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Photos top to bottom: (1) Multiplex amplicon PCR technology for detection of RVF (2) Through song and dance children learned good handwashing habits to protect from communicable diseases in DRC (3) The main drivers of antibiotic resistance, photo by WHO

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META-ANALYSIS

Dengue Virus and Blood Safety: A Mini-Review of Research Publications

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ABSTRACT

The growing demand for donated whole blood and blood products to save lives has both health benefits and health risks for blood recipients at the same time. Dengue virus, a re-emerging viral disease poses a threat to blood safety, and it has spread to over 128 countries in the world. Several studies have documented transfusion-transmitted (TT) dengue, with the first cases being reported in China in 2002 and Singapore in 2008. To understand the magnitude and broader picture of the dengue virus and blood safety, we conducted a mini-review of published literature from the Scopus database. The review focused on the number of publications related to the dengue virus among blood donors. Using keywords 'Dengue virus' AND 'Blood safety', ' Dengue virus' AND 'Blood donors' and 'Emerging infectious diseases' AND "Blood safety" were used to extract data from the Scopus database which was downloaded as a CSV Excel file covering a period 2004 to 2021. This was followed by a data-cleaning exercise and a descriptive analysis to generate the frequency of the number of publications. Most studies, as can be seen in the review, were concentrated in tropical regions of the world. Globally, South America and the Asian regions had the largest number of publications; while at the country level, Brazil and India had the highest number. More research output was witnessed during the years 2014 and 2018. The regions that experienced more frequent outbreaks of the disease, with the exception Africa, published most of the research work. Therefore, much more research work is needed to protect the safety of blood donors in Africa.

BACKGROUND INFORMATION

Emerging infectious diseases such as the dengue virus (DENV), chikungunya virus, and many others are believed to threaten blood safety and availability throughout the world.[1] Most of these threats arise from the rapid spreading and mutating of viral infections.[2] To address this challenge, the WHO regularly publishes epidemiological reports on emerging infectious diseases in blood safety through fact sheets to its member countries. Member countries can access these reports on an online Global Database on Blood Safety program created by the WHO and its partners.[3] These reports assess data from different countries, highlight achievements, and address the challenges facing blood safety. It is worth noting that not all blood transfusion services have access to this database or follow the guidelines developed by the WHO.[4] Screening blood donors for known and unknown transfusion-transmitted infectious diseases to ensure blood safety around the world is the sole responsibility of all blood transfusion services.[5] It is therefore logical that these services do so much to protect blood recipients from emerging infectious diseases. Acquisition of infection by blood transfusion can be life-threatening and costly for various families struggling financially.

Dengue, a recurrent infection caused by DENV, poses a threat to blood safety, and has spread to more than 128 countries around the world.[6] It is estimated that half of the world's population is at a high risk of being infected with dengue virus.[7] Dengue is a vector-borne disease spread by an *Aedes* mosquito species that are widely distributed in the tropical and subtropical regions of the world.[8] An estimate of approximately 25,000 dengue-related deaths is reported annually from endemic regions of the world.[9] However, there is evidence that DENV can also be spread through blood and tissue transplantation.[10]

Several studies have documented transfusion-transmitted (TT) dengue whereby the first case was reported in China in 2002 and a second case in Singapore in 2008. This mini-review was part of a large study that sought to understand the seroprevalence of the DENV among blood donors and its implications for blood safety.[11] To understand the magnitude and broader picture of the DENV and blood safety, we conducted a mini-review of existing literature from the Scopus database. The database was chosen purposefully because it contains STM journal articles and the references found in them are accessible enabling both forward- and backward-looking searches. The review focused on the number of publications related to the DENV among blood donors and their sources around

the world. This was to help us synthesize the current discussion on whether blood donors coming from dengue-endemic regions need to undergo testing. Using keywords 'Dengue virus' AND 'Blood safety', 'Dengue virus' AND 'Blood donors' and 'Emerging infectious diseases' AND 'Blood safety', data were extracted from the Scopus database, which was downloaded as a Ms. Excel CSV file. This was followed by a data-cleaning exercise and a descriptive analysis to generate the frequency of the number of publications. Ms. Excel was also used to present the global distribution of studies that focus on the DENV among potential blood donors from a global perspective.

Blood Transfusion as A Route For Dengue Virus

The burden and implication of the DENV on blood safety in some tropical and subtropical regions where the virus is endemic are currently unknown. Therefore, there is quite limited information on DENV transfusion-transmitted cases to help countries improve their disease surveillance systems within blood transfusion services.[12,13] The current review provides a snapshot of what is happening globally and helps fill this gap. Blood transfusion services around the world, as recommended by the WHO, are required to analyse transfusion risks and determine whether emerging infectious diseases are threats to blood safety in their country.[14] However, most of these countries lack the capacity to implement these recommendations.[15] The dependence on the WHO and other international organizations for epidemiological data before acting is a limitation that requires immediate intervention from countries in low-middle income countries (LMIC). Most African countries fall into this category and have limited human and financial resources. A robust surveillance system for the DENV not only provides the information required to maintain a blood supply but also helps and supports a safe blood supply.[16]

There is evidence that all patients who received donated blood from asymptomatic blood donors develop dengue-related symptoms after a few days.[17] Studies in Brazil, India, and Singapore have shown a 0.5% rate of dengue viremia among asymptomatic blood donors during DENV outbreaks.[18,19] Another study among healthy blood donors in Saudi Arabia has shown a seroprevalence between 1-7% for the DENV-NS1 antigen, the anti-DENV IgM antibody, and the anti-DENV IgG antibody.[9] Similarly, a study conducted in the northeast part of Mexico among blood donors using the enzyme-linked immunosorbent assay (ELISA) technique found 59% and 2% for IgG and IgM, respectively.[20] In India, a country that is the most affected by the DENV, a study conducted in the Pune region of western India showed seropositivity of 0.64% and 6.4% for NS1 and IgM respectively in 2017.[21] Despite all this evidence, very little is being done to implement mandatory screening for the DENV among blood donors to ensure blood safety.[22]

In the Sub-Sahara region, very little is known about the prevalence of DENV among blood donors, and the likelihood that transfusion-transmitted Dengue fever has never been reported.[23] Limited studies conducted in a few countries (Figure 1) have shown evidence of dengue seromarkers in asymptomatic blood donors. For example, a study conducted among Cameroonian blood donors showed a seropositivity rate of 5% for all serological markers using a simple immunochromatographic (IM) diagnostic kit.[24] In Tanzania, a similar study finding showed a seroprevalence of 50.6% DENV IgG among blood donors in Zanzibar.[25] Thousands of efforts and investments are required to conduct research studies on emerging infectious diseases and blood safety in Africa.[26]

Blood transfusion services (BTS) in most developing countries are faced with numerous challenges that hinder their ability to provide safe blood to their blood recipients. One of the challenges is the lack of financial support from their national government to enable them to perform their functions.[27] Consequently, there is an inadequate supply of blood products, and much worse infection-contaminated blood supplies. This situation, hinders the performance and delivery of BTS services as a state organ with the mandate to supply safer blood to its citizens. Providing quality blood products to patients who urgently need blood transfusion is a fundamentally essential element of a functional blood transfusion service for any country.[28] Therefore, this mini-review aimed to analyse the available literature and draw a conclusion on whether it was necessary to include DENV as a mandatory test in all countries with the presence of the disease.

Current Evidence of Post-Transfusion Dengue Fever

It is an accepted principle that population growth and the increasing incidence of diseases raise the probability that blood products from viraemic individuals could be provided to vulnerable blood recipients.[29,30] Previous studies in Hong Kong and Singapore have documented dengue transmitted by transfusions through blood derived from asymptomatic individuals.[31,32] In regions with frequent outbreaks of the DENV, blood services would be required to assess whether urgent steps are needed to ensure blood availability.

In a population where dengue is widespread, the possibility of receiving blood from asymptomatic viral donors is also not resolved by symptom-based exclusion.[33] To date, studies show that there is at least a duration of infectivity of 1-2 days before symptoms develop and therefore a donor donating his/her blood during this period could pose a threat to people who would receive the blood.[34] During the dengue outbreak, the occurrence of viremia among asymptomatic people, including blood donors, is unknown.[35] For example, in a study in Hong Kong and Singapore, four recipients who acquired the virus by blood transfusion endured a relatively mild course of the disease and eventually recovered with very minimal sequelae.[36] More details are still needed to establish a concrete conclusion on whether blood transfusion could be an alternative route of viral transmission. However, fear always comes from the loss of a potential blood donor pool due to deferral.

Global Distributions of Research Work Around the Dengue Virus among Blood Donors

Part of this review was to understand the distribution of research articles related to DENV among blood donors. As shown in Figure 1, most of the studies were concentrated in the tropical regions of the world. This shows a pattern similar to that seen in the distribution of the DENV in epidemiological studies.[37-41] South America and the Asian regions had the largest number of

publications; and at the country level, Brazil and India had the most publications. One of the explanations for this phenomenon is the increased number of dengue outbreaks in these regions in the recent past.[42] Few countries in these regions have implemented additional tests for the DENV in their test algorithms.[36,43-51] Thus, this raises the question as to why other regions do not implement dengue screening among blood donors, even with the predominant evidence. There are a number of reasons; first, several studies have documented the presence of viral markers (IgG, IgM, NS1, and RNA) among healthy donors in dengue-endemic regions.[36,48,52] It is therefore a major challenge among transfusion experts in deciding whether to screen or not, given that viral markers can also be detected among healthy subjects in the endemic regions.[53-55] Secondly, financial constraints and the cost of blood transfusion are some of the reasons in the literature to explain why there is a lack of predonation screening for the DENV.[56,57]

As shown in this review, very minimal research activities were observed in the African region regardless of the presence of dengue on the continent. A similar observation has also been made in a limited number of epidemiological research activities on the continent.[58] For example, single studies have been conducted in Tanzania, Cameroon, Ghana, Nigeria, Egypt, Burkina Faso, and Sudan.[24,59-62] Therefore, very little is known about the implications of the DENV among blood donors in tropical regions of Africa.[63] A simple intervention for Africa would be to implement a voluntary call system after a blood donation is made to the blood transfusion service about the appearance of clinical symptoms consistent with the infectious disease. This intervention is called a post-donation illness report (PDIR), which would be cheaper and more affordable in most poor countries. The passive approach of voluntary reporting does not require BTS to screen donors but requires the donors to report post-donation illness. Studies to assess the effectiveness of PDIR as a mitigation for viral agents, such as DENV, would help improve blood safety.[64] In addition, there is an opportunity for African researchers to conduct studies with the aim to protect the safety of blood recipients.[65]

Most of the investigations in the reviewed articles showed a high seroprevalence of dengue viral markers (IgM/IgG) among potential blood donors who were asymptomatic.[66-74] Therefore, it is essential to practice meticulous preventive techniques to ensure the safety of blood transfusions and to prevent the spread of the DENV in an endemic area.[75]

Distribution of Published Work on the Dengue Virus among Blood Donors Around the World

In recent years, the number (84) of publications related to the DENV among blood donors has increased drastically. As part of this mini-review and to understand research output, we purposed to establish the number of publications since the year 2004 to 2021. Between 5 and 12 research outputs were observed between 2014 and 2018 as shown in Figure 2. The main reason for this increase in production is probably related to the increased number of reported dengue outbreaks in endemic regions.[76] The impact of the COVID-19 pandemic on research output can also be seen in the decrease in the number of publications between 2019 and 2020. The reduction in the number of publications can be attributed to a change of focus to address the pandemic. However, a resumption of research output is also observed in 2021. This could have been due to understanding the current pandemic, which had an initial reaction full of uncertainties. This is a true reflection of what happens when there is a disease outbreak or a pandemic. The emergence of new viral infections can affect all aspects of our lives, including blood safety and availability.

