targets. Hower, these resources do not necessarily map to metabolic pathways and the targets are not involved in dormancy. In this study, we specifically identify drug resistance pathways to allow known drug resistant mutations in one target to be offset by inhibiting another enzyme of the same metabolic pathway. One of the putative targets, Rv1712, was analysed by modelling its three dimensional structure and docking potential inhibitors.

RESULTS: We mapped 18 TB drug resistance gene products to 15 metabolic pathways critical for mycobacterial growth and latent TB by screening publicly available microarray data. Nine putative targets, Rv1712, Rv2984, Rv2194, Rv1311, Rv1305, Rv2195, Rv1622c, Rv1456c and Rv2421c, were found to be essential, to lack a close human homolog, and to share >67 % sequence identity and >87 % query coverage with mycobacterial orthologs. A structural model was generated for Rv1712, subjected to molecular dynamic simulation, and identified 10 compounds with affinities better than that for the ligand cytidine-5'-monophosphate (C5P). Each compound formed more interactions with the protein than C5P.

CONCLUSIONS: We focused on metabolic pathways associated with bacterial drug resistance and proteins unique to pathogenic bacteria to identify novel putative drug targets. The ten compounds identified in this study should be considered for experimental studies to validate their potential as inhibitors of Rv1712.

PMCID: PMC4745158 **Free PMC Article**
PMID: 26856535 [PubMed - indexed for MEDLINE]

3. **Identification of patients who could benefit from bedaquiline or delamanid: a multisite MDR-TB cohort study.**

Bonnet M[1], Bastard M[2], du Cros P[3], Khamraev A[4], Kimenye K[5], Khurkhumal S[6], Hayrapetyan A[7], Themba D[8], Telnov A[9], Sanchez-Padilla E[2], Hewison C[10], Varaine F[10].

Int J Tuberc Lung Dis. 2016 Feb;20(2):177-86. doi: 10.5588/ijtld.15.0962.

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[8]Ministry of Health of Swaziland, Mbabane, Swaziland.
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[10]MSF, Paris, France.

**Comment in:**

- Using existing data to illustrate--and close--the gap in access to new anti-tuberculosis drugs. [Int J Tuberc Lung Dis. 2016]

**ABSTRACT**

BACKGROUND: The World Health Organization recommends adding bedaquiline or delamanid to multidrug-resistant tuberculosis (MDR-TB) regimens for which four effective drugs are not available, and delamanid for patients at high risk of poor outcome.

OBJECTIVE: To identify patients at risk of unfavourable outcomes who may benefit from the new drugs.

METHODS: Retrospective cohort study of treatment outcomes involving four to five effective drugs for 15-24 months in programmes in Uzbekistan, Georgia, Armenia, Swaziland and Kenya between 2001 and 2011.

RESULTS: Of 1433 patients, 48.5% had body mass index (BMI) <18.5 kg/m(2), 72.9% had a high bacillary load, 16.7% were resistant to two injectables, 2.9% were resistant to ofloxacin (OFX) and 3.0% had extensively drug-resistant TB (XDR-TB). Treatment success ranged from 59.7% (no second-line resistance) to 27.0% (XDR-TB). XDR-TB (aOR 8.16, 95%CI 3.22-20.64), resistance to two injectables (aOR 1.90, 95%CI 1.00-3.62) or OFX (aOR 5.56, 95%CI 2.15-14.37), past incarceration (aOR 1.88, 95%CI 1.11-3.2), history of second-line treatment (aOR 3.24, 95%CI 1.53-6.85), low BMI (aOR 2.22, 95%CI 1.56-3.12) and high bacillary load (aOR 2.32, 95%CI 1.15-4.67) were associated with unfavourable outcomes. Patients started on capreomycin rather than kanamycin were more likely to have an unfavourable outcome (aOR 1.54, 95%CI 1.04-2.28).

CONCLUSION: In our cohort, patients who may benefit from bedaquiline and delamanid represented up to two thirds of all MDR-TB patients.

PMID: 26792469 [PubMed - indexed for MEDLINE]

4. **Lab-on-Chip-Based Platform for Fast Molecular Diagnosis of Multidrug-Resistant Tuberculosis.**

Cabibbe AM[1], Miotto P[1], Moure R[2], Alcaide F[2], Feuerriegel S[3], Pozzi G[4], Nikolayevskyy V[5], Drobniewski F[5], Niemann S[3], Reither K[6], Cirillo DM[7]; TM-REST Consortium; TB-CHILD Consortium.

J Clin Microbiol. 2015 Dec;53(12):3876-80. doi: 10.1128/JCM.01824-15. Epub 2015 Aug 5.

Collaborators: (13)
Di Pietro P, San Biagio F, Alessi E, Barbuzzi TG, Tafaj S, Bachiyska E, Kontsevaya I, Balabanova Y, Lazzeri E, Sserunkuma J, Aloi F, Nsubuga M, Sasamalo M.

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**Comment in:**

- The Race Is On To Shorten the Turnaround Time for Diagnosis of Multidrug-Resistant Tuberculosis. [J Clin Microbiol. 2015]

**ABSTRACT**

We evaluated the performance of the molecular lab-on-chip-based VerePLEX Biosystem for detection of multidrug-resistant tuberculosis (MDR-TB), obtaining a diagnostic accuracy of more than 97.8% compared to sequencing and MTBDRplus assay for Mycobacterium tuberculosis complex and rifampin

and isoniazid resistance detection on clinical isolates and smear-positive specimens. The speed, user-friendly interface, and versatility make it suitable for routine laboratory use.

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PMCID: PMC4652106 **Free PMC Article**
PMID: 26246486 [PubMed - indexed for MEDLINE]

5. **Assessing the effect of decentralisation of laboratory diagnosis for drug-resistant tuberculosis in Kenya.**

Sharma A[1], Musau S[2], Heilig CM[3], Okumu AO[2], Opiyo EO[2], Basiye FL[4], Miruka FO[4], Kioko JK[5], Sitienei JK[6], Cain KP[7].

Int J Tuberc Lung Dis. 2015 Nov;19(11):1348-53. doi: 10.5588/ijtld.15.0328.

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[7]Division of Tuberculosis Elimination, CDC, Kisumu, Kenya.

**ABSTRACT**

SETTING: Drug susceptibility testing (DST) is recommended in Kenya to identify multidrug-resistant tuberculosis (MDR-TB) in persons registered for tuberculosis (TB) retreatment. DST is performed at a central laboratory with a two-step growth-based process and a regional laboratory with a simultaneous molecular- and growth-based process.

OBJECTIVE: To compare proportions of retreatment cases who underwent DST and turnaround times for hospitals referring to the central vs. regional laboratory.

DESIGN: Cases were persons registered for TB retreatment from 1 January 2012 to 31 December 2013. Records of 11 hospitals and 7 hospitals referring patients to the regional and central laboratories, respectively, were reviewed.

RESULTS: Respectively 238/432 (55%) and 88/355 (25%) cases from hospitals referring to the regional and central laboratories underwent DST. The mean time from case registration to receipt of DST results and initiation of MDR-TB treatment was quicker in hospitals referring to the regional laboratory. The

time required for the transportation of specimens, specimen testing and receipt of DST results at hospitals was shorter for the regional laboratory ($P < 0.05$).

CONCLUSION: Testing was faster and more complete at hospitals referring to the regional laboratory. A comprehensive review of MDR-TB detection in Kenya is required to increase the proportion of cases receiving DST.

PMCID: PMC5017007 **Free PMC Article**
PMID: 26467587 [PubMed - indexed for MEDLINE]

6. **Transcriptional Adaptation of Drug-tolerant Mycobacterium tuberculosis During Treatment of Human Tuberculosis.**

Walter ND[1], Dolganov GM[2], Garcia BJ[3], Worodria W[4], Andama A[4], Musisi E[4], Ayakaka I[4], Van TT[2], Voskuil MI[5], de Jong BC[6], Davidson RM[7], Fingerlin TE[8], Kechris K[9], Palmer C[9], Nahid P[10], Daley CL[11], Geraci M[12], Huang L[13], Cattamanchi A[10], Strong M[7], Schoolnik GK[2], Davis JL[10].

J Infect Dis. 2015 Sep 15;212(6):990-8. doi: 10.1093/infdis/jiv149. Epub 2015 Mar 11.

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**ABSTRACT**

BACKGROUND: Treatment initiation rapidly kills most drug-susceptible Mycobacterium tuberculosis, but a bacterial subpopulation tolerates prolonged drug exposure. We evaluated drug-tolerant bacilli in

human sputum by comparing messenger RNA (mRNA) expression of drug-tolerant bacilli that survive the early bactericidal phase with treatment-naive bacilli.

METHODS: M. tuberculosis gene expression was quantified via reverse-transcription polymerase chain reaction in serial sputa from 17 Ugandans treated for drug-susceptible pulmonary tuberculosis.

RESULTS: Within 4 days, bacterial mRNA abundance declined >98%, indicating rapid killing. Thereafter, the rate of decline slowed >94%, indicating drug tolerance. After 14 days, 16S ribosomal RNA transcripts/genome declined 96%, indicating slow growth. Drug-tolerant bacilli displayed marked downregulation of genes associated with growth, metabolism, and lipid synthesis and upregulation in stress responses and key regulatory categories-including stress-associated sigma factors, transcription factors, and toxin-antitoxin genes. Drug efflux pumps were upregulated. The isoniazid stress signature was induced by initial drug exposure, then disappeared after 4 days.

CONCLUSIONS: Transcriptional patterns suggest that drug-tolerant bacilli in sputum are in a slow-growing, metabolically and synthetically downregulated state. Absence of the isoniazid stress signature in drug-tolerant bacilli indicates that physiological state influences drug responsiveness in vivo. These results identify novel drug targets that should aid in development of novel shorter tuberculosis treatment regimens.

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PMCID: PMC4548467 **Free PMC Article**
PMID: 25762787 [PubMed - indexed for MEDLINE]

7. **Tuberculosis retreatment 'others' in comparison with classical retreatment cases; a retrospective cohort review.**

Nabukenya-Mudiope MG[1], Kawuma HJ[2], Brouwer M[3], Mudiope P[4], Vassall A[5].

BMC Public Health. 2015 Sep 2;15:840. doi: 10.1186/s12889-015-2195-2.

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**ABSTRACT**

BACKGROUND: Many of the countries in sub-Saharan Africa are still largely dependent on microscopy as the mainstay for diagnosis of tuberculosis (TB) including patients with previous history of TB treatment. The available guidance in management of TB retreatment cases is focused on bacteriologically confirmed TB retreatment cases leaving out those classified as retreatment 'others'. Retreatment 'others' refer to all TB cases who were previously treated but with unknown outcome of that previous treatment or who have returned to treatment with bacteriologically negative pulmonary or extra-pulmonary TB. This study was conducted in 11 regional referral hospitals (RRHs) serving high burden TB districts in Uganda to determine the profile and treatment success of TB retreatment 'others' in comparison with the classical retreatment cases.

METHODS: A retrospective cohort review of routinely collected National TB and Leprosy Program (NTLP) facility data from 1 January to 31 December 2010. This study uses the term classical retreatment cases to refer to a combined group of bacteriologically confirmed relapse, return after failure and return after loss to follow-up cases as a distinct group from retreatment 'others'. Distribution of categorical characteristics were compared using Chi-squared test for difference between proportions. The log likelihood ratio test was used to assess the independent contribution of type of retreatment, human immunodeficiency virus (HIV) status, age group and sex to the models.

RESULTS: Of the 6244 TB cases registered at the study sites, 733 (11.7%) were retreatment cases. Retreatment 'others' constituted 45.5% of retreatment cases. Co-infection with HIV was higher among retreatment 'others' (70.9%) than classical retreatment cases (53.5%). Treatment was successful in 410 (56.2%) retreatment cases. Retreatment 'others' were associated with reduced odds of success (AOR = 0.44, 95% CI 0.22,0.88) compared to classical cases. Lost to follow up was the commonest adverse outcome (38% of adverse outcomes) in all retreatment cases. Type of retreatment case, HIV status, and age were independently associated with treatment success.

CONCLUSION: TB retreatment 'others' constitute a significant proportion of retreatment cases, with higher HIV prevalence and worse treatment success. There is need to review the diagnosis and management of retreatment 'others'.

PMCID: PMC4556407 **Free PMC Article**
PMID: 26330223 [PubMed - indexed for MEDLINE]

8. **Routine use of Xpert® MTB/RIF in areas with different prevalences of HIV and drug-resistant tuberculosis.**

Page AL[1], Ardizzoni E[2], Lassovsky M[3], Kirubi B[4], Bichkova D[5], Pedrotta A[6], Lastrucci C[7], de la Tour R[8], Bonnet M[1], Varaine F[7].

Int J Tuberc Lung Dis. 2015 Sep;19(9):1078-83, i-iii. doi: 10.5588/ijtld.14.0951.

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**ABSTRACT**

SETTING: Despite the widespread introduction of Xpert(®) MTB/RIF in developing countries, reports of its use and value in routine conditions remain limited.

OBJECTIVE: To describe Xpert results in relation to microscopy, treatment initiation, cost and workload under routine conditions at four sites in Cambodia, Georgia, Kenya and Swaziland.

DESIGN: Laboratory and clinical information on presumed TB patients were obtained from routine registers over a period of at least 6 months between March and November 2012.

RESULTS: Among the 6086 presumed TB patients included in the analysis, Xpert testing increased the number of biologically confirmed cases by 15% to 67% compared to microscopy. Up to 12% of the initial Xpert results were inconclusive. Between 56% and 83% of patients were started on treatment based on microscopy and/or Xpert results, with median delays of 1-16 days. Rifampicin resistance was detected in 3-19% of Xpert-positive patients.

CONCLUSION: Despite the additional numbers of cases detected by Xpert compared to microscopy, large proportions of patients are still started on treatment empirically in routine practice. Patient and specimen flow should be optimised to reduce delays in treatment initiation. Simple, non-sputum-based point-of-care tests with high sensitivity are needed to improve TB diagnosis and management.
PMID: 26260829 [PubMed - indexed for MEDLINE]

9. **Disseminated tuberculosis in an HIV-infected child: rifampicin resistance detected by GeneXpert in a lymph node aspirate but not in cerebrospinal fluid.**

Gamell A[1], Ntamatungiro AJ[2], Battegay M[3], Letang E[1].

BMJ Case Rep. 2015 Aug 3;2015. pii: bcr2014207997. doi: 10.1136/bcr-2014-207997.

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**ABSTRACT**

A 9-year-old HIV-infected child previously treated with inadequate doses of antitubercular drugs based on weight was admitted 5 months after initial tuberculosis (TB) diagnosis with acute hemiplegia and inguinal lymphadenopathies in a rural hospital in Tanzania. He was diagnosed with TB meningitis and lymphadenitis using Xpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) assay. Rifampicin resistance was detected in the lymph node aspirate but not in the cerebrospinal fluid. His TB therapy was optimised based on available medications and antiretroviral treatment was initiated 6 weeks later. Despite these efforts, the clinical evolution was poor and the child died 12 weeks after admission.

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PMID: 26240094 [PubMed - indexed for MEDLINE]

10. **Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis.**

Dawson R[1], Diacon AH[2], Everitt D[3], van Niekerk C[4], Donald PR[5], Burger DA[6], Schall R[6], Spigelman M[7], Conradie A[4], Eisenach K[8], Venter A[9], Ive P[10], Page-Shipp L[11], Variava E[12], Reither K[13], Ntinginya NE[14], Pym A[15], von Groote-Bidlingmaier F[16], Mendel CM[7].