FIGURE 1: Global Distributions of Research Work on Dengue Virus and Blood Safety

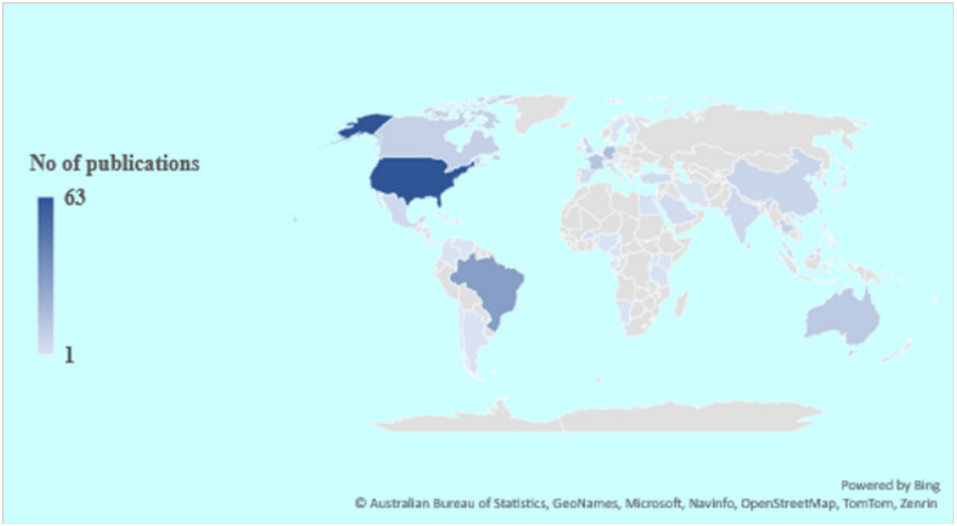
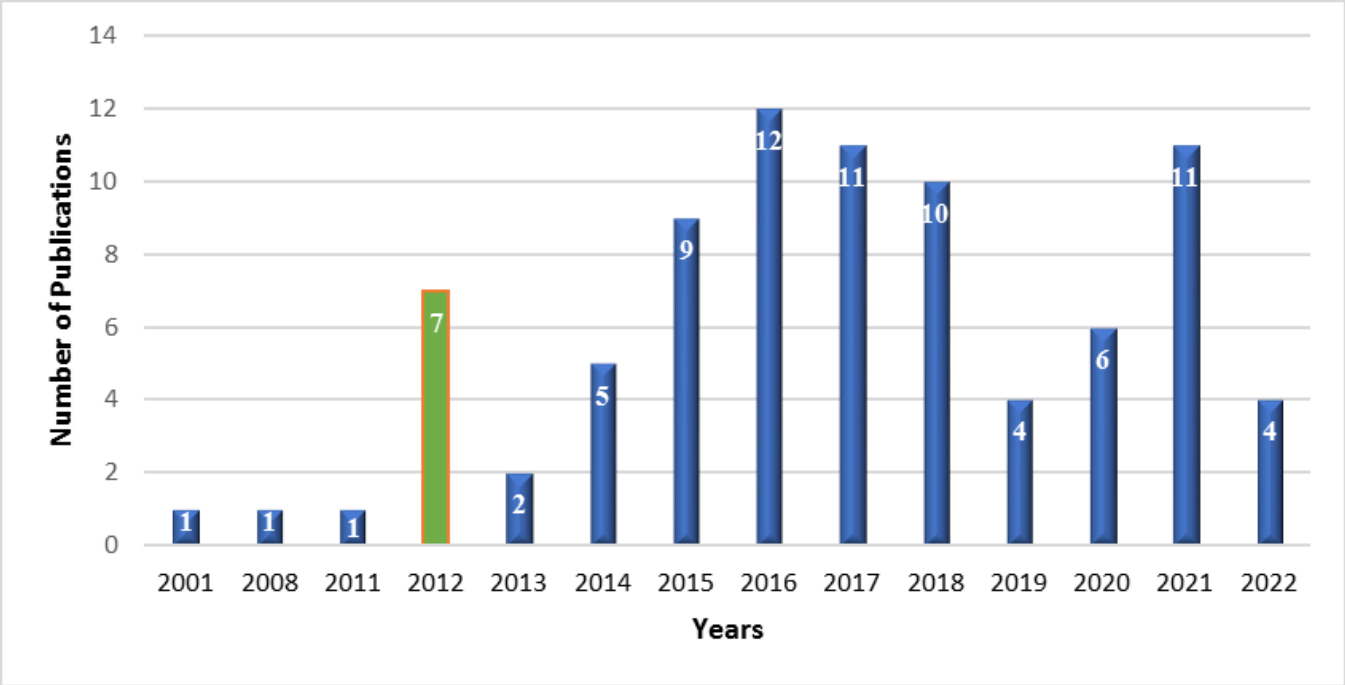


FIGURE 2: Distribution of Published Work From 2001 to 2022



CONCLUSION

This mini-review has shown that there is evidence that asymptomatic blood donors can transmit the DENV to blood recipients. Most of the research work was conducted in regions that experience frequent outbreaks of diseases, except in regions on the African continent. Therefore, much work is needed to protect the safety of blood donors in Africa.

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ORIGINAL ARTICLE

Use of Hair Samples for Monitoring of Antiretroviral Therapy Adherence

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ABSTRACT

Introduction: Measurement of antiretrovirals (ARVs) drug concentration in biological matrices such as blood and urine has been used previously for monitoring adherence. Unfortunately, they only reflect ARV doses taken within 1 to 2 days of sampling. Hair testing has become the most preferred tool to determine chronic exposure to some drugs, especially drugs of abuse, because of its long detection window.
Objective: This study, evaluated the utility of hair samples in therapeutic drug monitoring (TDM) as an indicator of ART adherence.
Methods: This study used nevirapine (NVP), an ARV integral component of the first line ART in Kenya, for many years. Matched hair and blood samples were obtained from 234 and 328 consenting HIV patients on first line ART with virologic failure (viral load >1000 copies/mL) and suppressed viral load (VL<1000 copies/mL) respectively. The ARV plasma and hair concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: The calculated median; interquartile range (IQR) of NVP levels in hair and plasma samples were 36.8ng/mL and 19.32ng/mL respectively. There was no significant difference between the level of NVP in hair and matched plasma samples (Wilcoxon signed rank test; Z = -0.93, $P > 0.05$).
Conclusion: The study has demonstrated that analysis of ARV drugs in the hair can determine drug exposure as an alternative to conventional plasma drug analysis, especially in our settings where laboratory facilities and skilled personnel to do phlebotomy are few or lacking.

INTRODUCTION

The treatment of HIV is complex, and for a successful clinical outcome, adherence to the Antiretroviral Therapy (ART) must be considered. ART is a critical component in reducing the HIV epidemic.[1] Globally, progress has been made in the HIV research and treatment cascade. Antiretroviral therapy has been used globally by 67% (25.4 million) of people living with HIV at the end of 2019.[2] In Kenya, 74% (1,112,254) of adults and 73% (71,500) of children in need of ART were receiving antiretroviral (ARVs) by the end of 2019.[3]

A remarkable proportion of these patients (68%) were virally suppressed.[2] At the time of the study, the first-line ART in Kenya for children, youth, and adults included two nucleoside reverse transcriptase inhibitors (NRTIs) plus one non-nucleoside reverse transcriptase inhibitor (NNRTI). The common NRTIs were zidovudine (AZT), stavudine (d4T), tenofovir (TDF), or lamivudine (3TC), NNRTIs were nevirapine (NVP) and efavirenz (EFV).[4] The first line of ART was a combination of one NNRTI (NVP or EFV) and two NRTIs (3TC, AZT).[4]

Analysis of ARVs in the blood of PLWH is a direct technique used to monitor PLWH's adherence to ART. Measurement of ARV levels in PLWH on ARVs is the foremost step in monitoring ARV drugs' adherence.[5] Unfortunately, the whole process involving the analysis of drugs in blood is expensive and unaffordable in most resource limited settings.
Hair is becoming an essential biological specimen for drug testing besides urine and blood. Cocaine, opiates, and amphetamines have been analysed in the hair.[5] The hair sample is obtained quickly, does not require specialized personnel to collect, and is stored at room temperature while awaiting analysis in the laboratory.[5] These features provide obvious cost and feasibility advantages for hair collection and storage over plasma samples. Further, hair analysis provides an advantage over blood or urine testing in assessing long term adherence.[5,6] This study, therefore, evaluated the utility of hair samples in TDM as an indicator of ART adherence to be used as an alternative method to ARVs analysis in blood. Hair analysis represents a cheaper option and is easier to adapt to the hospital setting for monitoring adherence to ART among people living with HIV

(PLWHIV) in low resource settings or where skilled personnel to perform plasma analysis are few or lacking.[7]

METHODS

Study Design and Site

A cross sectional study design was used in the sample collection. The consenting participants were recruited at two regular HIV care clinics Nairobi and Kisumu Counties. This study recruited patients who had health records available and had been on ART treatment for at least 12 months and were deemed to have attained steady state drug plasma concentrations. The PLWHIV with viral loads of more than 1000copies/mL was 81.7% of the study participants, while those whose viral load was less than 1000copies/mL after 12 months of being on a first line ART regimen were 62.2% of the study participants. A total of 268 and 145 participants were recruited from the Nairobi and Kisumu counties, making a total of 413 participants. The levels of HIV viral loads were extracted from the participants' medical folders.

The Inclusion Criteria for Cases and Controls

Eligible study participants were PLWHIV aged above 18 years on nevirapine (NVP) for over 12 months who attended the two HIV treatment care clinics in Kisumu and Nairobi and were willing to give written informed consent voluntarily. In addition, eligible study participants were required to have viral load results at month 12 of treatment. Participants were stratified into two groups, those with non-viral suppression with HIV-1 RNA viral load (VL) >1000 copies per ml of blood after being on treatment for a period of not less than 12 months and those with suppressed viral loads with HIV-1 RNA viral load (VL) <1000 copies. Participants' hair samples were taken from those who had not used hair dyes or permanent hair products in the previous three months and had hair length of not less than 1cm long.

Sample Collection

Plasma sample

The Blood was drawn from the vein located on the inside of the elbow of the participants. Approximately 5ml of whole blood was collected in plasma preparation tubes (PPT) with ethylenediaminetetraacetic acid (EDTA). The blood was centrifuged to obtain plasma aliquots and stored at -80˚C. The plasma samples were transported on dry ice to the KEMRI Centre of microbiology laboratory for storage and laboratory testing then stored at -80˚C at the central laboratory until NVP extraction.[8]

Hair Samples

The hair samples of the participants were cut with scissors from the human occipital scalp. The hair samples were covered in aluminums foil and placed in zip lock plastic bags with labels. They were delivered to the KEMRI HIV laboratory in sealed envelopes at room temperature and batched for testing.[9]

Materials and Reagents

Jomo Kenyatta University of Agriculture and Technology generously donated the NVP drug and carbamazepine as an internal standard (IS). The used HPLC grade acetonitrile, methanol (MeOH), analytical grade trifluoroacetic acid (TFA), Di-Methyl ether, ethyl acetate and ammonium acetate were purchased from Sigma Aldrich/Merck.

Preparation of Standard Solution for hair samples

A stock solution of 1mg/ml of both nevirapine and internal standard carbamazepine were prepared in methanol. The internal standard solution was prepared by diluting the stock solution with methanol to give 100ng/mL solution. Standard solutions were prepared by subsequent dilution of the stock solution with methanol to acetonitrile (20:80, v/v) concentrations ranging between 10ng/mL to 500ng/mL, with 100µL of carbamazepine was added to each solution.

Preparation of Standard Solution for Plasma Samples

A stock solution of nevirapine was prepared at a concentration of 1mg/ml in methanol. The working standard solutions were prepared in water: Acetronitrile (20:80, v/v) from stock solution. Standard solutions were prepared by subsequent dilution of the stock solution with methanol to concentrations ranging between 10ng/mL to 1000ng/mL.[10]

Extraction of NVP from Hair Samples

Hair samples of 10mg from each participant were weighed, cut into approximately 3mm, and placed into a glass test tube (16mm diameter x 125mm height). Internal standard (IS) was added to each sample at a 100ng/mL concentration. The extraction of NVP from hair was done by adding 2mL of methanol/trifluoroacetic acid (9/1, v/v ratio) solution to the samples and shaking in a reciprocal shaker for 16 hours.[11] The organic solvent was then evaporated to dryness by nitrogen gas. The extracted drugs were cleaned up further by liquid-liquid (Liq-Liq) extraction as described below. Briefly, 0.5ml of 0.2M sodium phosphate buffer (pH 9.4) was added to the sample and vortex mixed for 30 seconds. Three (3mL) dimethyl ether/ethyl acetate (1:1) measuring was added, and the mixture vortexed three times, each for 1 minute. This was then centrifuged at 3000 rotations per minute (rpm) for 10 minutes. The sample was frozen in dry ice before transferring the supernatant layer to a fresh test tube (13mm diameter x 100mm height). One hundred microliters (100µL) of 1% trifluoroacetic acid in methanol were added to the supernatant layer, and the sample evaporated to dryness using nitrogen gas. Reconstitution was done with 0.5ml of acetonitrile/water 1:1, and vortex mixing followed, each for 30 sec. The extracts were filtered using 0.45µm microfilters and then transferred into auto-sampler vials, and a 10µL aliquot was injected into the LC-MS/MS for analysis.

Extraction of from Plasma Samples

The protein precipitation method was used to extract NVP from plasma samples, followed by a liquid-liquid extraction process.[7] Plasma samples (0.2mL) were transferred to 2mL of Eppendorf tubes, and 0.2mL of organic solvent (50mM ammonium acetate solution: acetonitrile in the ratio1:6) was added for protein precipitation then vortex for 3 minutes. One (1mL) ethyl acetate was added to the above samples to extract analytes into the organic layer and hold the endogenous plasma materials water-soluble in the aqueous layer to decrease the matrix influence. The resulting samples were vortexed for 5 minutes and centrifuged for 10 minutes at 4°C at 12,000 rpm. The

supernatant was transferred into a new glass tube and evaporated at 37°C under nitrogen. After evaporation, a second ethyl acetate extraction was performed. For the dried extract, the organic layer was added and evaporated. Aliquots of 500µL made up of HPLC grade water and acetonitrile in a ratio of 1:15 of acetonitrile was used to reconstitute the residue, and 10µL aliquot was injected into the LC-MS/MS systems.[8]

NVP Quantification using LC-MS/MS

A very sensitive, selective, and reproducible LC-MS/MS method developed by[11] was validated and used to analyse hair samples and a LC-MS/MS method developed by [8]was validated and used to analyse plasma samples. The LC/MS/MS analysis was conducted under positive MRM mode, and the compounds were identified using ion mass and retention times. The triple quadrupole 6410 LC/MS and a Agilent HPLC system (Agilent) 1100 A were used in the study. The columns used for separation were Kinetex Evo C-18 (3mm x 100mm), 5µm for hair samples, and C18 4.6mm by 150mm), 5µm particle size for plasma samples. Mobile phases' flow rate was 0.45mL/minute. The temperature of the autosampler was 15°C, and the injection volume was 10µl. The mobile phase was made of 0.1% formic deionised water (mobile phase A) and 0.1% formic Acetonitrile (mobile phase B). The isocratic mode was used in the ratio of 20:80 of A to B; the column's temperature was set at 30°C. The total run time for LC was 10 minutes. The analytes and carbamazepine (CBZ) detection were done on a triple quadrupole mass spectrometer, equipped with an electro ion spray ionisation mode and positive ion mode. Masslynx software was used to control all LC and MS parameters. Transition ions and optimal conditions used to obtain a relative abundance of product ions were as follows: The cone voltage during the analysis of NVP and CBZ were 36V and 32V respectively. The collision temperatures were 25 and 20 °C for the analysis of NVP and CBZ respectively.

Multiple reaction monitoring (MRM) was used to quantify both NVP and IS. Precursor ions and product ions were optimized by directly injecting 1000ng/ml solutions of NVP and IS into the MS in a suitable mass range, respectively, in positive and negative polarity modes using the atmospheric technique. The highest intensity for [M+H] + ions was found in positive mode for NVP and IS, both accepted protons. The compounds and their relative molecular mass, precursor ions, and product ions are 267>226 and 237>197 for NVP and CBZ respectively. The LC-MS/MS method has high selectivity for the only precursor. The product ions of the analyte of interest monitored by MRM mode supported the precursor m/z and its fragment m/z (MRM transition) for every analyte.

Statistical Analysis

The concentrations of ARVs in hair samples were back calculated from the calibration curve of the NVP/CBZ ratio against concentration. The concentrations of ARVs in plasma samples were back calculated from the calibration curve of the NVP area against concentration. The Kolmogorov-Smirnov test and the Shapiro-Wilk test were used to determine the normality of data. The Wilcoxon W Test was used to evaluate the hypothesis. The test involves ranking the absolute paired differences between of plasma and hair.

Ethical approval

This study was approved by the KEMRI Scientific and Ethics Review Committee SERU Protocol Number 3214. Prior consent was sought from the specific hospitals where the sample collection took place in the two County Governments of Kisumu and Nairobi. Participants were assured that all information obtained from them would be treated with maximum confidentiality, and no names would be published in any report. All biological waste was collected in biohazard bags, followed by incineration.

RESULTS AND DISCUSSION

Demographic Characteristics of the Study Population

Study Population (Table 1) shows the demographic characteristics of study participants. A total of 413 participants on NVP as part of their ARV regimen were recruited for the study. The women participants contributes 58.4% of the study sample size. The median age was 41 years and the median duration of treatment was 6 (range, 3-11) years. Hair samples of 223 participants were collected and presented to the laboratory. Only 105 participants donated enough hair quantity >10mg since some were willing to donate their hair samples, but their hair was very short. The 328 plasma samples were collected and submitted to the laboratory to quantify nevirapine (NVP). However, only 308 plasma samples were analysed for NVP level because some participants who donated blood samples were reluctant to grant their hair samples. Some donated hair and were unwilling to donate blood samples. Therefore, participants with the matched plasma and hair samples were only 94 which represented 22% of the participants. 51.4% of participants who doneted their samples for analysis missed taking current ARVs the whole day or more (Table 1).

Determination of Nevirapine Levels in the Plasma and Hair Samples

The *P* value of the Shapiro-Wilk Test and Kolmogorov-Smirnov Test were all less than 0.05, implying that the data significantly deviated from a normal distribution.
The NVP concentration medians for hair and plasma samples were 36.8ng/mL for 19.3ng/mL, respectively. In 25% of participants, the NVP plasma and hair concentrations were less than or equal to 17.1ng/mL and 5.2ng/mL, respectively. Additionally, in 75% of participants, NVP hair and plasma concentrations were less than or equal to 102.3ng/mL and 562.6ng/mL respectively. The hair samples' interquartile range (IQR) was (17.10 to 102.30ng/mL), which means that 50% of the hair NVP concentrations were between 17.10 and 102.30ng/mL. The interquartile range (IQR) for the plasma samples were (5.23 to 562.65ng/mL). This also means that 50% of the plasma NVP concentrations were between 5.23 and 562.65ng/mL.

The hair NVP concentration ranged between 5.4 to 1211.5ng/mL while the plasma (Figure 1). NVP concentrations ranged between 0 to 5000ng/mL. The plasma concentration was greatly dispersed as compared to the hair concentration. This was in line with the report by [8] that high upper concentration limits of NVP's plasma concentration extended up to 9000ng/mL. Low plasma NVP concentrations can arise due to poor adherence.[12] However, some patients can still have low plasma levels of NVP, even while still adhering to the ART regimen. This

could result from some factors such as interpatient variability in exposure to NVP, drug interactions, and interaction of drug with food.[12]

The variance of the NVP levels in the hair and plasma samples were 14793.4 and 197158.0, respectively. There was a high variability of NVP levels amongst the participants in hair as compared to plasma samples. The high variability of NVP levels in plasma across could arise due to differences in patients' body mass index, age, race, rate of metabolism, and genetic.[7,13]

Comparison of NVP levels in the plasma and matched hair samples

The hair and matched plasma samples were analysed and compared to determine whether there is a significant difference in the levels of NVP between the two samples. The representative chromatograms of hair and matched plasma samples are as shown in (Figure 2). The mean NVP concentration in hair was 104.9ng/mL, while the mean NVP concentration in matched plasma samples was 525.4ng/mL. Wilcoxon signed-rank test was used to test the hypothesis and revealed that there was no significant difference between the level of NVP in hair and matched plasma samples (Table 3). These results matched what Gandhi's and others found $P > 0.05$: a significant difference between efavirenz concentrations in the hair and plasma samples.[14,15]

Nevirapine and efavirenz are two of the most commonly prescribed NNRTIs. The WHO recommends regimens consisting of a NNRTI backbone with either efavirenz or nevirapine.[16] This showed that the drug deposition in the hair depends on its concentration in the plasma and thus concluded that NVP concentrations in hair samples are, therefore, a strong measure of adherence just like the plasma samples.[17]