Lancet. 2015 May 2;385(9979):1738-47. doi: 10.1016/S0140-6736(14)62002-X. Epub 2015 Mar 18.

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**Comment in:**

- New drug combination may shorten tuberculosis treatment, study says. [BMJ. 2015]
- New effective antituberculosis regimens. [Lancet. 2015]

**ABSTRACT**

BACKGROUND: New antituberculosis regimens are urgently needed to shorten tuberculosis treatment. Following on from favourable assessment in a 2 week study, we investigated a novel regimen for efficacy and safety in drug-susceptible and multidrug-resistant (MDR) tuberculosis during the first 8 weeks of treatment.

METHODS: We did this phase 2b study of bactericidal activity--defined as the decrease in colony forming units (CFUs) of Mycobacterium tuberculosis in the sputum of patients with microscopy smear-positive pulmonary tuberculosis-at eight sites in South Africa and Tanzania. We enrolled treatment-naive patients with drug-susceptible, pulmonary tuberculosis, who were randomly assigned by computer-generated sequences to receive either 8 weeks of moxifloxacin, 100 mg pretomanid (formerly known as PA-824), and pyrazinamide (MPa100Z regimen); moxifloxacin, 200 mg pretomanid, and pyrazinamide (MPa200Z regimen); or the current standard care for drug-susceptible pulmonary tuberculosis, isoniazid, rifampicin, PZA, and ethambutol (HRZE regimen). A group of patients with MDR tuberculosis received MPa200Z (DRMPa200Z group). The primary outcome was bactericidal activity measured by the mean daily rate of reduction in M tuberculosis CFUs per mL overnight sputum collected once a week, with joint Bayesian non-linear mixed-effects regression modelling. We also assessed safety and tolerability by monitoring adverse events. This study is registered with ClinicalTrials.gov, number NCT01498419.

FINDINGS: Between March 24, 2012, and July 26, 2013 we enrolled 207 patients and randomly assigned them to treatment groups; we assigned 60 patients to the MPa100Z regimen, 62 to the MPa200Z regimen, and 59 to the HRZE regimen. We non-randomly assigned 26 patients with drug-resistant tuberculosis to the DRMPa200Z regimen. In patients with drug-susceptible tuberculosis, the bactericidal activity of MPa200Z (n=54) on days 0-56 (0·155, 95% Bayesian credibility interval 0·133-

0·178) was significantly greater than for HRZE (n=54, 0·112, 0·093-0·131). DRMPa200Z (n=9) had bactericidal activity of 0·117 (0·070-0·174). The bactericidal activity on days 7-14 was strongly associated with bactericidal activity on days 7-56. Frequencies of adverse events were similar to standard treatment in all groups. The most common adverse event was hyperuricaemia in 59 (29%) patients (17 [28%] patients in MPa100Z group, 17 [27%] patients in MPa200Z group, 17 [29%] patients. in HRZE group, and 8 [31%] patients in DRMPa200Z group). Other common adverse events were nausea in (14 [23%] patients in MPa100Z group, 8 [13%] patients in MPa200Z group, 7 [12%] patients in HRZE group, and 8 [31%] patients in DRMPa200Z group) and vomiting (7 [12%] patients in MPa100Z group, 7 [11%] patients in MPa200Z group, 7 [12%] patients in HRZE group, and 4 [15%] patients in DRMPa200Z group). No on-treatment electrocardiogram occurrences of corrected QT interval more than 500 ms (an indicator of potential of ventricular tachyarrhythmia) were reported. No phenotypic resistance developed to any of the drugs in the regimen.

INTERPRETATION: The combination of moxifloxacin, pretomanid, and pyrazinamide, was safe, well tolerated, and showed superior bactericidal activity in drug-susceptible tuberculosis during 8 weeks of treatment. Results were consistent between drug-susceptible and MDR tuberculosis. This new regimen is ready to enter phase 3 trials in patients with drug-susceptible tuberculosis and MDR-tuberculosis, with the goal of shortening and simplifying treatment.

FUNDING: Global Alliance for TB Drug Development.

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11. **Multi drug and other forms of drug resistant tuberculosis are uncommon among treatment naïve tuberculosis patients in Tanzania.**

Nagu TJ[1], Aboud S[2], Mwiru R[3], Matee M[2], Fawzi W[4], Mugusi F[5].

PLoS One. 2015 Apr 7;10(4):e0118601. doi: 10.1371/journal.pone.0118601. eCollection 2015.

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**ABSTRACT**

BACKGROUND: Surveillance and effective management of drug resistance is important to sustaining tuberculosis (TB) control efforts. We aimed to determine resistance rates to first line anti tuberculosis drugs and to describe factors associated with the resistance to any of the first line anti tuberculosis drugs in Dar es Salaam Tanzania.

MATERIALS: Newly diagnosed, TB patients with neither history of tuberculosis treatment nor isoniazid prophylaxis were included into the study. Sputum specimens were cultured on either mycobacteria growth indicator tube 960 (MGIT 960) or Lowenstein Jenstein (LJ) medium supplemented with either glycerol (GLJ) or pyruvate (PLJ). Drug susceptibility for isoniazid, rifampicin, streptomycin and ethambutol was determined by either Lowenstein-Jensen (LJ) medium or mycobacteria growth indicator tube 960 (MGIT 960).

RESULTS: A total of 933 newly diagnosed TB patients, were included into the study. Multi drug resistance (MDR) tuberculosis was detected among 2 (0.2%) patients. Resistance to any of the four tested drugs was detected among 54 (5.8%) patients. Mono-resistance to isoniazid, rifampicin, streptomycin and ethambutol were 21(2.3%), 3 (0.3%), 13 (1.4%), 9 (1.0%) respectively.

CONCLUSION: Primary resistance to first line anti tuberculosis drugs is still low in this setting. Continued vigilance including periodic national surveillance of anti-tuberculosis resistance is recommended.

PMCID: PMC4388561 **Free PMC Article**
PMID: 25849784 [PubMed - indexed for MEDLINE]

12. **Improvement in plasma drug activity during the early treatment interval among Tanzanian patients with multidrug-resistant tuberculosis.**

Ndusilo ND[1], Heysell SK[2], Mpagama SG[3], Gratz J[4], Segesela FH[1], Pazia SJ[1], Wang XQ[5], Houpt ER[6], Kibiki GS[1].

PLoS One. 2015 Mar 27;10(3):e0122769. doi: 10.1371/journal.pone.0122769. eCollection 2015.

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**ABSTRACT**

BACKGROUND: Individual pharmacokinetic variability may be common in patients treated for multidrug-resistant tuberculosis (MDR-TB) but data are sparse from resource-limited settings and across the early treatment interval.

METHODS: Plasma drug activity, as measured by the TB Drug Activity (TDA) assay at 2 and 4 weeks of treatment with a standardized MDR-TB regimen was performed in patients with pulmonary MDR-TB from Tanzania. TDA values were correlated with measures of early treatment outcome including every two week collection of sputum for time-to-positivity (TTP) in liquid culture from the MGIT 960 automated system. Patients were evaluated at 24 weeks and those surviving without delayed sputum culture conversion (>8 weeks), culture reversion after previously negative, or weight loss were defined as having a favorable outcome.

RESULTS: Twenty-five patients were enrolled with a mean age of 37 ±12 years. All were culture positive from the pretreatment sputum sample with a mean TTP in MGIT of 257 ±134 hours, and the median time to culture conversion on treatment was 6 weeks. Twenty patients (80%) had an increase in TDA, with the overall mean TDA at 2 weeks of 2.1 ±0.7 compared to 2.4 ±0.8 at 4 weeks (p = 0.005). At 2 weeks 13 subjects (52%) had a TDA value > 2-log killing against their own M. tuberculosis isolate compared to 17 subjects (68%) at 4 weeks (McNemar's exact test p = 0.29). An interim treatment outcome was able to be determined in 23 patients (92%), of whom 7 had a poor outcome (30%). An increase in TDA from week 2 to week 4 was associated with favorable outcome, [unadjusted OR = 20.0, 95% CI: 1.61-247.98, exact p = 0.017 and adjusted OR = 19.33, 95% CI: 1.55-241.5, exact p = 0.023].

CONCLUSIONS: The majority of patients with MDR-TB in Tanzania had an increase in plasma drug activity from week 2 to week 4 of treatment as measured by the TDA assay. Understanding the etiology and full impact of this dynamic may inform therapeutic intervention.

PMCID: PMC4376785 **Free PMC Article**
PMID: 25816161 [PubMed - indexed for MEDLINE]

13. **The movement of multidrug-resistant tuberculosis across borders in East Africa needs a regional and global solution.**

Cain KP[1], Marano N[1], Kamene M[2], Sitienei J[2], Mukherjee S[3], Galev A[4], Burton J[5], Nasibov O[5], Kioko J[2], De Cock KM[1].

PLoS Med. 2015 Feb 24;12(2):e1001791. doi: 10.1371/journal.pmed.1001791. eCollection 2015.

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**ABSTRACT**

Kevin Cain and colleagues reflect on the cross border movement of people from Somalia with MDR-TB and the implications for MDR-TB programs in East Africa.

PMCID: PMC4339836 **Free PMC Article**
PMID: 25710472 [PubMed - indexed for MEDLINE]

14. **Low resistance to first and second line anti-tuberculosis drugs among treatment naive pulmonary tuberculosis patients in southwestern Uganda.**

Orikiriza P[1], Tibenderana B[2], Siedner MJ[3], Mueller Y[4], Byarugaba F[5], Moore CC[6], Evans EE[7], Bonnet M[8], Page AL[8], Bazira J[5], Boum Y 2nd[1].

PLoS One. 2015 Feb 6;10(2):e0118191. doi: 10.1371/journal.pone.0118191. eCollection 2015.

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**ABSTRACT**

BACKGROUND: There are limited data on region-specific drug susceptibility of tuberculosis (TB) in Uganda. We performed resistance testing on specimens collected from treatment-naive patients with pulmonary TB in Southwestern Uganda for first and second line anti-TB drugs. We sought to provide data to guide regional recommendations for empiric TB therapy.

METHODS: Archived isolates, obtained from patients at Mbarara Regional Referral Hospital from February 2009 to February 2013, were tested for resistance to isoniazid and rifampicin using the MTBDRplus and Xpert MTB/RIF assays. A subset of randomly selected isolates was tested for second line agents, including fluoroquinolones (FQs), aminoglycosides, cyclic peptides, and ethambutol using the MTBDRsl assay. We performed confirmatory testing for FQ resistance using repeated MTBDRsl, the Mycobacteria growth indicator tube (MGIT) assay, and sequencing of the gyrA and gyrB genes.

RESULTS: We tested isolates from 190 patients. The cohort had a median age of 33 years (IQR 26-43), 69% (131/190) were male, and the HIV prevalence was 42% (80/190). No isolates (0/190) were rifampicin-resistant and only 1/190 (0.5%) was isoniazid-resistant. Among 92 isolates tested for second-line drug resistance, 71 (77%) had interpretable results, of which none were resistant to aminoglycosides, cyclic peptides or ethambutol. Although 7 (10%) initially tested as resistant to FQs by the MTBDRsl assay, they were confirmed as susceptible by repeat MTBDRsl testing as well as by MGIT and gyrase gene sequencing.

CONCLUSION: We found no MDR-TB and no resistance to ethambutol, FQs, or injectable anti-TB drugs in treatment naïve patients with pulmonary TB in Southwestern Uganda. Standard treatment guidelines for susceptible TB should be adequate for most patients with TB in this population. Where possible, molecular susceptibility testing methods should be routinely validated by culture methods.

PMCID: PMC4320102 **Free PMC Article**
PMID: 25658921 [PubMed - indexed for MEDLINE]

15. **The Mycobacterium tuberculosis Uganda II family and resistance to first-line anti-tuberculosis drugs in Uganda.**

Ezati N[1], Lukoye D[2], Wampande EM[3,4], Musisi K[5], Kasule GW[6], Cobelens FG[7], Kateete DP[8], Joloba ML[9,10].

BMC Infect Dis. 2014 Dec 19;14:703. doi: 10.1186/s12879-014-0703-0.

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**ABSTRACT**

BACKGROUND: The global increase in the burden of multidrug-resistant tuberculosis (MDR-TB) underscores an urgent need for data on factors involved in generation and spread of TB drug resistance. We performed molecular analyses on a representative sample of Mycobacterium tuberculosis (MTB) isolates. Basing on findings of the molecular epidemiological study in Kampala, we hypothesized that the predominant MTB strain lineage in Uganda is negatively associated with anti-TB drug resistance and we set out to test this hypothesis.

METHODS: We extracted DNA from mycobacterial isolates collected from smear-positive TB patients in the national TB drug resistance survey and carried out IS6110-PCR. To identify MTB lineages/sub lineages RT-PCR SNP was performed using specific primers and hybridization probes and the 'melting curve' analysis was done to distinguish the Uganda II family from other MTB families. The primary outcome was the distribution of the Uganda II family and its associations with anti-TB drug resistance and HIV infection.

RESULTS: Out of the 1537 patients enrolled, MTB isolates for 1001 patients were available for SNP analysis for identification of Uganda II family, of which 973 (97%) had conclusive RT-PCR results. Of these 422 (43.4%) were of the Uganda II family, mostly distributed in the south west zone (55.0%; OR = 4.6 for comparison with other zones; 95% CI 2.83-7.57; $p < 0.001$) but occurred in each of the other seven geographic zones at varying levels. Compared to the Uganda II family, other genotypes as a group were more likely to be resistant to any anti-TB drug (OR(adj) =2.9; 95% CI 1.63-5.06; p = 0.001) or MDR (OR(adj) 4.9; 95% CI, 1.15-20.60; p = 0.032), even after adjusting for geographic zone, patient category, sex, residence and HIV status. It was commonest in the 25-34 year age group 159/330 (48.2%). No association was observed between Uganda II family and HIV infection.

CONCLUSION: The Uganda II family is a major cause of morbidity due to TB in all NTLP zones in Uganda. It is less likely to be resistant to anti-TB drugs than other MTB strain lineages.

PMCID: PMC4367914 **Free PMC Article**
PMID: 25523472 [PubMed - indexed for MEDLINE]

16. **Detection and management of drug-resistant tuberculosis in HIV-infected patients in lower-income countries.**

Ballif M, Nhandu V, Wood R, Dusingize JC, Carter EJ, Cortes CP, McGowan CC, Diero L, Graber C, Renner L, Hawerlander D, Kiertiburanakul S, Du QT, Sterling TR, Egger M, Fenner L; International epidemiological Databases to Evaluate AIDS (IeDEA).

Int J Tuberc Lung Dis. 2014 Nov;18(11):1327-36. doi: 10.5588/ijtld.14.0106.

Collaborators: (383)
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Bassabi CC, Sy HS, Ba A, Diagne A, Dior H, Faye M, Gueye RD, Mbaye AD, Albert CH, Patassi A, Kotosso A, Kariyare BG, Gbadamassi G, Komi A, Mensah-Zukong KE, Pakpame P, Lawson-Evi AK, Atakouma Y, Takassi E, Djeha A, Ephoévi-gah A, Djibril Sel-H, Dabis F, Bissagnene E, Arrive E, Coffie P, Ekouevi D, Jaquet A, Leroy V, Lewden C, Sasco AJ, Amani D, Azani JC, Balestre E, Bessekon S, Bohossou F, Gilbert C, Karcher S, Gonsan JM, Carrou JL, Lenaud S, Nchot C, Malateste K, Yao AR, Siloue B, Clouet G, Dosso M, Doring A, Kouakou A, Rabourdin E, Rivenc J, Anglaret X, Ba B, Essanin JB, Ciaranello A, Datte S, Desmonde S, Elvis JS, Gottlieb GS, GHoro A, Kangah SN, Malvy D, Meless D, Mounkaila- Harouna A, Ndondoki C, Shiboski C, Tchounga B, Thiébaut R, Wandeler G, McGowan C, Cahn P, Gotuzzo E, Grinsztejn B, Pape J, Padgett D, Madero JS.