FIGURE 1: Representative LC-MS/MS chromatogram of hair sample showing the compounds's and their precursor ions, and product ions are 267>226 and 237>197 for NVP and CBZ respectively

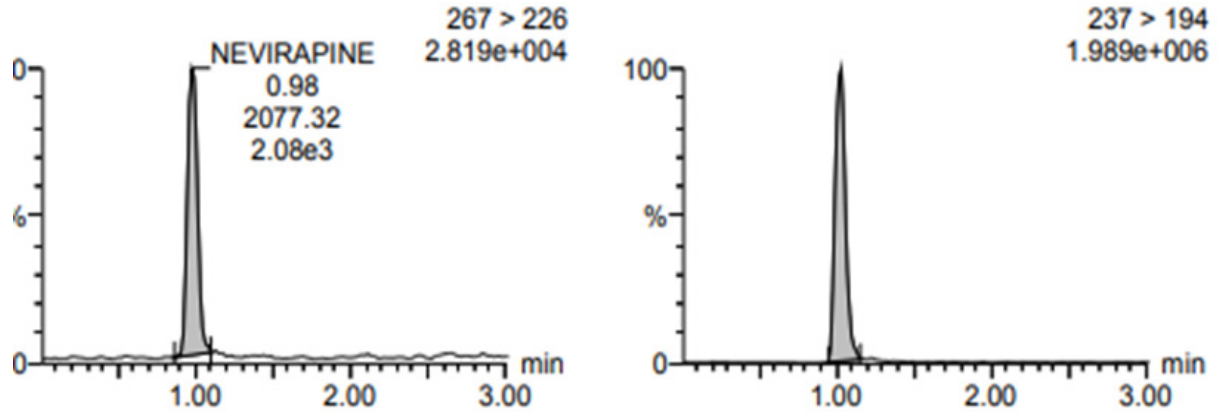

FIGURE 2: Representative LC-MS/MS chromatogram of matched plasma sample showing NVP precursor ions, and product ions, 267>226 respectively

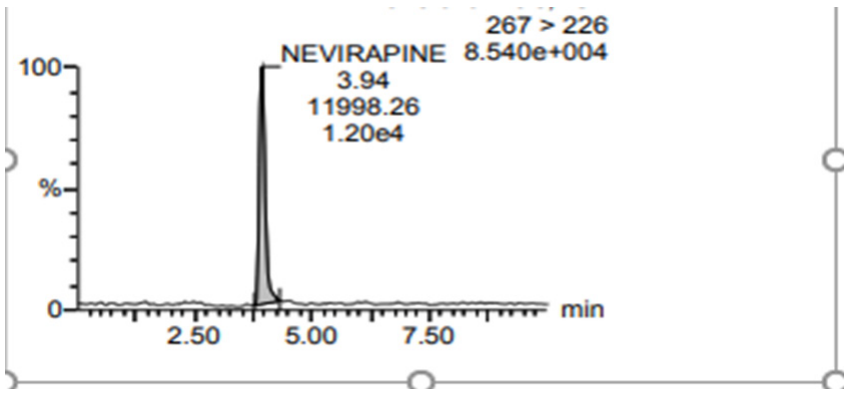

TABLE 1: Demographic Characteristics of Study Participants (N=413)

Variable		Frequency	Percentage
Age (years)	Median (range)	41 (34-49)	
	20-30	69	16.5
	31-40	135	32.6
	41-50	128	30.8
	>51	81	20.1
Gender	Female	242	58.4
	Male	171	41.6
Viral load	>1000copies/mL	210	50.8
	<1000copies/mL	47	11.2
samples types	Plasma samples	328	74.6
	Hair samples	223	25.4
Donated samples	Matched hair and plasma samples	94	22.0
	Donated only one sample type	329	78.0
Hair samples >10mg	Yes	105	47.1
	No	118	52.9
Plasma samples in good-conditions for analysis	Yes	308	93.9
	No	20	6.1
Duration on treatment	Median (IQR)	6	(3 - 11)
	1 - 5 Years	201	48.5
	5 - 10 Years	104	25.1
	>11 Years	108	26.4
Initial ARV type	lamivudine, nevirapine, stavudine	102	24.5
	lamivudine, efavirenz, tenofovir	194	46.8
	lamivudine, efavirenz, zidovudine	32	7.7
	lamivudine, nevirapine, tenofovir	26	6.3
	lamivudine, nevirapine, zidovudine	54	14.6
Changed initial ARV type	Yes	187	45.3
	No	226	54.7
Current ARV type	lamivudine, nevirapine, tenofovir	340	82.2
	lamivudine, nevirapine, zidovudine	72	17.3
	lamivudine, nevirapine, stavudine	1	0.5
Did not take ARV for a day or more	Yes	224	54.1
	No	189	45.9

TABLE 2: Mean, Median and Inter Quartile Range (IQR 25-75) Concentration of Nevirapine in Hair and Plasma Samples

Sample type	N	Mean (ng/mL)	Median (ng/mL)	IQR range (ng/mL)
Hair samples	105	106.1	36.8	17.1 – 102.3
Plasma samples	308	430.2	19.3	5.2 – 62.6

TABLE 3: Mean Concentration of Nevirapine in the Matched Hair and Plasma Samples

	N	Mean ± SE (ng/mL)
Hair concentration	94	104.90 ± 19.7
Plasma concentration	94	525.35 ± 96.2
Z = -0.928, *P>.05*		

CONCLUSION

There was no significant difference between the levels of NVP in hair and matched plasma samples. Therefore, as alternative to conventional plasma sample, hair sample can be used to monitor ART adherence, especially in our settings where laboratory facilities and skilled personnel to do phlebotomy are few or lacking. Determination of levels of other types of ARV drugs in hair and blood matched samples is highly recommended to ascertain whether hair sample can be used to monitor adherence to such drugs.

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EAHRC
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ORIGINAL ARTICLE

Seasonal Transmission Dynamics of Rift Valley Fever in Kilimanjaro Region, Tanzania

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ABSTRACT

Background: Rift Valley Fever (RVF) is a zoonotic disease that affects both animals and humans. Under reporting, misdiagnosis caused by the broad spectrum of symptoms presented by the disease, and limited access to rapid and accurate laboratory confirmation have led to an undefined burden of RVF. Reports are available that show the circulation of the virus during inter-epidemic periods, implying an endemic circulation of RVFV. This study aimed to determine RVFV transmission across annual seasons and demographic factors that are independently associated with exposure to RVFV.

Methodology: Repeated serosurveys were performed during the long rainy, short rainy, and dry seasons in Lower Moshi area of Moshi district, Kilimanjaro region from January to December 2020. The goal was to determine seroprevalence against RVFV antibodies in humans and factors associated with seropositivity. Detection of RVF antibody was performed by competitive Enzyme-Linked Immunosorbent Assays (cELISA) using serum samples. Stata statistical software version 15 was used for data analysis. Descriptive statistics was carried out, whereby categorical variables were summarised using frequencies and percentages. Numeric variables were summarised using median and interquartile range. Logistic regression was used to assess factors associated with RVF seropositivity.

Results: A total of 446 individuals were involved in the analysis. RVF seroprevalence was highest during rainy season (20.4%) and lowest in the dry season (4%). The overall annual seroprevalence of RVF was 12.8%. Season, participant age, and large number of residents in a given household were found to be significantly associated with RVF seropositivity ($p<.05$).

Conclusion: RVFV demonstrates an endemic circulation in Lower Moshi area of Kilimanjaro region, suggesting the site is a potential RVF hotspot. Based on this study's findings, we recommend close surveillance of RVF in the study area and other areas with similar ecology in Tanzania as a means of preparedness for future unpredicted RVF outbreaks.

BACKGROUND

Rift Valley Fever (RVF) is a Zoonotic disease that is caused by a Rift Valley Fever Virus (RVFV) belonging to family Bunyaviridae of genus Phlebovirus.[1] The disease primarily affects animals but can also infect humans.[2] Human transmission of RVF is through direct contact with infected animals mainly livestock such as; cattle, sheep, goats, buffalo, and camels, and bites from infected mosquitoes especially the Aedes and Culex species. Humans are at risk of being infected by RVFV when they live and engage in activities that bring them into contact with animals, animal products and vector mosquitoes.[3]

Due to under reporting, misdiagnosis caused by a broad spectrum of symptoms the infection can present with, and limited access to rapid and accurate laboratory confirmation, the burden of RVF remains undefined globally.[4] Reports show that RVF has mainly affected the Arabian Peninsula and Africa,[2,5] with over 3000 reported suspected and confirmed cases and approximately 1000 deaths from 2000 to 2007. In Tanzania, recent RVF outbreaks were reported in 2006 and 2007; Overall outbreaks occurred in 39.2% of the districts in Tanzania.[6] Such outbreaks may result in major societal impacts, including significant economic losses due to severe illnesses and abortions in domestic animals, which are significant income sources in different communities. Furthermore, RVF outbreaks have negative consequences on trade resulting from cross-border quarantines of domestic animal movements and animal products.[6]

Despite its rare occurrence and absence of its epidemics in recent years, studies in Tanzania continue to report the existence of RVF during the inter-epidemic period, in different regions of the country for both

animals and humans, hence showing evidence of virus circulation in Tanzania.[7–9] Most of the studies in Tanzania have attempted to determine the point prevalence of RVF with few or no studies that determine the period prevalence of RVF, showing the effect of seasonality on the transmission of RVFV as evidenced by the prevalence of RVF antibodies across seasons. Nonetheless, Regardless, key questions regarding the epidemiology of RVF remain to be; where the virus hides during the inter-epidemic periods, where RVF hotspots are, when will the next outbreak occur and whether seasonality affects RVFV transmission. This study aimed to determine RVFV transmission across annual seasons and demographic factors that are independently associated with exposure to RVFV.

METHODOLOGY

Study Design

The study involved comprehensive seasonal cross-sectional serosurveys to identify the seasonal inter-epidemic transmission dynamics of RVFV and its potential reservoirs across different seasons of the year 2020. The study conducted repeated seasonal cross-sectional serosurveys for a period of 12 months in a distinct ecological area with intimate contact between humans, livestock and vector mosquitoes.