**ABSTRACT**

SETTING: Drug resistance threatens tuberculosis (TB) control, particularly among human immunodeficiency virus (HIV) infected persons.

OBJECTIVE: To describe practices in the prevention and management of drug-resistant TB under antiretroviral therapy (ART) programs in lower-income countries.

DESIGN: We used online questionnaires to collect program-level data on 47 ART programs in Southern Africa (n = 14), East Africa (n = 8), West Africa (n = 7), Central Africa (n = 5), Latin America (n = 7) and the Asia-Pacific (n = 6 programs) in 2012. Patient-level data were collected on 1002 adult TB patients seen at 40 of the participating ART programs.

RESULTS: Phenotypic drug susceptibility testing (DST) was available in 36 (77%) ART programs, but was only used for 22% of all TB patients. Molecular DST was available in 33 (70%) programs and was used in 23% of all TB patients. Twenty ART programs (43%) provided directly observed therapy (DOT) during the entire course of treatment, 16 (34%) during the intensive phase only, and 11 (23%) did not follow DOT. Fourteen (30%) ART programs reported no access to second-line anti-tuberculosis regimens; 18 (38%) reported TB drug shortages.

CONCLUSIONS: Capacity to diagnose and treat drug-resistant TB was limited across ART programs in lower-income countries. DOT was not always implemented and drug supplies were regularly interrupted, which may contribute to the global emergence of drug resistance.

PMCID: PMC4323497 **Free PMC Article**
PMID: 25299866 [PubMed - indexed for MEDLINE]

17. **Rifampicin resistance mutations in the 81 bp RRDR of rpoB gene in Mycobacterium tuberculosis clinical isolates using Xpert® MTB/RIF in Kampala, Uganda: a retrospective study.**

Mboowa G[1], Namaganda C, Ssengooba W.

BMC Infect Dis. 2014 Sep 4;14:481. doi: 10.1186/1471-2334-14-481.

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**ABSTRACT**

BACKGROUND: Introduction of Xpert® MTB/RIF assay has revolutionalised the diagnosis of tuberculosis (TB) by simultaneously detecting the bacteria and resistance to rifampicin (rif), a surrogate marker for multi-drug resistant TB (MDR-TB) as well as one of the principal first-line anti-tuberculosis drugs. In general, rpoB mutations can be found in 96.1% of rif-resistant Mycobacterium tuberculosis (MTB) strains worldwide and these mutations usually are located in a region at the 507-533rd amino acid residuals (81 bp) in the MTB rpoB gene, which is referred to as Rifampicin-resistance-determining region (RRDR). In this study, we determined the frequency of MDR-TB in Kampala using Xpert® MTB/RIF in comparison with the agar proportion method using Middlebrook 7H11and further determined the frequency of probes for different rpoB gene mutations using Xpert® MTB/RIF assay in the 81 bp RRDR.

METHODS: A total of 1501 specimens received at Mycobacteriology laboratory, Makerere University for Xpert testing between May 2011 and May 2014 were analysed by Xpert® MTB/RIF assay. Specimens that were positive for both MTB and rifampicin resistance were further subjected to a complete first line anti-mycobacterial drug susceptibility testing using Middlebrook 7H11 agar proportion method (APM).

RESULTS: Xpert® MTB/RIF assay detected 313 MTB positive specimens and out of which 12 specimens had both MTB and rifampicin- resistance conferred by four different rpoB gene mutations in the 81 bp-RRDR of MTB, further one (1/12), specimen was found to be rifampicin mono-resistant on APM while the 11 were found to be MDR-TB. Probes associated with the observed rif- resistance were as follows: E (7/12), B (3/12), A (1/12), D (1/12) and no rif-resistance was associated with probe C. No specimen yielded rif-resistance associated with more than one probe failure (mutation combinations). Probe D was associated with rifampicin mono-resistant.

CONCLUSIONS: MDR-TB was at 3.5% in the studied population. Mutations associated with Probe E (58%) also known as codons 531and 533 are the commonest rpoB gene mutation identified by Xpert® MTB/RIF assay in this setting and mutations identified by probe E of the assay, turned out to be MDR-TB strains by agar proportion method antimicrobial susceptibility testing. No mutation was detected in the codon 522.

PMCID: PMC4164707 **Free PMC Article**
PMID: 25190040 [PubMed - indexed for MEDLINE]

18. **The T2 Mycobacterium tuberculosis genotype, predominant in Kampala, Uganda, shows negative correlation with antituberculosis drug resistance.**

Lukoye D[1], Katabazi FA[2], Musisi K[3], Kateete DP[2], Asiimwe BB[2], Okee M[2], Joloba ML[4], Cobelens FG[5].

Antimicrob Agents Chemother. 2014 Jul;58(7):3853-9. doi: 10.1128/AAC.02338-13. Epub 2014 Apr 28.

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**ABSTRACT**

Surveillance of the circulating Mycobacterium tuberculosis complex (MTC) strains in a given locality is important for understanding tuberculosis (TB) epidemiology. We performed molecular epidemiological studies on sputum smear-positive isolates that were collected for anti-TB drug resistance surveillance to establish the variability of MTC lineages with anti-TB drug resistance and HIV infection. Spoligotyping was performed to determine MTC phylogenetic lineages. We compared patients' MTC lineages with drug susceptibility testing (DST) patterns and HIV serostatus. Out of the 533 isolates, 497 (93.2%) had complete DST, PCR, and spoligotyping results while 484 (90.1%) participants had results for HIV testing. Overall, the frequency of any resistance was 75/497 (15.1%), highest among the LAM (34.4%; 95% confidence interval [CI], 18.5 to 53.2) and lowest among the T2 (11.5%; 95% CI, 7.6 to 16.3) family members. By multivariate analysis, LAM (adjusted odds ratio [OR(adj)], 5.0; 95% CI, 2.0 to 11.9; P < 0.001) and CAS (OR(adj), 2.9; 95% CI, 1.4.0 to 6.3; P = 0.006) families were more likely to show any resistance than was T2. All other MTC lineages combined were more likely to be resistant to any of the anti-TB drugs than were the T2 strains (OR(adj), 1.7; 95% CI, 1.0 to 2.9; P = 0.040). There were no significant associations between multidrug resistance and MTC lineages, but numbers of multidrug-resistant TB strains were small. No association was established between MTC lineages and HIV status. In conclusion, the T2 MTC lineage negatively correlates with anti-TB drug resistance, which might partly explain the reported low levels of anti-TB drug resistance in Kampala, Uganda. Patients' HIV status plays no role with respect to the MTC lineage distribution.

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PMCID: PMC4068514 **Free PMC Article**
PMID: 24777100 [PubMed - indexed for MEDLINE]

19. **Clinical and epidemiological characteristics of individuals resistant to M. tuberculosis infection in a longitudinal TB household contact study in Kampala, Uganda.**

Ma N, Zalwango S, Malone LL, Nsereko M, Wampande EM, Thiel BA, Okware B, Igo RP Jr, Joloba ML, Mupere E, Mayanja-Kizza H, Boom WH, Stein CM[1]; Tuberculosis Research Unit (TBRU).

BMC Infect Dis. 2014 Jun 27;14:352. doi: 10.1186/1471-2334-14-352.

[1]Department of Epidemiology & Biostatistics, Case Western Reserve University, 2103 Cornell Rd, Wolstein Research Building room 1316, Cleveland, OH 44106, USA. catherine.stein@case.edu.

**ABSTRACT**

BACKGROUND: Despite sustained exposure to a person with pulmonary tuberculosis (TB), some M. tuberculosis (Mtb) exposed individuals maintain a negative tuberculin skin test (TST). Our objective was to characterize these persistently negative TST (PTST-) individuals and compare them to TST converters (TSTC) and individuals who are TST positive at study enrollment.

METHODS: During a TB household contact study in Kampala, Uganda, PTST-, TSTC, and TST + individuals were identified. PTST- individuals maintained a negative TST over a 2 year observation period despite prolonged exposure to an infectious tuberculosis (TB) case. Epidemiological and clinical characteristics were compared, a risk score developed by another group to capture risk for Mtb infection was computed, and an ordinal regression was performed.

RESULTS: When analyzed independently, epidemiological risk factors increased in prevalence from PTST- to TSTC to TST+. An ordinal regression model suggested age ($p < 0.01$), number of windows ($p < 0.01$) and people ($p = 0.07$) in the home, and sleeping in the same room ($p < 0.01$) were associated with PTST- and TSTC. As these factors do not exist in isolation, we examined a risk score, which reflects an accumulation of risk factors. This compound exposure score did not differ significantly between PTST-, TSTC, and TST+, except for the 5-15 age group ($p = 0.009$).

CONCLUSIONS: Though many individual factors differed across all three groups, an exposure risk score reflecting a collection of risk factors did not differ for PTST-, TSTC and TST + young children and adults. This is the first study to rigorously characterize the epidemiologic risk profile of individuals with persistently negative TSTs despite close exposure to a person with TB. Additional studies are needed to characterize possible epidemiologic and host factors associated with this phenotype.

PMCID: PMC4091673 **Free PMC Article**
PMID: 24970328 [PubMed - indexed for MEDLINE]

20. **"Home is where the patient is": a qualitative analysis of a patient-centred model of care for multi-drug resistant tuberculosis.**

Horter S[1], Stringer B, Reynolds L, Shoaib M, Kasozi S, Casas EC, Verputten M, du Cros P.

BMC Health Serv Res. 2014 Feb 21;14:81. doi: 10.1186/1472-6963-14-81.

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**ABSTRACT**

BACKGROUND: Ambulatory, community-based care for multi-drug resistant tuberculosis (MDR-TB) has been found to be effective in multiple settings with high cure rates. However, little is known about patient preferences around models of MDR-TB care. Médecins Sans Frontières (MSF) has delivered home-based MDR-TB treatment in the rural Kitgum and Lamwo districts of northern Uganda since 2009 in collaboration with the Ministry of Health and the National TB and Leprosy Programme. We conducted a qualitative study examining the experience of patients and key stakeholders of home-based MDR-TB treatment.

METHODS: We used semi-structured interviews and focus-group discussions to examine patients' perceptions, views and experiences of home-based treatment and care for MDR-TB versus their perceptions of care in hospital. We identified how these perceptions interacted with those of their families and other stakeholders involved with TB. Participants were selected purposively following a stakeholder analysis. Sample size was determined by data saturation being reached within each identified homogenous category of respondents: health-care receiving, health-care providing and key informant. Iterative data collection and analysis enabled adaptation of topic guides and testing of emerging themes. The grounded theory method of analysis was applied, with data, codes and categories being continually compared and refined.

RESULTS: Several key themes emerged: the perceived preference and acceptability of home-based treatment and care as a model of MDR-TB treatment by patients, family, community members and health-care workers; the fear of transmission of other infections within hospital settings; and the identification of MDR-TB developing through poor adherence to and inadequate treatment regimens for DS-TB.

CONCLUSIONS: Home-based treatment and care was acceptable to patients, families, communities and health-care workers and was seen as preferable to hospital-based care by most respondents. Home-based care was perceived as safe, conducive to recovery, facilitating psychosocial support and allowing more free time and earning potential for patients and caretakers. These findings could contribute to development of an adaptation of treatment approach strategy at national level.

PMCID: PMC3943511 **Free PMC Article**
PMID: 24559177 [PubMed - indexed for MEDLINE]

21. **Plasma drug activity in patients on treatment for multidrug-resistant tuberculosis.**

Mpagama SG[1], Ndusilo N, Stroup S, Kumburu H, Peloquin CA, Gratz J, Houpt ER, Kibiki GS, Heysell SK.

Antimicrob Agents Chemother. 2014;58(2):782-8. doi: 10.1128/AAC.01549-13. Epub 2013 Nov 18.

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**ABSTRACT**

Little is known about plasma drug concentrations relative to quantitative susceptibility in patients with multidrug-resistant tuberculosis (MDR-TB). We previously described a TB drug activity (TDA) assay that determines the ratio of the time to detection of plasma-cocultured Mycobacterium tuberculosis versus control growth in a Bactec MGIT system. Here, we assess the activity of individual drugs in a typical MDR-TB regimen using the TDA assay. We also examined the relationship of the TDA to the drug concentration at 2 h (C2) and the MICs among adults on a MDR-TB regimen in Tanzania. These parameters were also compared to the treatment outcome of sputum culture conversion. Individually, moxifloxacin yielded superior TDA results versus ofloxacin, and only moxifloxacin and amikacin yielded TDAs equivalent to a -2-log killing. In the 25 patients enrolled on a regimen of kanamycin, levofloxacin, ethionamide, pyrazinamide, and cycloserine, the C2 values were found to be below the expected range for levofloxacin in 13 (52%) and kanamycin in 10 (40%). Three subjects with the lowest TDA result (<1.5, a finding indicative of poor killing) had significantly lower kanamycin C2/MIC ratios than subjects with a TDA of ≥1.5 (9.8 ± 8.7 versus 27.0 ± 19.1; P = 0.04). The mean TDAs were 2.52 ± 0.76 in subjects converting to negative in ≤2 months and 1.88 ± 0.57 in subjects converting to negative in >2 months (P = 0.08). In Tanzania, MDR-TB drug concentrations were frequently low, and a wide concentration/MIC range was observed that affected plasma drug activity ex vivo. An opportunity exists for pharmacokinetic optimization in current MDR-TB regimens, which may improve treatment response.

PMCID: PMC3910816 **Free PMC Article**
PMID: 24247125 [PubMed - indexed for MEDLINE]

22. **Epidemiology and genetic diversity of multidrug-resistant tuberculosis in East Africa.**

Kidenya BR[1], Webster LE[2], Behan S[2], Kabangila R[3], Peck RN[3], Mshana SE[4], Ocheretina O[5], Fitzgerald DW[2].
Tuberculosis (Edinb). 2014 Jan;94(1):1-7. doi: 10.1016/j.tube.2013.08.009. Epub 2013 Sep 7.

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**ABSTRACT**

Multidrug-resistant tuberculosis (MDR-TB) is an emerging problem in many parts of the world, and levels of MDR-TB among new TB patients are increasing in sub-Saharan Africa. We reviewed the prevalence and molecular epidemiology of MDR-TB in East Africa, including Burundi, Kenya, Rwanda, Tanzania, and Uganda. In 16 epidemiologic surveys, the prevalence of MDR among new cases ranges from 0.4% in Tanzania to 4.4% in Uganda, and among recurrent cases ranges from 3.9% in Tanzania to 17.7% in Uganda. There is a gap of 5948 cases between the estimated number of MDR-TB cases in East Africa and the number actually diagnosed. The only confirmed risk factors for MDR-TB are prior treatment for TB and refugee status. HIV has not been reported as a risk factor, and there are no reports of statistical association between spoligotype and drug resistance pattern. Increased capacity for diagnosis and treatment of MDR-TB is needed, with an emphasis on recurrent TB cases and refugees.

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PMCID: PMC3877177 **Free PMC Article**
PMID: 24215798 [PubMed - indexed for MEDLINE]

23. **Elucidating emergence and transmission of multidrug-resistant tuberculosis in treatment experienced patients by whole genome sequencing.**

Clark TG[1], Mallard K[2], Coll F[2], Preston M[3], Assefa S[4], Harris D[5], Ogwang S[6], Mumbowa F[7], Kirenga B[8], O'Sullivan DM[2], Okwera A[8], Eisenach KD[9], Joloba M[10], Bentley SD[5], Ellner JJ[11], Parkhill J[5], Jones-López EC[11], McNerney R[2].

PLoS One. 2013 Dec 11;8(12):e83012. doi: 10.1371/journal.pone.0083012. eCollection 2013.

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**ABSTRACT**

BACKGROUND: Understanding the emergence and spread of multidrug-resistant tuberculosis (MDR-TB) is crucial for its control. MDR-TB in previously treated patients is generally attributed to the selection of drug resistant mutants during inadequate therapy rather than transmission of a resistant strain. Traditional genotyping methods are not sufficient to distinguish strains in populations with a high burden of tuberculosis and it has previously been difficult to assess the degree of transmission in these settings. We have used whole genome analysis to investigate M. tuberculosis strains isolated from treatment experienced patients with MDR-TB in Uganda over a period of four years.

METHODS AND FINDINGS: We used high throughput genome sequencing technology to investigate small polymorphisms and large deletions in 51 Mycobacterium tuberculosis samples from 41 treatment-experienced TB patients attending a TB referral and treatment clinic in Kampala. This was a convenience sample representing 69% of MDR-TB cases identified over the four year period. Low polymorphism was observed in longitudinal samples from individual patients (2-15 SNPs). Clusters of samples with less than 50 SNPs variation were examined. Three clusters comprising a total of 8 patients were found with almost identical genetic profiles, including mutations predictive for resistance to rifampicin and isoniazid, suggesting transmission of MDR-TB. Two patients with previous drug susceptible disease were found to have acquired MDR strains, one of which shared its genotype with an isolate from another patient in the cohort.

CONCLUSIONS: Whole genome sequence analysis identified MDR-TB strains that were shared by more than one patient. The transmission of multidrug-resistant disease in this cohort of retreatment patients emphasises the importance of early detection and need for infection control. Consideration should be

given to rapid testing for drug resistance in patients undergoing treatment to monitor the emergence of resistance and permit early intervention to avoid onward transmission.

PMCID: PMC3859632 **Free PMC Article**
PMID: 24349420 [PubMed - indexed for MEDLINE]

24. **Application of quantitative second-line drug susceptibility testing at a multidrug-resistant tuberculosis hospital in Tanzania.**

Mpagama SG[1], Houpt ER, Stroup S, Kumburu H, Gratz J, Kibiki GS, Heysell SK.

BMC Infect Dis. 2013 Sep 14;13:432. doi: 10.1186/1471-2334-13-432.

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**ABSTRACT**

BACKGROUND: Lack of rapid and reliable susceptibility testing for second-line drugs used in the treatment of multidrug-resistant tuberculosis (MDR-TB) may limit treatment success.

METHODS: Mycobacterium tuberculosis isolates from patients referred to Kibong'oto National TB Hospital in Tanzania for second-line TB treatment underwent confirmatory speciation and susceptibility testing. Minimum inhibitory concentration (MIC) testing on MYCOTB Sensititre plates was performed for all drugs available in the second-line formulary. We chose to categorize isolates as borderline susceptible if the MIC was at or one dilution lower than the resistance breakpoint. M. tuberculosis DNA was sequenced for resistance mutations in rpoB (rifampin), inhA (isoniazid, ethionamide), katG (isoniazid), embB (ethambutol), gyrA (fluoroquinolones), rrs (amikacin, kanamycin, capreomycin), eis (kanamycin) and pncA (pyrazinamide).

RESULTS: Of 22 isolates from patients referred for second-line TB treatment, 13 (59%) were MDR-TB and the remainder had other resistance patterns. MIC testing identified 3 (14%) isolates resistant to ethionamide and another 8 (36%) with borderline susceptibility. No isolate had ofloxacin resistance, but 10 (45%) were borderline susceptible. Amikacin was fully susceptible in 15 (68%) compared to only 11 (50%) for kanamycin. Resistance mutations were absent in gyrA, rrs or eis for all 13 isolates available for sequencing, but pncA mutation resultant in amino acid change or stop codon was present in 6 (46%). Ten (77%) of MDR-TB patients had at least one medication that could have logically been modified based on these results (median 2; maximum 4). The most common modifications were a change from ethioniamide to para-aminosalicylic acid, and the use of higher dose levofloxacin.

CONCLUSIONS: In Tanzania, quantitative second-line susceptibility testing could inform and alter MDR-TB management independent of drug-resistance mutations. Further operational studies are warranted.

PMCID: PMC3848720 **Free PMC Article**
PMID: 24034230 [PubMed - indexed for MEDLINE]

25. **Multidrug- and isoniazid-resistant tuberculosis in three high HIV burden African regions.**

Sanchez-Padilla E[1], Ardizzoni E, Sauvageot D, Ahoua L, Martin A, Varaine F, Adatu-Engwau F, Akeche G, Salaniponi F, Bonnet M.

Int J Tuberc Lung Dis. 2013 Aug;17(8):1036-42. doi: 10.5588/ijtld.12.0842.

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**ABSTRACT**

SETTING: Despite major progress in the surveillance of drug-resistant tuberculosis (TB), data are lacking for many low-resource countries. World Health Organization estimates of multidrug-resistant TB (MDR-TB) rates in Africa are low, and based on very limited data from the African continent.

OBJECTIVE: To measure MDR-TB prevalence in sub-Saharan African regions with a high prevalence of human immunodeficiency virus (HIV).

METHOD: We conducted three anti-tuberculosis drug resistance surveys in sub-Saharan African regions with high HIV-TB coinfection prevalence: Homa Bay (Kenya), Chiradzulu (Malawi) and West Nile region (Uganda).

RESULTS: The prevalence of MDR-TB in new patients was found to be low in the three regions: 1.4% (95%CI 0.2-2.6) in Homa Bay, 2.0% (95%CI 0.4-3.6) in Chiradzulu and 0.6% (95%CI 0.0-1.5) in the West Nile region. We found no significant association between MDR-TB and HIV infection. Nonetheless, ≥ 10% of the new cases surveyed were resistant to isoniazid (INH).

CONCLUSION: The relatively high rate of resistance to INH highlights the need for rapid detection of INH resistance in addition to rifampicin (RMP) resistance, to allow rapid modification of treatment to avoid the acquisition of RMP resistance. Drug resistance should be monitored periodically.
PMID: 23827027 [PubMed - indexed for MEDLINE]

26. **Universal access to care for multidrug-resistant tuberculosis: an analysis of surveillance data.**

Falzon D[1], Jaramillo E, Wares F, Zignol M, Floyd K, Raviglione MC.

Lancet Infect Dis. 2013 Aug;13(8):690-7. doi: 10.1016/S1473-3099(13)70130-0. Epub 2013 Jun 4.

[1]Stop TB Department, World Health Organization, Geneva, Switzerland. falzond@who.int

**ABSTRACT**

BACKGROUND: The prospects for global tuberculosis control in the near future will be determined by the effectiveness of the response of countries to their burden of multidrug-resistant (MDR; resistance to, at least, isoniazid and rifampicin) tuberculosis. During the 2009 World Health Assembly, countries committed to achieve universal access to MDR-tuberculosis care by 2015. We assessed the progress towards the 2015 targets achieved by countries accounting for 90% of the estimated MDR-tuberculosis cases in the world in 2011.

METHODS: We analysed data reported to WHO by 30 countries expected to have more than 1000 MDR-tuberculosis cases among notified patients with pulmonary tuberculosis in 2011.

FINDINGS: In the 30 countries, 18% of the estimated MDR-tuberculosis cases were enrolled on treatment in 2011. Belarus, Brazil, Kazakhstan, Peru, South Africa, and Ukraine each detected and enrolled on treatment more than 50% of their estimated cases of MDR-tuberculosis. In Ethiopia, India, Indonesia, the Philippines, and Russia, enrolments increased steadily between 2009 and 2011 with a mean yearly change greater than 50%: however, in these countries enrolment in 2011 was low, ranging from 4% to 43% of the estimated cases. In the remaining countries (Afghanistan, Angola, Azerbaijan, Bangladesh, China, Democratic Republic of the Congo, Kenya, Kyrgyzstan, Moldova, Mozambique, Burma, Nepal, Nigeria, North Korea, Pakistan, South Korea, Thailand, Uzbekistan, and Vietnam) progress in detection and enrolment was slower. In 23 countries, a median of 53% (IQR 41-71) patients with MDR-tuberculosis successfully completed their treatment after starting it in 2008-09.

INTERPRETATION: Six countries (Belarus, Brazil, Kazakhstan, Peru, South Africa, and Ukraine) can achieve universal access to MDR-tuberculosis care by 2015 should they sustain their current pace of progress. In other countries a radical scale-up will be needed for them to have an effect on their MDR-tuberculosis burden. Unless barriers to diagnosis and successful treatment are urgently overcome, and new technologies in diagnostics and treatment effectively implemented, the global targets for 2015 are unlikely be achieved.

FUNDING: WHO.

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PMID: 23743044 [PubMed - indexed for MEDLINE]

27. **Anti-tuberculosis drug resistance among new and previously treated sputum smear-positive tuberculosis patients in Uganda: results of the first national survey.**

Lukoye D[1], Adatu F, Musisi K, Kasule GW, Were W, Odeke R, Kalamya JN, Awor A, Date A, Joloba ML.

PLoS One. 2013 Aug 1;8(8):e70763. doi: 10.1371/journal.pone.0070763. Print 2013.

[1]National Tuberculosis/Leprosy Program Ministry of Health, Kampala, Uganda.

**ABSTRACT**

BACKGROUND: Multidrug resistant and extensively drug resistant tuberculosis (TB) have become major threats to control of tuberculosis globally. The rates of anti-TB drug resistance in Uganda are not known. We conducted a national drug resistance survey to investigate the levels and patterns of resistance to first and second line anti-TB drugs among new and previously treated sputum smear-positive TB cases.

METHODS: Sputum samples were collected from a nationally representative sample of new and previously treated sputum smear-positive TB patients registered at TB diagnostic centers during December 2009 to February 2011 using a weighted cluster sampling method. Culture and drug susceptibility testing was performed at the national TB reference laboratory.

RESULTS: A total of 1537 patients (1397 new and 140 previously treated) were enrolled in the survey from 44 health facilities. HIV test result and complete drug susceptibility testing (DST) results were available for 1524 (96.8%) and 1325 (85.9%) patients, respectively. Of the 1209 isolates from new cases, resistance to any anti-TB drug was 10.3%, 5% were resistant to isoniazid, 1.9% to rifampicin, and 1.4% were multi drug resistant. Among the 116 isolates from previously treated cases, the prevalence of resistance was 25.9%, 23.3%, 12.1% and 12.1% respectively. Of the 1524 patients who had HIV testing 469 (30.7%) tested positive. There was no association between anti-TB drug resistance (including MDR) and HIV infection.

CONCLUSION: The prevalence of anti-TB drug resistance among new patients in Uganda is low relative to WHO estimates. The higher levels of MDR-TB (12.1%) and resistance to any drug (25.3%) among previously treated patients raises concerns about the quality of directly observed therapy (DOT) and adherence to treatment. This calls for strengthening existing TB control measures, especially DOT, routine DST among the previously treated TB patients or periodic drug resistance surveys, to prevent and monitor development and transmission of drug resistant TB.

PMCID: PMC3731251 **Free PMC Article**
PMID: 23936467 [PubMed - indexed for MEDLINE]

28. **Diagnosis and interim treatment outcomes from the first cohort of multidrug-resistant tuberculosis patients in Tanzania.**

Mpagama SG[1], Heysell SK, Ndusilo ND, Kumburu HH, Lekule IA, Kisonga RM, Gratz J, Boeree MJ, Houpt ER, Kibiki GS.

PLoS One. 2013 May 13;8(5):e62034. doi: 10.1371/journal.pone.0062034. Print 2013.

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**ABSTRACT**

SETTING: Kibong'oto National Tuberculosis Hospital (KNTH), Kilimanjaro, Tanzania.

OBJECTIVE: Characterize the diagnostic process and interim treatment outcomes from patients treated for multidrug-resistant tuberculosis (MDR-TB) in Tanzania.

DESIGN: A retrospective cohort study was performed among all patients treated at KNTH for pulmonary MDR-TB between November 2009 and September 2011.

RESULTS: Sixty-one culture-positive MDR-TB patients initiated therapy, 60 (98%) with a prior history of TB treatment. Forty-one (67%) were male and 9 (14%) were HIV infected with a mean CD4 count of 424 (±106) cells/µl. The median time from specimen collection to MDR-TB diagnosis and from diagnosis to initiation of MDR-TB treatment was 138 days (IQR 101-159) and 131 days (IQR 32-233), respectively. Following treatment initiation four (7%) patients died (all HIV negative), 3 (5%) defaulted, and the remaining 54 (89%) completed the intensive phase. Most adverse drug reactions were mild to moderate and did not require discontinuation of treatment. Median time to culture conversion was 2 months (IQR 1-3) and did not vary by HIV status. In 28 isolates available for additional second-line drug susceptibility testing, fluoroquinolone, aminoglycoside and para-aminosalicylic acid resistance was rare yet ethionamide resistance was present in 9 (32%).

CONCLUSION: The majority of MDR-TB patients from this cohort had survived a prolonged referral process, had multiple episodes of prior TB treatment, but did not have advanced AIDS and converted to culture negative early while completing an intensive inpatient regimen without serious adverse event. Further study is required to determine the clinical impact of second-line drug susceptibility testing and the feasibility of alternatives to prolonged hospitalization.

PMCID: PMC3652861 **Free PMC Article**
PMID: 23675411 [PubMed - indexed for MEDLINE]

29. **Multidrug-resistant tuberculosis, Somalia, 2010-2011.**

Sindani I[1], Fitzpatrick C, Falzon D, Suleiman B, Arube P, Adam I, Baghdadi S, Bassili A, Zignol M.

Emerg Infect Dis. 2013 Mar;19(3):478-80. doi: 10.3201/eid1903.121287.

[1]World Health Organization, Nairobi, Kenya.

**Erratum in:**

- Emerg Infect Dis. 2014 Nov;20(11):1961.

**ABSTRACT**

In a nationwide survey in 2011, multidrug-resistant tuberculosis (MDR TB) was found in 5.2% and 40.8% of patients with new and previously treated TB, respectively. These levels of drug resistance are among the highest ever documented in Africa and the Middle East. This finding presents a serious challenge for TB control in Somalia.

PMCID: PMC3647667 **Free PMC Article**
PMID: 23621911 [PubMed - indexed for MEDLINE]

30. **Anti-tuberculosis drug resistance pattern among pulmonary tuberculosis patients with or without HIV infection in Mwanza, Tanzania.**

Range N[1], Friis H, Mfaume S, Magnussen P, Chanualucha J, Kilale A, Mugomela A, Andersen AB.

Tanzan J Health Res. 2012 Oct;14(4):243-9.