Study Area

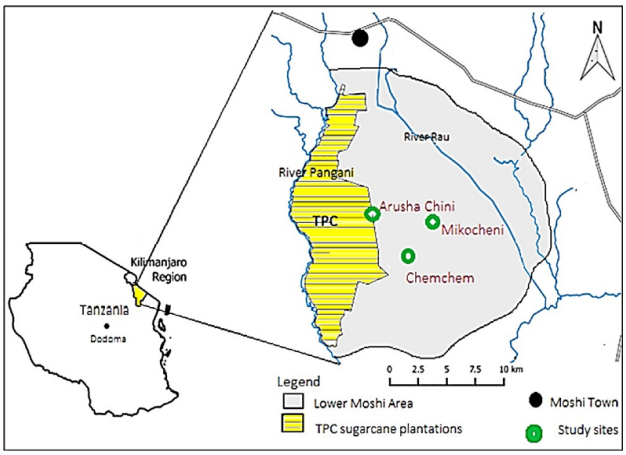
Figure 1 shows the study site as shown elsewhere.[10] The study was conducted in 3 villages, namely; Mikocheni, Chemchem, and Arusha Chini of Lower Moshi in Moshi Rural district of Kilimanjaro region of Tanzania. The site was purposively selected due to the presence of different RVFV hosts (Mosquitoes, humans, and ruminant animals) hence maximising the detection of the virus. Lower Moshi is located on the southern foothills of Mount Kilimanjaro, bordered by Kikuletwa River, Hai District, and Manyara Region on the West, while to the East, it borders Mwanga district. It's elevation ranges between 700 and 800 metres above sea level. Culex spp and Aedes spp are the main RVF vectors in this area.[11,12]

Lower Moshi has numerous water streams forming the irrigation channels for rice and sugar cane, covering an area of about 1100 hectares.[13] The area has 2 rainy seasons, the long rains which run from March to May and the short rainy season from October to December. The average annual rainfall is 900 mm and is highly seasonal with period March to May accounting for 70% of the annual precipitation. The remainder falls during the unpredictable short rains between October and December. Between these 2 rainy seasons is a hot dry season from January to February and a cool dry season from June to September. Aside from paddy production, residents of the area also grow vegetables, maize, peas, and beans. They also keep cattle, goats, sheep, and poultry.

Population and Eligibility Criteria

The study population included all residents of the 3-study village (males and females) aged 10 years and above who were involved in either crop farming or livestock keeping. Individuals who were absent during the time of data collection, who were critically ill, had cognitive impairment and those who had underlying health conditions that interfered with the drawing of blood, were excluded from the study. Consent to participate in the study was obtained directly from adults aged 18 years and above, whereas parents or legal guardians for participants aged below 18 years consented on their children's behalf. Also, before participation, the children assented to participate in the study.

FIGURE 1: Map of Tanzania Showing the Study Site



Source: (Chilongola et al, 2022)

Sample Size and Sampling Technique

The study area was purposively selected since the area has the interaction of different RVFV hosts, mosquitoes, humans and ruminant animals. Stratified random sampling was used to group households based on eligibility, followed by a simple random sample from each stratum. Convenient sampling based on inclusion criteria was used to sample study participants. Sampling was done 3 times in the year, once during each season; the long rainy season (March-May), dry season (June-September) and a short rainy season (October-December). A total of 446 participants in all 3 seasons were sampled through cross-sectional serosurveys such that; 124 participants in the dry season, 172 participants in the long rains season and 150 participants in the short rains season were recruited.

Data Collection Methods

Data collection involved the use of electronic forms designed using Open Data Kit (ODK) tools (https://opendatakit.org/) deployed in Android tablets for participants' demographic data collection, then followed by blood sample collection.

Blood Sample Collection

The blood sample collection was performed by a team of expert phlebotomists from Kilimanjaro Christian Medical Centre (KCMC). Venipuncture was used to draw 3 millilitres of blood from the median cubital vein. Each sample was split into two 1.5 ml aliquots, which were then put into plain and EDTA vacutainer tubes,

respectively. Each sample was placed in an EDTA tube with 4.5 ml of Tri Reagent (Zymo Research, Irvine, CA, USA), gently mixed by shaking for 1 minute, and then sent directly to the Kilimanjaro Clinical Research Institute (KCRI) biotechnology lab at 4°C for Ribonucleic acid (RNA) extraction and Polymerase Chain Reaction (PCR) analysis. After allowing the samples to clot for not more than 20 minutes at room temperature, they were spun at 2000g for 10 minutes in a refrigerator-based centrifuge to produce clear serum, which was then transferred to sterile, clean serum tubes. Immunoglobulin G (IgG) and Immunoglobulin M (IgM) to RVFV were screened for in serum samples.

RVFV Competitive ELISA

The ID Screen RVF Competition Multi-Species kit (ID-vet, Gables, France) was used to conduct a Competitive Enzyme-Linked Immunosorbent Assay (cELISA) to check for antibodies against RVFV in all blood samples. This kit can identify both IgG and IgM antibodies against the RVFV nucleoprotein (NP). The kit's sensitivity range from 91% to 100%, and it's specificity is 100%.[14] According to the manufacturer's directions and as previously mentioned, the cELISA technique was carried out.[8] The mean value of the 2 negative controls (ODNC) was calculated to control each plate's validity, and a plate was considered as being valid if the OD_{NC} was greater than 0.7. The mean value of the 2 positive controls divided by OD_{NC} needed to be 0.3 for a plate to be considered valid. The competition percentage for each sample was obtained by multiplying (OD_{sample}/OD_{NC}) by 100. A sample was considered positive if the result was less than 0.4 and negative if it was greater than 0.5.

Data Management

Each season had its dataset which was recorded in Microsoft Excel. The principal investigator ensured the confidentiality of the data throughout the study and data collected was only used for the study.

Statistical Analysis

The 3 datasets were initially integrated into a single Microsoft Excel file, with seasonal data indicated, for the examination of seasonal seroprevalence of RVF. Following that, the datasets were imported for analysis into STATA software 15. (Stata Corp LLC, College Station, Texas, USA). Data cleaning was performed to ensure consistence of the variables in the 3 datasets. Encoding, labelling, defining, recording, and variable generation were carried out to produce clean datasets. The variables in the dataset that had complete data were used in the analysis. A single, consolidated dataset was then created by combining the 3 original datasets.

A univariate logistic regression model was first fitted to obtain the crude odds ratios (cORs). Variables with a p-value <.05 were considered to be statistically significantly associated with the outcome variable. Akaike information criterion was used to assess the best form to fit the variable age and number of people living in the same household, either as a categorical or a numeric variable. A model with a lesser Akaike value was selected as a good fit model. A likelihood ratio test was conducted to select variables to be used in a multivariable logistic regression model. All the exposure variables were fitted one at a time with the exposure variable of interest (Seasonal distribution). When the test produced a p-value <.05, then the corresponding variable was entered into a multivariable logistic regression model. A multivariable logistic regression model was then performed on the selected variables to obtain the Adjusted OR (aOR) and variables with a p-value <.05 were considered statistically significantly associated with the outcome variable.

Ethical Considerations

Ethical clearance certificate PG 42/2021 was granted by Kilimanjaro Christian Medical University's College Research and Ethics Review Committee (CRERC). Relevant permissions to collect specimens from the field were obtained from the district and regional medical officers and respective district and regional administrative secretaries for Moshi CBD and Kilimanjaro region. In all cases, confidentiality was maintained.

RESULTS

Social Demographic Characteristics of the Study Participants

A total of 446 individuals who met the inclusion criteria were included for analysis in this study. The overall median age of the participants was 40 (IQR=26-54) whereby, 237 (53.6%) of the participants were aged between 21 and 50 years. Of all the participants, 286 (54.2%) were females (Table 1).

Seasonal Seroprevalence of RVF

As shown in Figure 2, the overall seroprevalence of RVF in the year 2020 in the Lower Moshi area of the Moshi district of the Kilimanjaro region was 12.8%. The long rainy season had the highest RVF seroprevalence of 20.4% whereas the dry season had the lowest (4%).

FIGURE 2: Seasonal Seroprevalence of RVF in Lower Moshi district (N=446)

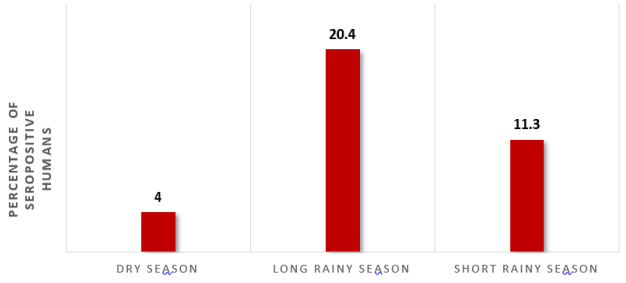


Figure 2 shows the overall RVF seroprevalence across each season. The y-axis shows the percentage for RVF seroprevalence whereas the x-axis presents seasons of the year. Participants were most seropositive (exposed) to RVFV in the long rainy season, but least exposed in the dry season.

Proportional difference of Participants' Demographic Characteristics with RVF Seropositivity

RVF seropositivity significantly differed across the seasons of the year, age of participant and education level. There were marginal associations between the number of

individuals living in the same house and travelling outside the residence area. A high proportion of participants who were RVF seropositive were in the long rainy season, (χ^2 =17.634, *p<.01*) and participants who were aged above 50 years were more seropositive (χ^2 =17.058, *p<.01*). Also, participants with primary education were more seropositive compare to other education groups (χ^2 =7.750, p<.01). Results are presented in Table 2.

Factors Associated with RVF Seropositivity

Results on the participants' factors that are associated with RVF seropositivity are summarised in Table 3. During the long rainy season of the year, participants had 6.08 times higher odds of being seropositive with RVF compared to the dry season. in the general population [OR:6.08, (95%CI, 2.31-16.0)], while participants in the short rainy season had 3.04 times higher odds of RVF seropositivity compared to the dry season [OR:3.04, (95% CI, 1.08-8.50)]. Age in numerical form was chosen by the Akaike information criterion as the optimum form, therefore, for every additional year of age, the odds of being seropositive for RVF in the general population rise by 1.03 times higher [(OR:1.03, (95% CI, 1.01-1.04)].

After performing the likelihood ratio test, the variables chosen for the adjusted logistic regression analysis were; season, sex, participant's age, and the number of persons residing in the same house. Except for sex, all other chosen factors in the final model had a statistically significant association with RVF seropositivity at a p-value <.05. We also found the variable 'season' to confound the variable "number of people living in the same house" by 29.2%. After adjusting for sex, age of the participant, and the number of people residing in the same household in the general population, participants in the long rainy season had 7.30 times higher odds of RVF seropositivity compared to the dry season [(OR:7.3, (95% CI, 2.46-21.67)], whereas, they had 3.35 times higher odds of being RVF seropositive during the short rainy season than they did during the dry season [(OR:3.35, (95% CI, 1.06-10.56)].

Additionally, after adjusting for a season, sex and the number of persons living in the same house, it was observed that, for every one-year increase in age, there was a significant increase of seropositivity to RVFV by 1.03 times higher odds [(OR:1.03, (95%CI 1.01-1.04)]. Individuals living in the same house at a number greater than 5 had 2.77 times higher odds of being RVF seropositive compared to those who shared a home with less than or equal to 5 persons [OR:2.77, (95% CI, 1.27–6.03)].