[1]National Institute for Medical Research, Muhimbili Medical Research Centre, Dar es Salaam, Tanzania. hrange08@gmail.com

**ABSTRACT**

Anti-tuberculosis drug resistance is a major problem in tuberculosis (TB) control, particularly multi-drug resistance TB (MDR-TB). The objective of this study was to determine the prevalence of primary and acquired anti-TB drug resistance among newly diagnosed pulmonary TB (PTB) and relapse cases. Sputa were collected from newly diagnosed and relapse PTB patients. Drug susceptibility tests (DST) were performed on sputum culture positive isolates of Mycobacterium tuberculosis using resistance ratio method on four first-line anti-TB drugs: rifampicin, isoniazid, ethambutol and streptomycin. Demographic and anthropometric information was collected and HIV status was determined. Of the 523 culture positive isolates, DST results were available for 503 (96%), 455 were new and 48 were relapse cases. Resistance to at least one of the four drugs was observed in 7.8% (39/503) of the

isolates, 7.3% (33/455) were new and 12.5% (6/48) were from relapse cases. Mono resistance to isoniazid was higher in both among new 45.5% (15/33) and relapse 50.0% (3/6) cases. Resistance to rifampicin and streptomycin alone was equal 4/33 (12.1%) and only among new cases. Resistance to ethambutol alone was only one among new cases. Overall MDR-TB prevalence was 2.4% (12/503), nine were new and three were relapse cases. MDR-TB was 17.9% (7/39) for rifampicin and isoniazid. Prevalence of HIV was 43.3% and was similar among new and relapse cases and not risk factor for drug resistance. Majority of PTB patients (52%) had BMI below 18 kg/m2. Those with BMI greater than 18 kg/m2 were more likely to develop drug resistance than those with BMI below 18 kg/m2 (P=0.004). With the resurgence of TB and the high prevalence of HIV among TB patients, prevalence of drug resistance is still low both among new and relapses cases. Despite the current low drug resistance, there is a need for continuous monitoring of the resistance.

PMID: 26591721 [PubMed - indexed for MEDLINE]

31. **Integration of HIV testing in tuberculosis drug resistance surveillance in Kazakhstan and Kenya.**

Klinkenberg E[1], van den Hof S, Tursynbayeva A, Kipruto H, Wahogo J, Pak S, Kutwa A, L'Herminez R.

Int J Tuberc Lung Dis. 2012 May;16(5):615-7. doi: 10.5588/ijtld.11.0262. Epub 2012 Mar 8.

[1]Regional Team Africa, KNCV Tuberculosis Foundation, The Hague, The Netherlands. klinkenberge@kncvtbc.nl

**ABSTRACT**

In Kenya and Kazakhstan, integration of human immunodeficiency virus (HIV) testing results into the routine surveillance of multidrug-resistant tuberculosis (MDR-TB) proved feasible and useful. The integration process improved overall data quality and data validation capacity, and integrated data are a useful addition to routine cohort and treatment outcome data. Besides their importance for individual patient care, they provide trends on the association of MDR-TB and HIV in the routine programme setting. They also form a useful epidemiological basis for more specific studies, such as on nosocomial outbreaks. Whether the system itself is sensitive enough to monitor possible outbreaks needs further investigation.

PMID: 22409816 [PubMed - indexed for MEDLINE]

32. **Multidrug resistance among new tuberculosis cases: detecting local variation through lot quality-assurance sampling.**

Hedt BL[1], van Leth F, Zignol M, Cobelens F, van Gemert W, Nhung NV, Lyepshina S, Egwaga S, Cohen T.

Epidemiology. 2012 Mar;23(2):293-300. doi: 10.1097/EDE.0b013e3182459455.

[1]Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA 02115, USA. bethhedt@gmail.com

**ABSTRACT**

BACKGROUND: Current methodology for multidrug-resistant tuberculosis (MDR TB) surveys endorsed by the World Health Organization provides estimates of MDR TB prevalence among new cases at the national level. On the aggregate, local variation in the burden of MDR TB may be masked. This paper investigates the utility of applying lot quality-assurance sampling to identify geographic heterogeneity in the proportion of new cases with multidrug resistance.

METHODS: We simulated the performance of lot quality-assurance sampling by applying these classification-based approaches to data collected in the most recent TB drug-resistance surveys in Ukraine, Vietnam, and Tanzania. We explored 3 classification systems- two-way static, three-way static, and three-way truncated sequential sampling-at 2 sets of thresholds: low MDR TB = 2%, high MDR TB = 10%, and low MDR TB = 5%, high MDR TB = 20%.

RESULTS: The lot quality-assurance sampling systems identified local variability in the prevalence of multidrug resistance in both high-resistance (Ukraine) and low-resistance settings (Vietnam). In Tanzania, prevalence was uniformly low, and the lot quality-assurance sampling approach did not reveal variability. The three-way classification systems provide additional information, but sample sizes may not be obtainable in some settings. New rapid drug-sensitivity testing methods may allow truncated sequential sampling designs and early stopping within static designs, producing even greater efficiency gains.

CONCLUSIONS: Lot quality-assurance sampling study designs may offer an efficient approach for collecting critical information on local variability in the burden of multidrug-resistant TB. Before this methodology is adopted, programs must determine appropriate classification thresholds, the most useful classification system, and appropriate weighting if unbiased national estimates are also desired.

PMCID: PMC3276714 **Free PMC Article**
PMID: 22249242 [PubMed - indexed for MEDLINE]

33. **Resistance patterns of Mycobacterium tuberculosis isolates from pulmonary tuberculosis patients in Nairobi.**

Ndung'u PW[1], Kariuki S, Ng'ang'a Z, Revathi G.

J Infect Dev Ctries. 2012 Jan 12;6(1):33-9.

[1]Institute of Tropical Medicine and Infectious Diseases (ITROMID), Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya. perpetualndungu@yahoo.com

**ABSTRACT**

INTRODUCTION: In Kenya, which ranks thirteenth of 27 high tuberculosis burden countries, diagnosis is based on Ziehl-Neelsen staining alone and patients are treated without information on sensitivity patterns. This study aimed to determine resistance patterns of Mycobacterium tuberculosis isolated from pulmonary samples.

METHODOLOGY: Pulmonary tuberculosis patients in Nairobi were randomly sampled after informed consent and recruited into the study using a structured questionnaire. Specimens were cultured in liquid and solid media, and drug susceptibility tests were performed for first-line drugs including (isoniazid, rifampin, streptomycin, ethambutol and pyrazinamide).

RESULTS: Eighty-six (30%) of 286 isolates were resistant to at least one of five antibiotics tested. Thirty-seven (30.2%) isolates were resistant to isoniazid; 15 (11.6%) to streptomycin; 13 (4.5%) to ethambutol; four (1.4%) to rifampin ; and 30 (10.4%) to pyrazinamide. Double resistance was seen as follows: four (1.4%) isolates were resistant to both isoniazid and pyrazinamide; four (1.4%) to streptomycin and isoniazid; and one (0.3%) to rifampin and streptomycin. Two isolates (0.7%) were multidrug resistant, and one was triple resistant with an additional resistance to ethambutol. Results also showed 88.7% of patients were below the age of 40 years, while 26.3% were HIV positive. The majority of the patients (66.5%) were unemployed or self-employed in small businesses, with 79.4% earning less than 100 USD per month.

CONCLUSION: The high resistance observed in isoniazid, which is a first-line drug, could result in an increase in multidrug resistance unless control programs are strengthened. Poverty should be addressed to reduce infection rates.

**Free Article**

PMID: 22240426 [PubMed - indexed for MEDLINE]

34. **Low rate of fluoroquinolone resistance in Mycobacterium tuberculosis isolates from northern Tanzania.**

van den Boogaard J[1], Semvua HH, van Ingen J, Mwaigwisya S, van der Laan T, van Soolingen D, Kibiki GS, Boeree MJ, Aarnoutse RE.

J Antimicrob Chemother. 2011 Aug;66(8):1810-4. doi: 10.1093/jac/dkr205. Epub 2011 Jun 3.

[1]Radboud University Nijmegen Medical Centre, University Centre for Chronic Diseases Dekkerswald, PO Box 66, 6560 AB, Groesbeek, The Netherlands. jossyvandenboogaard@gmail.com

**ABSTRACT**

OBJECTIVES: Fluoroquinolones are used in second-line treatment of tuberculosis (TB) and have a potential role in shortening TB treatment duration. The wide use of fluoroquinolones in the treatment of other infections, including respiratory tract infections in patients with (undiagnosed) active TB, could result in fluoroquinolone-resistant Mycobacterium tuberculosis. We determined the rate of fluoroquinolone resistance in M. tuberculosis isolates obtained from Tanzanian patients and linked this to previous fluoroquinolone exposure and mycobacterial resistance to rifampicin and isoniazid.

METHODS: A total of 291 M. tuberculosis isolates were obtained between April 2009 and June 2010 from patients with smear-positive pulmonary TB and tested for susceptibility to ciprofloxacin, moxifloxacin, rifampicin and isoniazid. Information on previous fluoroquinolone use was obtained by interviewing patients and checking their medical files.

RESULTS: Only 2 (0.7%) of the 291 M. tuberculosis isolates were resistant to ciprofloxacin; 1 of which was intermediately resistant to moxifloxacin as well. These two isolates were susceptible to rifampicin and isoniazid. Twenty-two (8%) of the 291 patients had a history of fluoroquinolone use (median: 7 days; interquartile range: 5-10 days). The patients from whom the fluoroquinolone-resistant M. tuberculosis isolates were obtained had no known history of previous fluoroquinolone use.

CONCLUSIONS: Our findings indicate that the rate of fluoroquinolone-resistant M. tuberculosis in Tanzanian patients with TB is low and not related to previous, brief episodes of exposure to fluoroquinolones. The findings favour future application of fluoroquinolones in TB treatment regimens of shorter duration.

**Free Article**

PMID: 21642290 [PubMed - indexed for MEDLINE]

35. **Rates of anti-tuberculosis drug resistance in Kampala-Uganda are low and not associated with HIV infection.**

Lukoye D[1], Cobelens FG, Ezati N, Kirimunda S, Adatu FE, Lule JK, Nuwaha F, Joloba ML.

PLoS One. 2011 Jan 10;6(1):e16130. doi: 10.1371/journal.pone.0016130.

[1]Public Health Department, Kampala City Council, Kampala, Uganda.

**ABSTRACT**

BACKGROUND: Drug resistance among tuberculosis patients in sub-Saharan Africa is increasing, possibly due to association with HIV infection. We studied drug resistance and HIV infection in a representative sample of 533 smear-positive tuberculosis patients diagnosed in Kampala, Uganda.

METHODS/PRINCIPAL FINDINGS: Among 473 new patients, multidrug resistance was found in 5 (1.1%, 95% CI 0.3-2.5) and resistance to any drug in 57 (12.1%, 9.3-15.3). Among 60 previously treated patients this was 7 (11.7%, 4.8-22.6) and 17 (28.3%; 17.5-41.4), respectively. Of 517 patients with HIV results, 165 (31.9%, 27.9-36.1) tested positive. Neither multidrug (adjusted odds ratio (OR(adj)) 0.7; 95% CI 0.19-2.6) nor any resistance (OR(adj) 0.7; 0.43-1.3) was associated with HIV status. Primary resistance to any drug was more common among patients who had worked in health care (OR(adj) 3.5; 1.0-12.0).

CONCLUSION/SIGNIFICANCE: Anti-tuberculosis drug resistance rates in Kampala are low and not associated with HIV infection, but may be associated with exposure during health care.

PMCID: PMC3018425 **Free PMC Article**
PMID: 21249225 [PubMed - indexed for MEDLINE]

36. **National anti-tuberculosis drug resistance study in Tanzania.**

Chonde TM[1], Basra D, Mfinanga SG, Range N, Lwilla F, Shirima RP, van Deun A, Zignol M, Cobelens FG, Egwaga SM, van Leth F.

Int J Tuberc Lung Dis. 2010 Aug;14(8):967-72.

[1]National Tuberculosis and Leprosy Programme, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania. vanlethf@kncvtbc.nl

**ABSTRACT**

OBJECTIVE: To assess the prevalence of anti-tuberculosis drug resistance in a national representative sample of tuberculosis (TB) patients in Tanzania according to recommended methodology.

DESIGN: Cluster survey, with 40 clusters sampled proportional to size, of notified TB patients from all diagnostic centres in the country.

RESULTS: The survey enrolled 1019 new and 148 retreatment patients. The adjusted prevalence of Mycobacterium tuberculosis strains resistant to any of the four first-line drugs in new patients was 8.3%, while the prevalence of multidrug-resistant TB (MDR-TB) was 1.1%. In retreatment patients, the crude prevalence for any resistance and for MDR-TB was respectively 20.6% and 3.9%. The prevalence of drug resistance did not differ in relapse patients compared to failure patients. These estimates are among the lowest in those African countries with an estimated level of drug resistance in the last 5 years.

CONCLUSION: The low levels of drug resistance in Tanzania are likely due to a well performing TB control programme and the absence of noticeable involvement of the private sector in TB treatment.
PMID: 20626940 [PubMed - indexed for MEDLINE]

37. **Evaluation of seven tests for the rapid detection of multidrug-resistant tuberculosis in Uganda.**

Bwanga F[1], Joloba ML, Haile M, Hoffner S.

Int J Tuberc Lung Dis. 2010 Jul;14(7):890-5.

[1]Department of Medical Microbiology, College of Health Sciences, Makerere University, Kampala, Uganda.

**ABSTRACT**

SETTINGS: National Tuberculosis (TB) Reference Laboratory and Department of Medical Microbiology, College of Health Sciences, Makerere University, Kampala, Uganda.

OBJECTIVE: To evaluate head-to-head rapid tests for drug susceptibility testing (DST) of Mycobacterium tuberculosis against rifampicin (RMP) and isoniazid (INH) in a resource-limited setting.

METHODS: Thirty-one well-characterised strains of M. tuberculosis were tested with the nitrate reductase assay (NRA), microscopic observation drug susceptibility (MODS), MGIT 960 (Mycobacterium Growth Indicator Tube 960), Genotype MTBDRplus, Alamar blue, MTT and resazurin assays. The proportion method on Löwenstein-Jensen medium was used as the reference test.

RESULTS: NRA correctly identified the resistant strains, with 100% sensitivity and specificity. MGIT 960 detected all multidrug-resistant strains but missed one RMP-monoresistant strain. Genotype MTBDRplus detected all RMP-resistant strains, but the sensitivity for detection of INH resistance was lower (88%). Sensitivity and specificity ranged from 86% to 100% for MODS and from 57% to 100% for the Alamar blue, MTT and resazurin assays. Test results were obtained within 2-14 days.

CONCLUSION: In the study setting, NRA, MGIT 960 and Genotype MTBDRplus gave excellent detection of multidrug-resistant tuberculosis, with significantly shorter time to results compared to conventional testing.
PMID: 20550774 [PubMed - indexed for MEDLINE]

38. **Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology.**

Helb D[1], Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R, Banada P, Safi H, Blakemore R, Lan NT, Jones-López EC, Levi M, Burday M, Ayakaka I, Mugerwa RD, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins MD, Persing DH, Alland D.

J Clin Microbiol. 2010 Jan;48(1):229-37. doi: 10.1128/JCM.01463-09. Epub 2009 Oct 28.

[1]Department of Medicine, New Jersey Medical School, University of Medicine and Dentistry, New Jersey, Newark, New Jersey 07103, USA.