TABLE 1: Participant's Social Demographics Characteristics (N=446)

Variable	The dry season (n=124) n (%)	The long rainy season (n=172) n (%)	The short rainy season (n=150) n (%)	Total (N=446) n (%)
Sex				
Female @	51 (41.1)	105 (61.4)	85 (56.7)	241 (54.2)
Male	73 (58.9)	66 (38.6)	65 (43.3)	204 (45.8)
Age (years) #				
< 20	1 (0.8)	19 (11.1)	46 (30.9)	66 (14.9)
21-50	94 (77.7)	77 (44.8)	66 (44.3)	237 (53.6)
>50	26 (21.5)	76 (44.1)	37 (24.8)	139 (31.5)
Median (IQR)	41 (32-49)	48 (29-60.5)	35 (16-50)	40 (26-54)
Number of people in the same household δ				
≤ 5	58 (46.8)	83 (48.5)	9 (6.0)	150 (33.7)
> 5	66 (53.2)	88 (51.5)	141 (94.0)	295 (66.3)
Median (IQR)	5 (3-6)	5 (3-6)	8 (7-11)	6 (4-8)
Travelled outside domicile				
No	76 (61.3)	114 (66.3)	126 (84.0)	316 (70.9)
Yes	48 (38.7)	58 (33.7)	24 (16.0)	130 (29.1)
Area of destination (n=130)				
Rural	15 (31.2)	22 (37.9)	8 (33.3)	45 (34.6)
Peri/Urban destination	33 (68.8)	36 (62.1)	16 (66.7)	85 (65.4)
Education level				
No formal education	44 (35.5)	42 (24.4)	26 (17.3)	112 (25.1)
Primary education	12 (9.7)	116 (67.4)	106 (70.7)	234 (52.5)
Tertiary education	68 (54.8)	14 (8.2)	18 (12.0)	100 (22.4)

The symbols @, # and δ represent the following: @= One missing value on sex on the long rainy season; #= Four missing values on age (3 during the dry season and 1 during

TABLE 2: Proportional of RVF Seropositivity Across the Study Participants' Characteristics (N=446)

Variable	Total	Rift Valley Fever n (%)	Seropositivity 95% CI	$\chi 2$	P-value
Season					
Dry Season	124 (27.8)	5 (4.0)	1.6-9.3	17.634	<.001
Long rainy season	172 (38.6)	35 (20.4)	15.0-27.0		
Short rainy season	150 (33.6)	17 (11.3)	7.1-17.5		
Sex*					
Female	241 (54.2)	25 (10.4)	7-14.9	2.336	.126
Male	204 (45.8)	31 (15.2)	10.9-20.8		
Age of Participants (Years)#					
<20	66 (13.9)	6 (9.1)	4.1-18.9	17.058	<.001
21-50	237 (55.6)	19 (8.0)	5.2-12.3		
>50	139 (29.8)	31 (22.3)	16.1-30.0		
Number of people living in the same household"					
≤ 5	150 (33.7)	13 (8.7)	5.1-14.4	3.476	.062
> 5	295 (66.3)	44 (15.0)	11.3-19.5		
Travelled outside domicile					
No	316 (70.9)	45 (14.2)	10.8-18.6	2.074	.150
Yes	130 (29.1)	12 (9.2)	5.2-15.6		
Area of travel (n=130)					
Rural destination	45 (11.7)	7 (15.6)	7.5-29.6	3.286	.070
Peri/Urban destination	85 (20.4)	5 (5.9)	2.4-13.5		
Highest education level					
No formal education	112 (22.4)	14 (12.5)	7.5-20.1	7.950	.019
Primary education	234 (53.0)	38 (16.2)	12.0-21.6		
Tertiary education	100 (24.6)	5 (5)	2.1-11.5		

Table 2 presents associations between participant characteristics and RVF seropositivity. Rows displays the total number of participants in each variable level, the frequency and proportion of RVF-positive individuals in each category with their 95% CI, the chi-square test (2) value, and their corresponding P-values for statistical significance. Column show independent categorical variables assessed. The symbols *, #, and " each show one missing value for the sex column, four missing values for the age column, and one missing value for the number of individuals residing in the same household

TABLE 3: Factors Associated with RVF Seropositivity

Variable	cOR (95%CI)	P-value	aOR (95%CI)	P-value
Season				
Dry Season	Ref		Ref	
Long rainy season	6.08 (2.31-16.01)	<.001	7.30 (2.46-21.67)	<.001
Short rainy season	3.04 (1.09-8.50)	.034	3.35(1.06-10.56)	.039
Sex				
Female	Ref		Ref	
Male	1.55 (0.88-2.72)	.128	1.42 (0.77-2.63)	.261
Age of Participant	1.03 (1.01-1.04)	<.001	1.03 (1.01-1.04)	.001
Number of people living in the same				
≤ 5	Ref		Ref	
> 5	1.85 (0.96-3.54)	.065	2.77 (1.27-6.03)	.01

Continue

TABLE 3: Continued

Variable	cOR (95%CI)	P-value	aOR (95%CI)	P-value
Travelled outside domicile				
No	Ref			
Yes	0.61 (0.31-1.20)	.153	-	-
Area of travel				
Rural destination	2.95 (0.88-9.89)	.08	-	-
Peri/Urban destination	Ref			
Highest education level				
No formal education	Ref			
Primary education	1.36 (0.70-2.62)	.364	-	-
Tertiary education	0.37 (0.13-1.06)	.065	-	-

Table 3 summarises the analysis of factors associated with RVF seropositivity. The column shows the factors that are being assessed while the row shows the crude odds ratio (cOR) and adjusted odds ratio (aOR) with their 95%CI and P-values to confirm any statistically significant associations of the factors with RVF seropositivity.

DISCUSSION

The overarching aim of this study was to determine the seasonal variations of RVF seroprevalence and identify demographic factors that are likely to influence RVF seropositivity in humans. The study reports an overall annual RVF seroprevalence in the Lower Moshi area of the Kilimanjaro region in Tanzania to be 12.8%, with the rainy season having the highest seroprevalence at 20.4% against 4% in the dry season. This prevalence suggests that despite the apparent seasonal fluctuations in RVF seroprevalence, individuals are continuously exposed to RVFV throughout the year, even in the absence of an outbreak. Several previous studies have reported endemic RVF transmission during inter-epidemic/epizootic periods.[5,8,9,11,12,15–27] The mechanisms for RVFV maintenance have been described in previous studies.[2,12,28–31]

Climate is known to affect the geographic, temporal distribution, life cycles of arthropod vectors, and the spread and evolution of the viruses they transmit. It also defines the efficiency with which arboviruses are transmitted from arthropods to vertebrate hosts.[32] Climatic variables indirectly affect vector abundance and distribution, and their ability to vector arboviral diseases.[33] Lower Moshi sugarcane and paddy irrigation area have no previous reports on RVF outbreaks. However, our results suggest an endemic prevalence of the disease in the area, which labels it as a potential hotspot for RVF transmission in north-eastern Tanzania.

Lower Moshi is characterised by having numerous water streams with abundant populations of Culex spp and Aedes spp mosquitoes,[18] and these are the main RVF vectors.[11] The epidemiology of RVF in East Africa is closely associated with the ecological factors prevalent in the Great Rift Valley, which spans Ethiopia, Kenya, and northern Tanzania. Usually, the wet and marshy environments within East Africa cause the transovarially infected dormant eggs of Aedes mosquitoes in the soil to hatch, making Aedes mosquitoes the principal vector responsible for RVFV maintenance.[34] Hatched infectious Aedes mosquitoes transmit the virus to nearby livestock and wildlife vertebrate hosts, which serve as amplifiers of the virus, infecting more mosquitoes, and thereafter secondary vectors of the virus (Culex, Anopheles and Mansonia mosquitoes) amplify the transmission of the virus to non-infected domestic animals and humans.[35] Accordingly, the presence of irrigation schemes, abundant vector mosquitoes, and close interaction between ruminants and persons in our study site makes the site an ideal environment for RVFV maintenance.

Consistent with previous findings, old age and large number of household members were associated with higher seropositivity to RVF.[36,37] As mentioned in studies conducted elsewhere, older male persons are more likely to have been previously exposed to RVFV as a result of their long-term involvement in milking, animal herding and contact with infected vector mosquitoes. Old age predisposition suggests an endemic circulation of RVFV in the study area, rather than a single outbreak event, as a reason for the detected seroprevalence.[8,9] With this information, lower Moshi can be considered as a potential area for future outbreaks of RVF. Therefore, concerns in controlling the spread of the disease should be taken into consideration. The association between seasonality and RVF will raise awareness on where to concentrate and aid in resource allocation towards the prevention and control of the disease in this area. Furthermore, the study has helped to shade light on the possibility that the study areas continue being infected with RVF during the inter-epidemic period despite the absence of previous reports on outbreaks of the disease.

CONCLUSIONS

We observed the highest RVF seroprevalence during the long rainy season compared to other seasons. Moreover, seasonal distribution was found to be significantly associated with RVF seropositivity. An increase in age and the number of people living in the same household also

increased the chances of RVF seropositivity in human population residing in Lower Moshi district. Furthermore, the study has raised awareness of the possibility of RVF circulation during the inter-epidemic period, even in areas that have not been previously reported with RVF outbreaks.

Study limitations

The study used cross-sectional data and thus we could not establish the temporality or causality of associations between seasonality or any other variable with RVF.

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ORIGINAL ARTICLE

Seroprevalence of *Toxoplasma gondii* and Associated Risk factors Among Pregnant Women Attending Antenatal Care in Ilala Municipality, Dar es Salaam, Tanzania

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ABSTRACT

Background: *Toxoplasma gondii (T. gondii)* infection during pregnancy is associated with various complications for the mother and baby. In Tanzania, there is a paucity of data on exposure to *T. gondii* infection among pregnant women and the associated risk factors. Therefore, this study investigated the seroprevalence of *T. gondii* and associated factors among pregnant women attending antenatal care in Ilala Municipality, Dar es Salaam.

Methods: A cross sectional study was carried out among 383 pregnant women attending antenatal health care. A five mL of blood sample was collected from each recruited pregnant woman, processed to obtain serum, and tested for the presence of IgG and IgM anti *T. gondii* specific antibodies. A structured questionnaire was used to gather information on the risk factors predisposing pregnant women to the infection. Data analysis was performed using descriptive statistics and logistic regression.

Results: Of the 383 participants, 104 (27.2%) were positive for antibodies specific to T. gondii; 102 (26.63%) were positive only for IgG, and 2 (0.52%) were positive for both IgM and IgG antibodies. Significant risk factors for *T. gondii* infection were maternal age of 34-39 years (AOR:3.71;95% CI:1.52-9.06), eating unwashed fruits (AOR:7.39;95% CI:3.99-13.66), not washing hand with soap after meat preparation (AOR:7.53; 95% CI:3.40-16.64), consumption of undercooked meat (AOR:3.75; 95% CI:1.95-7.21), and consumption of raw vegetable (AOR: 1.99; 95% CI: 1.04-3.80). Cat ownership was not statistically significantly associated with toxoplasmosis (AOR:1.90; 95% CI: 0.89-4.08).

Conclusions: The seroprevalence of *T. gondii* infection (27.2%) indicates ongoing transmission, hence the need for regular screening during antenatal care and establishment of a control programme.