**ABSTRACT**

Current nucleic acid amplification methods to detect Mycobacterium tuberculosis are complex, labor-intensive, and technically challenging. We developed and performed the first analysis of the Cepheid Gene Xpert System's MTB/RIF assay, an integrated hands-free sputum-processing and real-time PCR system with rapid on-demand, near-patient technology, to simultaneously detect M. tuberculosis and rifampin resistance. Analytic tests of M. tuberculosis DNA demonstrated a limit of detection (LOD) of 4.5 genomes per reaction. Studies using sputum spiked with known numbers of M. tuberculosis CFU predicted a clinical LOD of 131 CFU/ml. Killing studies showed that the assay's buffer decreased M. tuberculosis viability by at least 8 logs, substantially reducing biohazards. Tests of 23 different commonly occurring rifampin resistance mutations demonstrated that all 23 (100%) would be identified as rifampin resistant. An analysis of 20 nontuberculosis mycobacteria species confirmed high assay specificity. A small clinical validation study of 107 clinical sputum samples from suspected tuberculosis cases in Vietnam detected 29/29 (100%) smear-positive culture-positive cases and 33/39 (84.6%) or 38/53 (71.7%) smear-negative culture-positive cases, as determined by growth on solid medium or on both solid and liquid media, respectively. M. tuberculosis was not detected in 25/25 (100%) of the culture-negative samples. A study of 64 smear-positive culture-positive sputa from retreatment tuberculosis cases in Uganda detected 63/64 (98.4%) culture-positive cases and 9/9 (100%) cases of rifampin resistance. Rifampin resistance was excluded in 54/55 (98.2%) susceptible cases. Specificity rose to 100% after correcting for a conventional susceptibility test error. In

conclusion, this highly sensitive and simple-to-use system can detect M. tuberculosis directly from sputum in less than 2 h.

PMCID: PMC2812290 **Free PMC Article**
PMID: 19864480 [PubMed - indexed for MEDLINE]

39. **Comparison of rapid tests for detection of rifampicin-resistant Mycobacterium tuberculosis in Kampala, Uganda.**

Ogwang S[1], Asiimwe BB, Traore H, Mumbowa F, Okwera A, Eisenach KD, Kayes S, Jones-López EC, McNerney R, Worodria W, Ayakaka I, Mugerwa RD, Smith PG, Ellner J, Joloba ML.

BMC Infect Dis. 2009 Aug 26;9:139. doi: 10.1186/1471-2334-9-139.

[1]Department of Medical Microbiology, Makerere University College of Health Sciences, Kampala, Uganda. ogwangsam@yahoo.com

**ABSTRACT**

BACKGROUND: Drug resistant tuberculosis (TB) is a growing concern worldwide. Rapid detection of resistance expedites appropriate intervention to control the disease. Several technologies have recently been reported to detect rifampicin resistant Mycobacterium tuberculosis directly in sputum samples. These include phenotypic culture based methods, tests for gene mutations and tests based on bacteriophage replication. The aim of the present study was to assess the feasibility of implementing technology for rapid detection of rifampicin resistance in a high disease burden setting in Africa.

METHODS: Sputum specimens from re-treatment TB patients presenting to the Mulago Hospital National TB Treatment Centre in Kampala, Uganda, were examined by conventional methods and simultaneously used in one of the four direct susceptibility tests, namely direct BACTEC 460, Etest, "in-house" phage test, and INNO- Rif.TB. The reference method was the BACTEC 460 indirect culture drug susceptibility testing. Test performance, cost and turn around times were assessed.

RESULTS: In comparison with indirect BACTEC 460, the respective sensitivities and specificities for detecting rifampicin resistance were 100% and 100% for direct BACTEC and the Etest, 94% and 95% for the phage test, and 87% and 87% for the Inno-LiPA assay. Turn around times ranged from an average of 3 days for the INNO-LiPA and phage tests, 8 days for the direct BACTEC 460 and 20 days for the Etest. All methods were faster than the indirect BACTEC 460 which had a mean turn around time of 24 days. The cost per test, including labour ranged from $18.60 to $41.92 (USD).

CONCLUSION: All four rapid technologies were shown capable of detecting rifampicin resistance directly from sputum. The LiPA proved rapid, but was the most expensive. It was noted, however, that the LiPA test allows sterilization of samples prior to testing thereby reducing the risk of accidental laboratory transmission. In contrast the Etest was low cost, but slow and would be of limited assistance when treating patients. The phage test was the least reproducible test studied with failure rate of 27%.

The test preferred by the laboratory personnel, direct BACTEC 460, requires further study to determine its accuracy in real-time treatment decisions in Uganda.

PMCID: PMC2744678 **Free PMC Article**
PMID: 19709423 [PubMed - indexed for MEDLINE]

40. **Anti-TB drug resistance levels and patterns among Mycobacterium tuberculosis isolated from newly diagnosed cases of pulmonary tuberculosis in Dar es Salaam, Tanzania.**

Matee M[1], Mfinanga S, Holm-Hansen C.

APMIS. 2009 Apr;117(4):263-7. doi: 10.1111/j.1600-0463.2008.02429.x.

[1]Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania.

**ABSTRACT**

Anti-tuberculosis drug resistance levels and patterns of Mycobacterium tuberculosis (Mtb) isolated from newly diagnosed tuberculosis (TB) patients in Temeke district in Dar es Salaam, Tanzania were investigated. A total of 226 Mtb isolates from 564 TB suspects with no previous history of anti-TB treatment were tested for drug resistance against rifampicin, isoniazid, streptomycin and ethambutol on Lowenstein Jensen (LJ) medium using the proportion method. Of the 226 isolates, 22 (9.7%) were resistant to any one of the four anti-TB drugs; nine (3.99%) isolates were isoniazid mono-drug resistant and eight (3.54%) isolates were streptomycin mono-drug resistant. Multi-drug resistance, defined as resistance to both rifampicin and isoniazid, was observed in three (1.3%) isolates and two were also resistant to streptomycin and ethambutol. One (0.44%) isolate had poly resistance to isoniazid and streptomycin. The level of anti-TB drug resistant Mtb in Temeke, an HIV endemic area, remained constant between 1995 and 2007. The level of resistance to any one of the four anti-TB drugs was between 9.0% and 10%, resistance to individual drugs <4% and multi-drug resistance <2%.
PMID: 19338514 [PubMed - indexed for MEDLINE]

41. **Implementation of a national anti-tuberculosis drug resistance survey in Tanzania.**

Chonde TM[1], Doulla B, van Leth F, Mfinanga SG, Range N, Lwilla F, Mfaume SM, van Deun A, Zignol M, Cobelens FG, Egwaga SM.

BMC Public Health. 2008 Dec 30;8:427. doi: 10.1186/1471-2458-8-427.

[1]National Tuberculosis and Leprosy Control Program, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania. tmchondes@yahoo.com

**ABSTRACT**

BACKGROUND: A drug resistance survey is an essential public health management tool for evaluating and improving the performance of National Tuberculosis control programmes. The current manuscript describes the implementation of the first national drug resistance survey in Tanzania.

METHODS: Description of the implementation process of a national anti-tuberculosis drug resistance survey in Tanzania, in relation to the study protocol and Standard Operating Procedures.

RESULTS: Factors contributing positively to the implementation of the survey were a continuous commitment of the key stakeholders, the existence of a well organized National Tuberculosis Programme, and a detailed design of cluster-specific arrangements for rapid sputum transportation. Factors contributing negatively to the implementation were a long delay between training and actual survey activities, limited monitoring of activities, and an unclear design of the data capture forms leading to difficulties in form-filling.

CONCLUSION: Careful preparation of the survey, timing of planned activities, a strong emphasis on data capture tools and data management, and timely supervision are essential for a proper implementation of a national drug resistance survey.

PMCID: PMC2628900 **Free PMC Article**
PMID: 19116022 [PubMed - indexed for MEDLINE]

42. **Rate and amplification of drug resistance among previously-treated patients with tuberculosis in Kampala, Uganda.**

Temple B[1], Ayakaka I, Ogwang S, Nabanjja H, Kayes S, Nakubulwa S, Worodria W, Levin J, Joloba M, Okwera A, Eisenach KD, McNerney R, Elliott AM, Smith PG, Mugerwa RD, Ellner JJ, Jones-López EC.

Clin Infect Dis. 2008 Nov 1;47(9):1126-34. doi: 10.1086/592252.

[1]Medical Research Council-Uganda Virus Research Institute, Uganda Research Unit on AIDS, Entebbe, Uganda.

**ABSTRACT**

BACKGROUND: Drug-resistant Mycobacterium tuberculosis has emerged as a global threat. In resource-constrained settings, patients with a history of tuberculosis (TB) treatment may have drug-resistant disease and may experience poor outcomes. There is a need to measure the extent of and risk factors for drug resistance in such patients.

METHODS: From July 2003 through November 2006, we enrolled 410 previously treated patients with TB in Kampala, Uganda. We measured the prevalence of resistance to first- and second-line drugs and analyzed risk factors associated with baseline and acquired drug resistance.

RESULTS: The prevalence of multidrug-resistant TB was 12.7% (95% confidence interval [95% CI], 9.6%-16.3%). Resistance to second-line drugs was low. Factors associated with multidrug-resistant TB at enrollment included a history of treatment failure (odds ratio, 23.6; 95% CI, 7.7-72.4), multiple previous TB episodes (odds ratio, 15.6; 95% CI, 5.0-49.1), and cavities present on chest radiograph (odds ratio, 5.9; 95% CI, 1.2-29.5). Among a cohort of 250 patients, 5.2% (95% CI, 2.8%-8.7%) were infected with M. tuberculosis that developed additional drug resistance. Amplification of drug resistance was associated with existing drug resistance at baseline (P <.01) and delayed sputum culture conversion (P <.01).

CONCLUSIONS: The burden of drug resistance in previously treated patients with TB in Uganda is sizeable, and the risk of generating additional drug resistance is significant. There is an urgent need to improve the treatment for such patients in low-income countries.

PMCID: PMC2883442 **Free PMC Article**
PMID: 18808360 [PubMed - indexed for MEDLINE]

43. **Mycobacterium tuberculosis spoligotypes and drug susceptibility pattern of isolates from tuberculosis patients in peri-urban Kampala, Uganda.**

Asiimwe BB[1], Ghebremichael S, Kallenius G, Koivula T, Joloba ML.

BMC Infect Dis. 2008 Jul 28;8:101. doi: 10.1186/1471-2334-8-101.

[1]Department of Medical Microbiology, Makerere University Medical School, Kampala, Republic of Uganda. benon.asiimwe@ki.se

**ABSTRACT**

BACKGROUND: The poor peri-urban areas of developing countries with inadequate living conditions and a high prevalence of HIV infection have been implicated in the increase of tuberculosis (TB). Presence of different lineages of Mycobacterium tuberculosis has been described in different parts of the world. This study determined the predominant strain lineages that cause TB in Rubaga division, Kampala, Uganda, and the prevalence of resistance to key anti-tuberculosis drugs in this community.

METHODS: This was a cross-sectional study of newly diagnosed sputum smear-positive patients aged >or= 18 years. A total of 344 isolates were genotyped by standard spoligotyping and the strains were compared with those in the international spoligotype database (SpolDB4). HIV testing and anti-tuberculosis drug susceptibility assays for isoniazid and rifampicin were performed and association with the most predominant spoligotypes determined.

RESULTS: A total of 33 clusters were obtained from 57 spoligotype patterns. According to the SpolDB4 database, 241 (70%) of the isolates were of the T2 family, while CAS1-Kili (3.5%), LAM9 (2.6%), CAS1-Delhi (2.6%) were the other significant spoligotypes. Furthermore, a major spoligotype pattern of 17 (4.5%) strains characterized by lack of spacers 15-17 and 19-43 was not identified in SpolDB4. A total of 92 (26.7%) of the patients were HIV sero-positive, 176 (51.2%) sero-negative, while 76 (22.1%) of the patients did not consent to HIV testing. Resistance to isoniazid was found in 8.1% of strains, while all 15 (4.4%) strains resistant to rifampicin were multi-drug resistant. Additionally, there was no association between any strain types in the sample with either drug resistance or HIV sero-status of the patients.

CONCLUSION: The TB epidemic in Kampala is localized, mainly caused by the T2 family of strains. Strain types were neither associated with drug resistance nor HIV sero-status.

PMCID: PMC2519071 **Free PMC Article**
PMID: 18662405 [PubMed - indexed for MEDLINE]

44. **Low levels of second-line drug resistance among multidrug-resistant Mycobacterium tuberculosis isolates from Rwanda.**

Umubyeyi A[1], Rigouts L, Shamputa IC, Dediste A, Struelens M, Portaels F.

Int J Infect Dis. 2008 Mar;12(2):152-6. Epub 2007 Oct 18.

[1]Department of Microbiology, CHU St Pierre, Brussels, Belgium. alainenyaruhirira@hotmail.com

**ABSTRACT**

BACKGROUND: Multidrug-resistant tuberculosis (MDR-TB) has become a therapeutic problem in many parts of the world, necessitating the inclusion of second-line anti-tuberculosis drugs in specific treatment regimens.

METHODS: We studied the susceptibility of 69 MDR Mycobacterium tuberculosis isolates from Rwanda to second-line drugs by the BACTEC 460 method.

RESULTS: The results showed that 62 (89.9%) were resistant to rifabutin while a low rate (4.3%) of resistance was registered for ofloxacin; there was one case (1.4%) of resistance each for para-aminosalicylic acid, kanamycin, ethionamide, and clarithromycin.

CONCLUSIONS: This information is important for devising an appropriate treatment regimen for MDR-TB patients in order to stop the spread of MDR strains and contain the acquisition of additional drug resistance in Rwanda.

**Free Article**
PMID: 17950021 [PubMed - indexed for MEDLINE]

45. **Evidence of 'amplifier effect' in pulmonary multidrug-resistant tuberculosis: report of three cases.**

Umubyeyi A[1], Shamputa IC, Rigouts L, Dediste A, Struelens M, Portaels F.

Int J Infect Dis. 2007 Nov;11(6):508-12. Epub 2007 Mar 21.

[1]Mycobacteriology Unit, Institute of Tropical Medicine, B-2000 Antwerp, Belgium; Service of Microbiology, CHU St Pierre, Brussels, Belgium. alainenyaruchirira@hotmail.com

**ABSTRACT**

INTRODUCTION: A cluster of three related cases of tuberculosis (TB) with primary multidrug resistance was investigated at the Centre Hospitalier Universitaire of Kigali (CHUK) in Rwanda. The patients were HIV-1/2 seronegative. Patients 1 and 2 were hospitalized in the same room of CHUK for one month. Patient 3 was a younger sibling of patient 2.

METHODS: Drug susceptibility of two consecutive Mycobacterium tuberculosis isolates from each patient was tested by the BACTEC 460 radiometric method. DNA fingerprinting was performed using spoligotyping and mycobacterial interspersed repetitive units of variable numbers of tandem repeats (MIRU-VNTR) analysis. All patients initially received the World Health Organization category I regimen.

RESULTS: The isolates collected during the first TB episode were resistant to isoniazid, rifampin and ethambutol. After subsequent retreatment regimens with rifampin, isoniazid, streptomycin, pyrazinamide (8 months) and rifampin, isoniazid, streptomycin, pyrazinamide, ciprofloxacin (21 months), patients 1 and 2 developed additional resistance to streptomycin and quinolones. Patient 3 received only the category I regimen and consecutive isolates retained the initial drug susceptibility pattern. All isolates were genetically indistinguishable by spoligotyping and MIRU-VNTR, indicating the same origin.