BACKGROUND

Toxoplasmosis is a zoonotic disease caused by an intracellular protozoan called *Toxoplasma gondii* (*T. gondii*).[1] Approximately over 60% of the world population has been exposed to *T. gondii* infection, with seropositivity rates ranging from less than 10% to over 90% in different parts of the world or within regions in the same country.[2–4] Acquisition of *T. gondii* is through the ingestion of tissue cysts in meat, ingestion of food, water, or soil contaminated with sporulated oocysts, and directly from the cat feces.[5] Additionally, transfusion of *T. gondii* unscreened blood and organ transplant permit the dissemination of *T. gondii* tachyzoites to a large variety of body organs, causing congenital diseases during pregnancy.[6–8]

The majority of healthy individuals with *T. gondii* infections are asymptomatic. However, immune-compromised individuals, immunosuppressive drugs users, and pregnant women who acquired toxoplasmosis during pregnancy suffer severe infection and high mortality.[6,9] The primary infection during gestation age determines the risk of maternal-fetal transmission, which ranges from (10% to 24%) in the first trimester to (60% to 90%) in the third trimester of which the risk of congenital defect become more severe with earlier infections.[10–13]

The circulation of *T. gondii* parasites across the fetal placenta barrier results in miscarriage, preterm delivery, death in utero, neonatal growth retardation, hydrocephalus, cerebral calcification, neurological or ophthalmic disease in the new-born, during childhood or adolescence.[3,6,9,14–16] The seroprevalence of *T. gondii* infection ranges from 4% to 78% among pregnant women in endemic countries.[17,18] Variation in prevalence's depends on local environmental factors, especially temperature and moisture, kitchen habits, and hygienic standards.[4] Routine maternal screening through serological tests to monitor acute

and latent *T. gondii* infections during pregnancy reduces the possibility of fetal infections and substantial damages. However, in most of the resource-limited countries, including Tanzania, screening of the *T. gondii* is not done. Hence, the pregnant women remain undiagnosed.[17–19]

In Tanzania, there is a paucity of information on the current seroprevalence of *T. gondii* infection and associated risk factors among pregnant women. Also, there is limited information on the level of knowledge on *T. gondii* infection, transmission, prevention, and effects of toxoplasmosis on the fetus among pregnant women. Therefore, this study aimed to determine the current seroprevalence of *T. gondii* and associated factors among pregnant women attending antenatal care in Ilala Municipality, Dar es Salaam. The findings will provide basic information on the current burden of the disease and associated risk factors that might be used to develop appropriate control interventions for the prevention and treatment of toxoplasmosis in pregnant women.

METHODS

Study Design and Settings

A quantitative facility based cross sectional study was conducted in August 2020 to determine the seroprevalence of *T. gondii* and associated risk factors among pregnant women attending public antenatal clinics in Ilala municipality. Ilala municipal council is the regional headquarter for the Dar es Salaam Region (Figure 1). Dar es Salaam region has one municipal council (Ilala) and four district councils (Kinondoni, Kigamboni, Temeke, and Ubungo). The Ilala municipal lies between the longitudes of 39° and 40° east and latitude of 60° and 70° south of the equator, having an area of 1,393 km^2.[20] The municipal council has approximately a population of 1,220,611 whereby males are 595,928 and females are 624,683.[21] The Ilala municipal has a total of 36 wards with 2 public health centers and 24 public dispensaries.[20] Ilala municipal was selected because it's among the endemic area for Toxoplasmosis with the highest population of women in Dar es Salaam (Figure 1).

Study Population

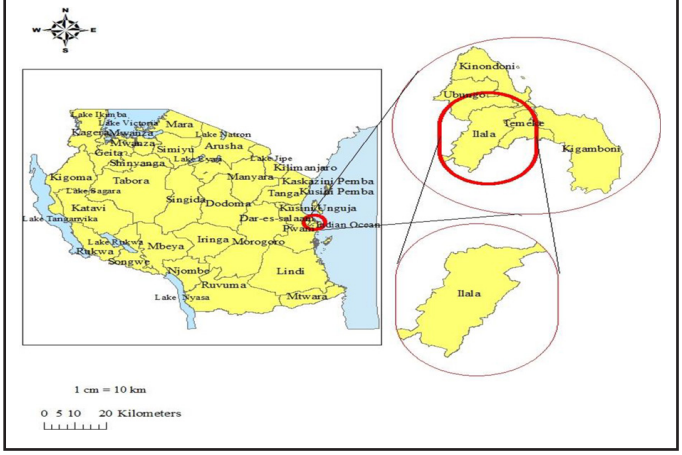
The study population was the pregnant women attending public antenatal clinics in the Ilala municipality. Only pregnant women who agreed and signed informed consent were enrolled in this study.

Sample Size Determination and Sampling Procedures

The minimum sample size for seroprevalence determination was estimated using the formula for a cross-sectional survey: $n = Z^2 P (100-P)/\varepsilon^2$, whereby; n=minimum sample size required, Z= standard normal deviate of 1.96 using a 95% confidence interval, p= expected proportion of *T. gondii* (35%) from the previous study done in Dar es Salaam, Tanzania[10] and ε = margin of error (5%). Through computation with the above formula, a minimum sample size of 349 was obtained. Considering 5% of the sample size for non-response rate and the design effect of 1.5.[22] Therefore, the total sample obtained was 549 pregnant women.

A multi-stage sampling technique was applied to enroll the 549 pregnant women in this study. In the 1st stage, one health center and four dispensaries were selected by simple random sampling technique. In the second stage, a probability proportional to size (PPS) was applied to obtain the sample for recruitment in each facility selected. For the PPS sampling, the preceding month's attendance for ANC services in the selected facilities was extracted from the registries of each facility and summed up. Then the attendance for the preceding month of each facility was divided by the facilities sum attendances and multiplied by the overall sample size (n) to obtain the sample to recruited per selected facility. In the selected facilities, recruitment of pregnant women attending ANC services was done according to daily catchment, where the daily catchment was less than the expected daily eligible recruitment; consecutive pregnant women were recruited until sample for the facility was achieved. Where daily catchment exceeded the expected daily recruitment, systematic sampling was used to recruit until completion of the sample size for the facility. In a month, a total of 118, 75, 72, 58, and 60 pregnant women from Chanika health center, Buyuni, Kinyerezi, Kitunda, and Tabata A dispensaries, respectively accepted to be enrolled in the study by signing the informed consent.

FIGURE 1: A Map Showing the Study Area, Ilala Municipal Council in Dar Es Salaam



Data Collection Tool

The structured questionnaire adapted and modified from Mwambe et al, Paul et al, and Teweldemedhin et al[17,18] was used to collect the required information. The questionnaire had four sections; section A was used to collect information on socio-demographic characteristics such as age, marital status, occupation, level of education, gravidity, trimester of pregnancy, section B collected information on the risk exposures to *T. gondii* infection such as cat ownership, history of contact with cats, history of consumption of undercooked meat, eating unwashed fruits, drinking of raw milk and unboiled water and domestic/ household gardening, and section C focused on awareness and knowledge about toxoplasmosis causes, mode of transmission, symptoms, effects, and preventive measures. All participants (383) were asked awareness questions. However, in the knowledge section, only forty participants who were

aware of toxoplasmosis were interviewed.

Blood Collection and Serological Analysis

Following completion of the structured interview at antenatal clinics, trained laboratory technologists aseptically collected 5mL of venous blood from each study participant using a sterile vacutainer tube and dispensed it into a sterile tube. Collected samples were uniquely labeled with code numbers and then transported from collection sites to Muhimbili National Hospital (MNH) for examination. The collected blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum samples. Then the serums samples were examined for anti-*T. gondii* immunoglobulin M and G using the Abbot Architect analyzer. The architect Toxo IgG and IgM analyzer had sensitivity of 97.5% and 89.9%, respectively and specificity of 99.1% and 99.8%, respectively.[23,24]

The internal quality control and respondents immunoglobulin results were interpreted as per manufacture instruction as IgG with <1.6, 1.6-3, and ≥3 considered as non-reactive (negative), gray zone, and reactive (positive) antibody results respectively, while with IgM<0.50,0.50-1 and ≥1 were regarded as non-reactive (negative), gray zone and reactive (positive) antibody respectively.[19] The labeled aliquots of examined serum samples were stored in the freezer at -20°C or colder at MNH.

Data Analysis

The obtained results were entered and analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0 software produced by IBM Corporation, Armonk, NY, USA. Descriptive statistics were used to summarize and describe the variables in the frequency tables with their proportions. Pearson's chi-square test was used to compare proportions and to assess the association between a *T. gondii* seroprevalence and independent variables. Knowledge was assessed using five questions, each answer was given a mark of one for a correct, zero for the incorrect, and then the total score for each participant was calculated. Subsequently, the mean score was calculated, aiding in the classification of the levels of knowledge. The obtained mean score was 2.3. Hence, a score >2 was classified as a high level of knowledge and a score ≤2 as a low level of knowledge. Univariate logistic regression was used to identify the variables for multivariable logistic regression. All independent variables with a P value <0.25 in the univariate analysis were subjected to the multivariable analysis to adjust potential confounders. The P values <0.05 were considered statistically significant.

Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board (IRB) of the Muhimbili University of Health and Allied Sciences (IRB#: MUHAS-REC-07-2020-390). Permission was obtained from the Ilala municipality administrative authorities in all respective study sites before beginning the study. Signed informed consent was obtained from each study participant before blood collection, and the collected information was kept confidential.

RESULTS

Socio-Demographic Characteristics of the Study Participants

A total of 383 participants were recruited, with a response rate of 69.76%. Of the 383 pregnant women recruited, more than half (55.1%) were aged <27 years and had primary education (56.7%). The majority of the pregnant women were married (88.8%), with less than half (40.2%) in the second trimester (Table 1).

Seroprevalence of *T. gondii* Infection among Pregnant Women

The overall prevalence of *T. gondii* infection among pregnant women was 104 (27.2%), with 102 (26.6%) positive for *T. gondii* specific IgG while 2 (0.52%) tested positive for both *T. gondii* specific IgG and IgM antibodies (Figure 2).

TABLE 1: Socio-demographic Information of Study Participants (N=383)

Socio-Demographics	Categories	n (%)
Age (years)		
	≤ 27	211 (55.1)
	28-33	119 (31.1)
	34-39	44 (11.5)
	≥ 40	09 (2.3)
Marital status		
	Single	36 (9.4)
	Divorced/separated	07 (1.8)
	Married	340 (88.8)
Occupational		
	Student	04 (1.0)
	Housewife	152 (39.7)
	Peasant	27 (7.0)
	Businesswomen	188 (49.1)
	Employed	12 (3.1)
Education level		
	None	14 (3.7)
	Primary school	217 (56.7)
	Secondary & above	152 (39.7)
Gravidity		
	Primigravid	91 (23.8)
	Multigravid	292 (76.2)
Trimester of pregnancy		
	First trimester	80 (20.9)
	Second trimester	154 (40.2)
	Third trimester	149 (38.9)
Health facilities		
	Chanika health center	117(30.5)
	Buyuni dispensary	73 (19.1)
	Kinyerezi dispensary	75 (19.6)
	Tabata A dispensary	60 (15.7)
	Kitunda dispensary	58 (15.1)

Association of Socio-Demographic Characteristics with *T. gondii* Infection

The prevalence of *T. gondii* was higher among the pregnant women in the first trimester (31.3), aged 34 -39 years (45.5%), married (27.6%), businesswomen (28.7%), and multigravid (28.4%). Also, there was a statistically significant association between the age groups of the pregnant women and the prevalence of *T. gondii* infection (Table 2).