CONCLUSIONS: These observations highlight the risk of nosocomial transmission of multidrug-resistant (MDR) TB and the possible selection of secondary resistance to second-line drugs if a single new drug is added at the time of retreatment of MDR TB patients.

**Free Article**

PMID: 17376726 [PubMed - indexed for MEDLINE]

46. **Implementation validation performed in Rwanda to determine whether the INNO-LiPA Rif.TB line probe assay can be used for detection of multidrug-resistant Mycobacterium tuberculosis in low-resource countries.**

Quezada CM[1], Kamanzi E, Mukamutara J, De Rijk P, Rigouts L, Portaels F, Ben Amor Y.

J Clin Microbiol. 2007 Sep;45(9):3111-4. Epub 2007 Jul 11.

[1]Laboratory of Structural Microbiology, Rockefeller University, New York, NY, USA.

**ABSTRACT**

We validated the implementation of the INNO-LiPA Rif.TB line probe assay, a diagnostic test for rapid detection of multidrug-resistant tuberculosis (MDR-TB), in Rwanda. No substantial difference was found between results obtained in Rwanda and results obtained in Belgium with the same samples. This rapid diagnostic test for MDR-TB can therefore be reliably implemented in a resource-poor setting.

PMCID: PMC2045290 **Free PMC Article**
PMID: 17626172 [PubMed - indexed for MEDLINE]

47. **Molecular investigation of recurrent tuberculosis in patients from Rwanda.**

Umubyeyi AN[1], Shamputa IC, Rigouts L, Dediste A, Karita E, Struelens MJ, Portaels F.

Int J Tuberc Lung Dis. 2007 Aug;11(8):860-7.

[1]Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium.
alainenyaruhirira@hotmail.com

**ABSTRACT**

SETTING: Pulmonary tuberculosis (TB) patients enrolled in four provinces of Rwanda.

OBJECTIVE: To determine the cause of recurrent TB.

DESIGN: Serial Mycobacterium tuberculosis isolates obtained from patients with recurrent TB from January 2002 to September 2005 were genotyped by spoligotyping and mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) typing. Drug resistance was determined by phenotypic susceptibility testing and sequencing of rpoB, katG, inhA and embB genes.

RESULTS: Among 710 culture-positive TB patients enrolled in the study, initial drug susceptibility testing results were available for 638. Sixty-nine of these had multidrug-resistant (MDR) TB and 569 were non-MDR-TB. Among the MDR-TB patients, 22 had follow-up isolates after cure (n = 12) or chronic infection

(n = 10). The DNA patterns of sequential isolates from 4 of the 12 previously cured MDR-TB patients were different, indicating re-infection. DNA patterns of isolates from the remaining 8 previously cured and 10 chronic MDR-TB patients were identical, suggesting reactivation and treatment failure, respectively. Among the non-MDR-TB patients, disease recurrence was observed in one case; this was determined to be due to reactivation after initial mixed infection.

CONCLUSION: These results document a high treatment failure/reactivation rate for MDR-TB and suggest that re-infection within 2 years may not be a common cause of recurrent TB in this setting.
PMID: 17705951 [PubMed - indexed for MEDLINE]

48. **M. tuberculosis genotypic diversity and drug susceptibility pattern in HIV-infected and non-HIV-infected patients in northern Tanzania.**

Kibiki GS[1], Mulder B, Dolmans WM, de Beer JL, Boeree M, Sam N, van Soolingen D, Sola C, van der Zanden AG.

BMC Microbiol. 2007 May 31;7:51.

[1]Department of Internal Medicine, Endoscopy Unit, Kilimanjaro Christian Medical Centre, Tumaini University, Moshi, Tanzania. gkibiki@gmail.com gkibiki@gmail.com

**ABSTRACT**

BACKGROUND: Tuberculosis (TB) is a major health problem and HIV is the major cause of the increase in TB. Sub-Saharan Africa is endemic for both TB and HIV infection. Determination of the prevalence of M. tuberculosis strains and their drug susceptibility is important for TB control.TB positive culture, BAL fluid or sputum samples from 130 patients were collected and genotyped. The spoligotypes were correlated with anti-tuberculous drug susceptibility in HIV-infected and non-HIV patients from Tanzania.

RESULTS: One-third of patients were TB/HIV co-infected. Forty-seven spoligotypes were identified. Fourteen isolates (10.8%) had new and unique spoligotypes while 116 isolates (89.2%) belonged to 33 known spoligotypes. The major spoligotypes contained nine clusters: CAS1-Kili 30.0%, LAM11- ZWE 14.6%, ND 9.2%, EAI 6.2%, Beijing 5.4%, T-undefined 4.6%, CAS1-Delhi 3.8%, T1 3.8% and LAM9 3.8%. Twelve (10.8%) of the 111 phenotypically tested strains were resistant to anti-TB drugs. Eight (7.2%) were monoresistant strains: 7 to isoniazid (INH) and one to streptomycin. Four strains (3.5%) were resistant to multiple drugs: one (0.9%) was resistant to INH and streptomycin and the other three (2.7%) were MDR strains: one was resistant to INH, rifampicin and ethambutol and two were resistant to all four anti-TB drugs. Mutation in the katG gene codon 315 and the rpoB hotspot region showed a low and high sensitivity, respectively, as predictor of phenotypic drug resistance.

CONCLUSION: CAS1-Kili and LAM11-ZWE were the most common families. Strains of the Beijing family and CAS1-Kili were not or least often associated with resistance, respectively. HIV status was not associated with spoligotypes, resistance or previous TB treatment.

PMCID: PMC1913919 **Free PMC Article**
PMID: 17540031 [PubMed - indexed for MEDLINE]

49. **Limited fluoroquinolone resistance among Mycobacterium tuberculosis isolates from Rwanda: results of a national survey.**

Umubyeyi AN[1], Rigouts L, Shamputa IC, Fissette K, Elkrim Y, de Rijk PW, Struelens MJ, Portaels F.

J Antimicrob Chemother. 2007 May;59(5):1031-3. Epub 2007 Feb 28.

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**ABSTRACT**

OBJECTIVES: There is an increasing interest in the possible role of fluoroquinolone antibiotics for the treatment of tuberculosis (TB), but widespread use of these antibiotics for the treatment of other bacterial infections may select for fluoroquinolone-resistant Mycobacterium tuberculosis strains.

METHODS: We evaluated fluoroquinolone susceptibility using the proportion method (ofloxacin, critical concentration 2.0 mg/L) in isolates from patients enrolled in a national drug resistance survey in Rwanda from November 2004 to February 2005.

RESULTS: Of the 701 M. tuberculosis isolates studied, 617 (88%) were susceptible to all first-line drugs, 32 (4.6%) were multidrug-resistant (MDR) and 52 (7.4%) were resistant to one or more first-line drugs but not MDR. Ofloxacin resistance was found in four (0.6%) of the isolates; three of them being MDR and one susceptible to all first-line drugs. Mutations in the gyrA gene were found in all ofloxacin-resistant strains at codons 80 and 94.

CONCLUSIONS: Our finding is not alarming for Rwanda, but highlights the general risk of producing resistance to fluoroquinolones, jeopardizing the potential for these drugs to be used as second-line anti-TB agents in the programmatic management of drug-resistant TB and creating incurable TB strains.

**Free Article**
PMID: 17329272 [PubMed - indexed for MEDLINE]

50. **[Primary and acquired resistance to antituberculous drugs in strains of Mycobacterium tuberculosis isolated in Rwanda].**

[Article in French]
Umubyeyi AN[1], Rigouts L, Zissis G, Kamanzi E, Pauwels P, Gasana M, Vandebriel G, Struelens M, Portaels F.

Med Trop (Mars). 2007 Apr;67(2):149-53.

[1]L'Unité de Mycobactériologie de l'Institut de Médecine Tropicale, Université Nationale du Rwanda. alainenyaruhirira@hotmail.com

**ABSTRACT**

This study was undertaken within the framework of a surveillance project on the resistance of Mycobacterium tuberculosis to first-line antituberculosis drugs in four provinces of Rwanda with a high prevalence of tuberculosis (TB). The purpose was to determine the prevalence of primary and acquired resistance of M. tuberculosis to major antituberculosis drugs. A cohort of patients (n=710) with pulmonary TB documented by positive microscopic examinations of exhaustive samples was recruited at 7 treatment centers. Sputum samples were cultured on Löwenstein-Jensen and Coletsos media. Sensitivity to antituberculosis drugs was tested using a BACTEC 460 radiometric system. M. tuberculosis was isolated in 644 of the 710 patients (90.7%). A total of 296 out of 573 tested for HIV infection (51.7%) were positive. Primary resistance to one, two, three or four antituberculosis drugs was observed in 3.5%, 2.9%, 1.4% and 5.7% respectively. The prevalence of acquired resistance to antituberculosis drugs was 11.2%. Primary monoresistance to streptomycin was the most prevalent (2.3%) followed by resistance to ethambutol (1%). The combined rate of multiresistance was 11.6% with 7% involving new cases and 25.5% involving retreatment. This study showed that the rates of primary and acquired resistance to first-line antituberculosis drugs were high and that TB was associated with HIV infection. The National TB Control Program must implement measures to coordinate diagnosis and management of TB and HIV infection.
PMID: 17691433 [PubMed - indexed for MEDLINE]

51. **Results of a national survey on drug resistance among pulmonary tuberculosis patients in Rwanda.**

Umubyeyi AN[1], Vandebriel G, Gasana M, Basinga P, Zawadi JP, Gatabazi J, Pauwels P, Nzabintwali F, Nyiramasarabwe L, Fissette K, Rigouts L, Struelens MJ, Portaels F.

Int J Tuberc Lung Dis. 2007 Feb;11(2):189-94.

[1]National University of Rwanda, Butare, Rwanda. alainenyaruhirira@hotmail.com

**Erratum in:**

- Int J Tuberc Lung Dis. 2007 Aug;11(8):936.

**ABSTRACT**

BACKGROUND: One of the principal objectives of tuberculosis (TB) control is to minimise the emergence of drug resistance. The first national survey was conducted in Rwanda to determine the prevalence of M. tuberculosis drug resistance.

METHODS: Sputum samples were collected from all new and retreatment cases in the health districts from November 2004 to February 2005. Drug susceptibility testing of isolates against first-line drugs was performed by the proportion method.

RESULTS: Of 616 strains from new cases, 6.2% were resistant to isoniazid, 3.9% to rifampicin and 3.9% were multidrug-resistant TB. Among 85 strains from previously treated cases, the prevalence of resistance was respectively 10.6%, 10.6% and 9.4% (MDR-TB strains). Eight MDR cases showed additional resistance to ethambutol and streptomycin.

CONCLUSION: The level of MDR-TB among TB patients in Rwanda is high. The main reasons of this emergence of MDR-TB can be attributed to the disorganisation of the health system, migration of the population during the 1994 civil war and poor success rates, with a high number of patients transferred out and lost to follow-up. On the other hand, the use of treatment regimens administered twice weekly during the continuation phase could be another important factor and merit further investigations.
PMID: 17263290 [PubMed - indexed for MEDLINE]

52. **Low-cost rapid detection of rifampicin resistant tuberculosis using bacteriophage in Kampala, Uganda.**

Traore H[1], Ogwang S, Mallard K, Joloba ML, Mumbowa F, Narayan K, Kayes S, Jones-Lopez EC, Smith PG, Ellner JJ, Mugerwa RD, Eisenach KD, McNerney R.

Ann Clin Microbiol Antimicrob. 2007 Jan 9;6:1.

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**ABSTRACT**

BACKGROUND: Resistance to anti-tuberculosis drugs is a serious public health problem. Multi-drug resistant tuberculosis (MDR-TB), defined as resistance to at least rifampicin and isoniazid, has been reported in all regions of the world. Current phenotypic methods of assessing drug susceptibility of M. tuberculosis are slow. Rapid molecular methods to detect resistance to rifampicin have been

developed but they are not affordable in some high prevalence countries such as those in sub Saharan Africa. A simple multi-well plate assay using mycobacteriophage D29 has been developed to test M. tuberculosis isolates for resistance to rifampicin. The purpose of this study was to investigate the performance of this technology in Kampala, Uganda.

METHODS: In a blinded study 149 M. tuberculosis isolates were tested for resistance to rifampicin by the phage assay and results compared to those from routine phenotypic testing in BACTEC 460. Three concentrations of drug were used 2, 4 and 10 microg/ml. Isolates found resistant by either assay were subjected to sequence analysis of a 81 bp fragment of the rpoB gene to identify mutations predictive of resistance. Four isolates with discrepant phage and BACTEC results were tested in a second phenotypic assay to determine minimal inhibitory concentrations.

RESULTS: Initial analysis suggested a sensitivity and specificity of 100% and 96.5% respectively for the phage assay used at 4 and 10 microg/ml when compared to the BACTEC 460. However, further analysis revealed 4 false negative results from the BACTEC 460 and the phage assay proved the more sensitive and specific of the two tests. Of the 39 isolates found resistant by the phage assay 38 (97.4%) were found to have mutations predictive of resistance in the 81 bp region of the rpoB gene. When used at 2 mug/ml false resistant results were observed from the phage assay. The cost of reagents for testing each isolate was estimated to be 1.3 US dollars when testing a batch of 20 isolates on a single 96 well plate. Results were obtained in 48 hours.

CONCLUSION: The phage assay can be used for screening of isolates for resistance to rifampicin, with high sensitivity and specificity in Uganda. The test may be useful in poorly resourced laboratories as a rapid screen to differentiate between rifampicin susceptible and potential MDR-TB cases.

PMCID: PMC1779803 **Free PMC Article**
PMID: 17212825 [PubMed - indexed for MEDLINE]

53. **Rifampicin mono-resistant Mycobacterium tuberculosis in Bujumbura, Burundi: results of a drug resistance survey.**

Sanders M[1], Van Deun A, Ntakirutimana D, Masabo JP, Rukundo J, Rigouts L, Fissette K, Portaelst F.

Int J Tuberc Lung Dis. 2006 Feb;10(2):178-83.

[1]Programme National de Lutte contre la Lèpre et la Tuberculose, Bujumbura, Burundi.

**ABSTRACT**

SETTING: Bujumbura, Burundi.

OBJECTIVES: To determine resistance levels of Mycobacterium tuberculosis (TB) to the main anti-tuberculosis drugs after 11 years of a DOTS programme using a WHO-recommended partially intermittent 6-month rifampicin (RMP) first-line regimen and fixed-dose drug combinations (FDCs).

DESIGN: Drug susceptibility testing of systematic samples of M. tuberculosis isolated from newly registered sputum smear-positive cases in the capital during a 15-month period (2002-2003).

RESULTS: Of 496 strains from new cases, 16.1% showed resistance to any drug, 6.3% to isoniazid (INH), 2.0% to RMP (1.4% multidrug-resistant TB [MDR-TB]), 13.3% to streptomycin and 1.6% to ethambutol. Among 69 strains from previously treated cases, the prevalence of resistance was 30%, 19%, 15% (12% MDR-TB strains), 25% and 6%, respectively.

CONCLUSION: Levels of drug resistance in Bujumbura are higher than average for Africa, despite long-term use of the DOTS strategy with FDCs and a ban on sales of TB drugs. Most worrying is the appearance of MDR-TB and RMP-resistant, INH-susceptible strains in new cases. Although a survey cannot prove that high HIV prevalence, elevated levels of resistance to some other drugs and irregular intake allowed acquisition of drug resistance, the effectiveness and safety of 6-month regimens with (partially) intermittent RMP throughout under such conditions should be investigated.
PMID: 16499257 [PubMed - indexed for MEDLINE]

54. **Isolation of multidrug-resistant tuberculosis strains in patients from private and public health care facilities in Nairobi, Kenya.**

Githui WA[1], Meme HK, Juma ES, Kinyanjui P, Karimi F, Chakaya JM, Kangangi J, Kutwa A.

Int J Tuberc Lung Dis. 2004 Jul;8(7):837-41.

[1]Centre for Respiratory Diseases Research, Kenya Medical Research Institute (KEMRI), Nairobi. wgithui@hotmail.com

**ABSTRACT**

SETTING: Health care facilities in Nairobi, Kenya.

OBJECTIVE: To document the presence of multidrug-resistant tuberculosis (MDR-TB) strains in patients from Nairobi between September 1999 and October 2001.

DESIGN: Descriptive study.

RESULTS: Of the 983 referred patients who submitted sputum for culture and drug susceptibility testing (DST), 59% were males. Two hundred and nine (21.3%) patients had a positive culture, of whom 15.2% had a request for DST against isoniazid, rifampicin, streptomycin and ethambutol. Of these, 65 (43.6%) had an isolate resistant to one or more drugs, while 17 (11.4%) had MDR-TB. Ten (59.0%) cases were referred from public health care facilities while seven (41%) were from the private sector. Sixteen isolates were resistant to all four drugs. All MDR-TB cases but one were from Nairobi.

CONCLUSION: The emergence of MDR-TB in Nairobi is a cause for concern. An outbreak would be catastrophic, creating not only increased morbidity and mortality but also a tremendous strain on already limited health care resources. Lack of policies for the treatment and management of MDR-TB and the unavailability of appropriate diagnostic facilities may increase its spread. Efforts to prevent outbreaks of MDR-TB should be emphasised.

PMID: 15260274 [PubMed - indexed for MEDLINE]

55. **[Resistant tuberculosis is spreading in Sweden. Molecular epidemiological strain identification by "fingerprinting" can make the infection tracing easier].**

[Article in Swedish]

Ghebremichael S[1], Koivula T, Hoffner S, Romanus V, Petrini B, Norén B, Sylvan S, Källenius G.

Lakartidningen. 2002 Jun 6;99(23):2618-9, 2622-3.

[1]Smittskyddsinstitutet, Solna.

**ABSTRACT**

Resistance of Mycobacterium tuberculosis to antibiotics is a world wide problem. A study is reported with the aim to analyse the spread of resistant isolates of M tuberculosis complex from patients with tuberculosis in Sweden. The study is based on a sample of 192 M tuberculosis complex isolates from patients with drug resistant tuberculosis during 1994-2000. All isolates resistant to at least one of the drugs streptomycin, isoniazid, ethambutol and rifampicin were included in the study. Restriction fragment length polymorphism (RFLP) was performed, using IS6110 as a probe for hybridisation. Visualised bands were analysed by Gel Compar software. The majority of the isolates were from patients born in high TB prevalence countries. During the years 1996-2000 there was one major cluster generated from 34 isolates. In 1996-1998 there were two isolates per year, in 1999 it increased to 20 isolates, and eight cases in 2000. All strains were resistant to isoniazid. All patients in this cluster were found to be from Africa. In comparing the pattern in the T-base the strains matched with strain BEA-000007341 isolated from a patient in Rwanda. The majority of patients with drug resistant tuberculosis in Sweden are immigrants from countries with high incidence of tuberculosis. Spread of disease to the Swedish born population is uncommon. However, an increasingly prevalent clone of isoniazid resistant tuberculosis was found among African immigrants, mainly living in the Stockholm area.

PMID: 12101614 [PubMed - indexed for MEDLINE]

56. **Laboratory methods for diagnosis and detection of drug resistant Mycobacterium tuberculosis complex with reference to developing countries: a review.**

Githui WA[1].

East Afr Med J. 2002 May;79(5):242-8.

[1]Centre for Respiratory Diseases Research, Kenya Medical Research Institute, P.O. Box 47855, Nairobi, Kenya.

**ABSTRACT**

OBJECTIVE: To outline principles, advantages and limitations of the currently available laboratory methods for diagnosis and detection of drug resistance of Mycobacterium tuberculosis complex.

DATA SOURCE: Published series of peer reviewed journals and manuals written on laboratory methods that are currently used for diagnosis and detection of drug resistance of Mycobacterium tuberculosis complex were reviewed using the index medicus, pubmed and medline search. Conventional bacteriological microscopy and culture, BACTEC, and molecular-based techniques were included. Basic principles, advantages and limitations of the cited techniques have been highlighted.

CONCLUSION: Conventional bacteriological microscopy and culture are usually used for diagnosis of tuberculosis (TB) particularly in developing countries. However, their limited sensitivity, specificity and delayed results make this provision inadequate. Despite the development of quicker and more sensitive novel diagnostic techniques, their complexity and high cost has limited their use in many poor-resource countries. Due to the rapidly growing TB problem in these countries, there is urgent need to assess promising alternative methodologies in settings with high disease prevalence.
PMID: 12638807 [PubMed - indexed for MEDLINE]

57. **Surveillance of drug-resistant tuberculosis and molecular evaluation of transmission of resistant strains in refugee and non-refugee populations in North-Eastern Kenya.**

Githui WA[1], Hawken MP, Juma ES, Godfrey-Faussett P, Swai OB, Kibuga DK, Porter JD, Wilson SM, Drobniewski FA.

Int J Tuberc Lung Dis. 2000 Oct;4(10):947-55.

[1]Centre for Respiratory Diseases Research, Kenya Medical Research Institute, Nairobi. wgithui@hotmail.com

**ABSTRACT**

SETTING: Three refugee camp complex clinics and an adjacent non-refugee treatment centre in North-Eastern Kenya.

OBJECTIVES: To use conventional and molecular epidemiology tools to determine: 1) the prevalence of drug resistance in newly diagnosed patients with smear-positive pulmonary tuberculosis in refugee and non-refugee populations; 2) risk factors for resistance in the two populations; and 3) whether IS6110 restriction fragment length polymorphism (RFLP) and spoligotyping showed similarities in DNA fingerprinting patterns of drug-resistant isolates that could infer transmission within and between the two populations.

RESULTS: Of 241 isolates from the camps, 44 (18.3%) were resistant to one or more drugs, seven of which (2.9%) were multidrug-resistant TB (MDR-TB). Of 88 isolates from the non-refugees, five (5.7%) were resistant to one or more drugs without MDR-TB. Drug resistance was higher in the camps than in the non-refugee population (OR = 3.7; 95%CI 1.42-9.68; P < 0.007). Resistance was significantly higher in one camp compared with the other two, despite a comparable ethnic distribution. Unusually, females were more associated with drug resistance than their male counterparts in both populations (OR = 2.3; 95%CI 1.2-4.8; P = 0.008). There was evidence of transmission of streptomycin-resistant strains in the refugee population. DNA fingerprints of resistant strains from the non-refugee population were unique and different from those in the refugee camps.

CONCLUSION: The observed high levels of drug resistance and MDR-TB, combined with evidence of transmission of strains resistant to streptomycin in the refugee population, suggest a need for strengthened TB control programmes in settings with a high risk of developing drug-resistant strains.
PMID: 11055762 [PubMed - indexed for MEDLINE]

58. **Anti-tuberculosis drug resistance surveillance in Uganda 1996-1997.**

Bretzel G[1], Aziz M, Wendl-Richter U, Adatu F, Aisu T, van Wijnen A, Sticht-Groh V.

Int J Tuberc Lung Dis. 1999 Sep;3(9):810-5.

[1]Armauer Hansen Institute/German Leprosy Relief Association, Würzburg. dahwd@geod.geonet.de

**ABSTRACT**

SETTING: Drug resistance surveillance conducted by the National Tuberculosis and Leprosy Control Programme (NTLP) Uganda from 1996-1997 in collaboration with the Armauer Hansen Institute/German Leprosy Relief Association (GLRA), Germany, for the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance.

OBJECTIVE: To determine the prevalence of primary and acquired anti-tuberculosis drug resistance in Uganda.

DESIGN: The survey area covered three GLRA-supported operational NTLP zones, corresponding to 50% of the Ugandan population. A representative random sampling of individual patients was chosen as sampling procedure. Altogether 586 smear-positive TB patients (537 new cases and 49 previously treated cases) were included in the survey.

RESULTS: For primary resistance the results were as follows: isoniazid (H) 6.7%, rifampicin (R) 0.8%, ethambutol (E) 6.1%, streptomycin (S) 13.4%, thioacetazone (T) 3.2%, pyrazinamide (Z) 0%, multidrug resistance (MDR) 0.5%; for acquired resistance they were: H 37.8%, R 4.4%, S 22.2%, E 11.1%, T 20.0%, Z 0%, and MDR 4.4%.

CONCLUSION: According to these data the NTLP Uganda has been effective in preventing high levels of primary drug resistance. If it is assumed that the sampling process reflects the distribution of new patients and previously treated patients in the study areas, the amount of acquired resistance (any resistance) in the community of smear-positive patients is approximately 5%. To further monitor programme performance the NTLP will embark on a nationwide survey in 1998/1999.
PMID: 10488890 [PubMed - indexed for MEDLINE]

59. **Drug resistant tuberculosis in sub-Saharan Africa: an estimation of incidence and cost for the year 2000.**

Carpels G[1], Fissette K, Limbana V, Van Deun A, Vandenbulcke W, Portaels F.

Tuber Lung Dis. 1995 Dec;76(6):480-6.

[1]Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium.

**ABSTRACT**

SETTING: Rwanda.

OBJECTIVES: To evaluate the tuberculosis (TB) drug resistance in Rwanda on smear positive sputa, collected prospectively at the start of the National TB programme, before the start of any treatment or retreatment. To adapt the scenarios of Schulzer et al (1992) to the data from Rwanda, in order to obtain an estimation of the number of drug resistant and multi-drug resistant (MDR) TB cases expected by the year 2000.

DESIGN: A total of 298 specimens (236 randomly selected new cases and 62 retreated cases), collected between January 1991 and June 1993, were sent to Belgium in 1% cetylpyridinium chloride. Drug resistance was determined using the proportion method.

RESULTS: MDR, i.e. resistance to at least rifampicin (R) and isoniazid (H), was observed in 3 (1.3%) out of 236 new cases and in 4 (6.5%) out of 62 treated cases. For new cases, single drug resistance to H, R and ethambutol (E) was 3%, 0.4% respectively; for treated cases it was 14.5%, 1.6% and 6.5 respectively. Based on the estimate of the size of the TB problem in sub-Saharan Africa by the year of 2000 (Schulzer), we calculated that the region should expect between 15,543 and 223, 417 cases of MDR, all forms combined (between 2.3 and 32.7 per 100,000 inhabitants), by the end of the century.

CONCLUSION: The results from Rwanda during the period studied do not appear dramatic. However, for some other developing countries, they may just represent the tip of the iceberg. Rapid recognition of resistance to the major antituberculosis agents is essential for control of TB. Integration of an MDR increase factor into the TB budget would not dramatically increase the total TB budget. Our data urgently point the the need for drug resistance surveys, followed by continuous drug resistance monitoring in high TB prevalence areas.
PMID: 8593367 [PubMed - indexed for MEDLINE]

60. **Anti-tuberculous initial drug resistance of Mycobacterium tuberculosis in Kenya: a ten-year review.**

Githui WA[1], Kwamanga D, Chakaya JM, Karimi FG, Waiyaki PG.

East Afr Med J. 1993 Oct;70(10):609-12.

[1]Respiratory Diseases Research Unit, Kenya Medical Research Institute, Nairobi.

**ABSTRACT**

Our experience at the Respiratory Diseases Research Unit (RDRU), over the last 10 years (1981-1990) on the initial drug resistance pattern, focusing on three drugs viz: isoniazid (H), streptomycin (S) and rifampicin (R) is presented. Records on all isolates of M. tuberculosis from one specimen of every newly diagnosed patient recruited countrywide between 1981-1990 were reviewed. We analyzed records of 6,514 isolates and found that total resistance to the three drugs had increased from 8.9% to 14.4%. Resistance to H alone increased from 6.8% to 10.2% while that of S alone from 0.8% to 1.8%. Resistance to R was between 0.1% and 0.3%. Generally, the increase in the resistance trend to both H and S was statistically significant (p = < 0.05 and 0.03, respectively). Although in our analysis we did not address the possible impact of HIV infection, we hope that these findings form a basis for evaluation of this and other possible factors on the emergence of anti-TB drug resistance in future studies.

PIP: A retrospective review of medical records of 6514 Mycobacterium tuberculosis isolates of newly diagnosed patients at the Respiratory Diseases Research Unit of the Kenya Medical Research Institute between 1981 and 1990 aimed to determine the initial drug-resistance pattern for isoniazid, streptomycin, and rifampicin. Overall resistance increased from 8.9 to 14.4% (p 0.001). The increase in the resistance trend to isoniazid and to streptomycin were statistically significant (6.8-10.2; p 0.05 and 0.8-1.8; p = 0.03, respectively) as well as the trend among isolates resistant to both isoniazid and streptomycin (1.2.4; p = 0.03). The trend was more pronounced during 1987-1990 than during 1981-1986. There was no trend in the resistance to rifampicin alone (0.1-0.3%). Just 4 isolates were resistant

to both isoniazid and rifampicin. Only 1 was resistant to both streptomycin and rifampicin. None were resistant to all 3 antibiotics. These first-line drugs are used widely in Kenya. These rates of initial resistance to the drugs are lower than those in other developing countries. The lower resistance rate is unlikely to continue, however, due to higher prevalence of HIV infection and the associated increase in tuberculosis incidence. These findings provide researchers a baseline with which to study M. tuberculosis drug resistance and other risk factors as drug resistance increases in Kenya.
PMID: 8187653 [PubMed - indexed for MEDLINE]

61. **Controlled clinical trial of a regimen of two durations for the treatment of isoniazid resistant pulmonary tuberculosis.**

Babu Swai O[1], Aluoch JA, Githui WA, Thiong'o R, Edwards EA, Darbyshire JH, Nunn AJ.

Tubercle. 1988 Mar;69(1):5-14.

[1]Kenya Medical Research Institute, Respiratory Diseases Research Centre, Nairobi.

**ABSTRACT**

Patients with pulmonary tuberculosis who were failures of primary chemotherapy with strains resistant to isoniazid or to isoniazid and streptomycin were allocated at random to receive a regimen of rifampicin and ethambutol for 6 (4RE) or 9 months (7RE), supplemented in both treatment series by streptomycin plus pyrazinamide for the first 2 months. The patients were treated in hospital for the first 2 months and thereafter treatment was supervised on a daily basis in the nearest health institution by an appointed member of staff or at home by responsible members of the community. A total of 306 patients was admitted and 226 patients remained for analysis at the end of chemotherapy, 179 with a strain resistant to isoniazid alone and 47 with a strain resistant to isoniazid and streptomycin. There were only two failures at the end of chemotherapy, one in the 6-month series who had resistance to both isoniazid and streptomycin pretreatment, and one in the 9-month series who had resistance to isoniazid alone. For the 144 patients with initial resistance to isoniazid alone assessed up to 30 months, the relapse rates were low in both series: 4% for the 72 patients in the 6-month series and 3% for the 72 patients in the 9-month series. However, for the 34 patients with resistance to both drugs, three of the 14 in the 6-month but none of 20 in the 9-month series relapsed.
PMID: 3051607 [PubMed - indexed for MEDLINE]

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