Risk Factors Associated with the *T. gondii* Infection among Pregnant Women

Few of the pregnant women (15.4 %) own domestic cats, and of which more than half (54.2%) had a history of cat contact. Also, nearly one-third (32.6%) reported eating unwashed fruits, while more than half reported eating raw vegetables (56.7%) and undercooked meat (50.7%). The prevalence of *T. gondii* infection was statistically significantly associated with owning the cat, eating unwashed fruits, handwashing practice before meat preparation and after household gardening, and history of consuming undercooked meat, and raw vegetables (Table 3).

Pregnant Women's Awareness on Toxoplasmosis

Out of 383 pregnant women surveyed, less than a quarter (10.4%) had heard of toxoplasmosis, while the rest had never heard of toxoplasmosis (89.6%). Of 40 study participants who were aware, most of them (40.0%) mentioned they heard of Toxoplasmosis on social media (WhatsApp, Facebook, and Instagram), followed by hospital/health clinics (27.5%), schools (17.5%), news media [television, radio, magazine] (10.0%) and few participants (5.0%) had heard from all sources.

Participants' awareness on toxoplasmosis varied with education. Pregnant women with secondary and above education were more aware of toxoplasmosis compared to pregnant women with primary education and none (Table 4).

Pregnant Women's Knowledge on Toxoplasmosis

Of the 40 participants who had heard of toxoplasmosis, nearly half (47.5%) of the pregnant women did not know the cause of toxoplasmosis. However, almost two-third (65%) knew the correct mode of toxoplasmosis transmission. Almost two-thirds (65%) of the pregnant women reported miscarriage as the complication of toxoplasmosis in pregnant women, and more than one-third (37.5%) correctly reported avoiding contact with cats as the preventive measure of acquiring toxoplasmosis (Table 5). Of the 40 participants, 17 (42.5%) had a low level of knowledge on toxoplasmosis, while the rest 23 (57.5%) had a high level of knowledge.

Association of Socio-demographic Factors with Knowledge on Toxoplasmosis Among Pregnant Women

A high level of knowledge was observed among the women aged 28-33 years, while a low level of knowledge was high among women aged 34-39 years compared to the rest of the age groups. There was a statistically significant association between the age of the participants and the level of education. A high level of knowledge was observed among the primigravid pregnant women (66.7%) compared to multigravid and pregnant women in the first trimester (61.5%) compared to other trimesters. However, the differences were not statistically significant (Table 6).

Factors Associated with *T. gondii* Seropositivity Among Pregnant Women

The results of bivariate logistic regression analysis show that maternal age, presence of a domestic cat at home, eating unwashed fruits, not washing hands with soap after meat preparation, not washing hands with soap after household gardening, consumption raw/undercooked meat, and consumption of raw/undercooked vegetable were significantly associated with *T. gondii* infection. However, upon adjusting for the confounders, the result of multivariate logistic regression analysis showed that age of 34-39 years, eating unwashed fruits, not washing hands with soap after meat preparation, consumption of raw/undercooked meat, and consumption of raw vegetable were the statistically significant risk factors of *T. gondii* infection (Table 7).

TABLE 2: Association of Socio-demographic Characteristics With *T. gondii* Infection Among Pregnant Women (N=383)

Variable	Categories	n	Seropositivity (%)	P value
Age group	≤ 27	211	45 (21.3)	.007*
	28-33	119	37 (31.1)	
	34-39	44	20 (45.5)	
	≥ 40	09	02 (22.2)	
Marital status	Single	36	09 (25.0)	.700
	Divorced/separated	07	01 (14.3)	
	Married	340	94(27.6)	
Occupational	Student	04	01 (25.0)	.669
	Housewife	152	42 (27.6)	
	Peasant	27	04 (14.8)	
	Businesswomen	188	54 (28.7)	
	Employed	12	03 (25.0)	

Continue

TABLE 2: Continued

Variable	Categories	n	Seropositivity (%)	P value
Education level	None	14	04 (28.6)	.659
	Primary school	217	55 (25.3)	
	Secondary and above	152	45 (29.6)	
Gravidity	Primigravid	91	21 (23.1)	.317
	Multigravid	292	83 (28.4)	
Trimester	First trimester	80	25 (31.3)	.651
	Second trimester	154	40 (26.0)	
	Third trimester	149	39 29.2)	
Health facilities	Chanika health center	117	30 (25.6)	.759
	Buyuni dispensary	73	17 (23.3)	
	Kinyerezi dispensary	75	24 (32.0)	
	Tabata A dispensary	60	18 (30.0)	
	Kitunda dispensary	58	15 (25.9)	

TABLE 3: Risk Factors Associated With *T. gondii* Infection Among Pregnant Women in Ilala Municipality of Dar es Salaam (N=383)

Variable	Categories	n	Seropositivity (%)	P value
Own domestic cats				
	Yes	59 (15.4)	27 (45.8)	.000*
	No	324 (84.6)	77 (23.8)	
History of cat contact				
	Yes	32 (54.2)	18 (56.2)	.078
	No	27 (45.8)	09 (33.3)	
Eat unwashed fruits				
	Yes	125 (32.6)	74 (59.2)	.000*
	No	258 (67.4)	30 (11.6)	
Wash hands after meat preparation				
	Yes	177 (46.2)	12 (6.8)	.000*
	No	206 (53.8)	92 (44.7)	
Wash hands after household gardening				
	Yes	173 (45.2)	29 (16.8)	.000*
	No	210 (54.8)	75 (35.7)	
Source of drinking water				
	Tape water	176 (46.0)	48 (27.3)	.387
	Well water	202 (52.7)	56 (27.7)	
	Mineral bottled water	05 (1.3)	00 (0.0)	
Boil drinking water				
	Yes	109 (28.5)	33 (30.3)	.386
	No	274 (71.5)	71 (25.9)	
Drinking raw milk				
	Yes	110 (28.7)	35 (31.8)	.193
	No	273 (71.3)	69 (25.3)	
History of consuming undercooked meat				
	Yes	194 (50.7)	82 (42.3)	.000*
	No	189 (49.3)	22 (11.6)	

Continue

TABLE 3: Continued

Variable	Categories	n	Seropositivity (%)	P value
History of consuming raw vegetable				
	Yes	217 (56.7)	78 (35.9)	.000*
	No	166 (43.3)	26 (15.7)	
Awareness				
	Yes	40 (10.4)	11 (27.5)	.959
	No	343(89.6)	93 (27.1)	
Level of Knowledge				
	High	23 (57.5)	06 (35.3)	.343

TABLE 4: Study Participants' Awareness on Toxoplasmosis According to Socio-demographic Characteristics (N=383)

Variable	Categories	n	Awareness status Yes (%)	P value
Age	≤ 27	211	23 (10.9)	.344
	28-33	119	15 (12.6)	
	34-39	44	02 (4.5)	
	≥ 40	09	00 (0.0)	
Marital status	Single	36	04 (11.1)	.657
	Divorced/separated	07	00 (0.0)	
	Married	340	36 (10.6)	
Occupation	Student	04	01 (25.0)	.092
	Housewife	152	13 (8.6)	
	Peasant	27	00 (0.0)	
	Businesswomen	188	23(12.2)	
	Employed	12	03 (25.0)	
Educational level	None	14	00 (0.0)	.000*
	Primary school	217	11 (5.1)	
	Secondary and above	152	29 (19.1)	
Gravidity	Primigravid	91	12 (13.2)	.327
	Multigravid	292	28 (9.6)	
Trimester	First trimester	80	13 (16.2)	.144
	Second trimester	154	15 (9.7)	
	Third trimester	149	12 (8.1)	

TABLE 5: Pregnant Women's Knowledge on Toxoplasmosis (N=40)

Variable	Categories	Respondent n (%)
Causative agent	Worms	02 (5.0)
	Plasmodium	07 (17.5)
	Toxoplasma	09 (22.5)
	Amoeba	03 (7.5)
	I don't know	19 (47.5)
Mode of transmission	Contact with infected person	03 (7.5)
	Drinking treated water	03 (7.5)
	Eating raw/undercooked meat	26 (65.0)
	Eating food poison	01 (2.5)
	Sexual intercourse	02 (5.0)
	I don't know	05 (12.5)
Symptom	Swollen glands	14 (35.0)
	Diarrhoea	03 (7.5)
	Legs swelling	06 (15.0)
	Nausea	04 (10.0)
	I don't know	13 (32.5)
Effects	Blindness	01 (2.5)
	Eclampsia	01 (2.5)
	Anaemia	00 (0.0)
	Gestational diabetes	03 (7.5)
	Miscarriage	26 (65.0)
	I don't know	09 (22.5)
Preventive measures	Avoid eating meat and fruits	06 (15.0)
	Avoid contact with cats	15 (37.5)
	Avoid hands shaking	04 (10.0)
	Avoid drinking untreated water	02 (5.0)
	Abstain from sexual intercourse	02 (5.0)
	I don't know	11 (27.5)

TABLE 6: Association of Pregnant Women's Socio-demographic Characteristics With Knowledge on Toxoplasmosis (N=40)

Variable	Categories	n	Low Level (%)	High Level (%)	P value
Age (years)	≤27	23	13 (56.5)	10 (43.5)	.008*
	28-33	15	02 (13.3)	13 (86.7)	
	34-39	02	02 (100.0)	-	
	≥40	-	-	-	
Marital status	Single	04	03 (75.0)	01 (25.0)	.166
	Divorced/separated	-	-	-	
	Married	36	14 (38.9)	22 (61.1)	
Occupation	Student	01	-	01 (100.0)	.934
	Housewife	13	05 (38.5)	08 (61.5)	
	Businesswomen	23	11 (47.8)	12 (52.2)	
	Employed	03	01 (33.3)	02 (66.7)	
Educational level	None	-	-	-	.096
	Primary school	11	07 (63.6)	04 (36.4)	
	Secondary and above	29	10 (34.5)	19 (65.5)	
Gravidity	Primigravid	12	04 (33.3)	08 (66.7)	.341
	Multigravid	28	13 (46.4)	15 (53.6)	
Trimester	First trimester	13	05 (38.5)	08 (61.5)	.849
	Second trimester	15	06 (40.0)	09 (60.0)	
	Third trimester	12	06 (50.0)	06 (50.0)	

TABLE 7: Factors Associated With *T. gondii* Seropositivity Among Pregnant Women

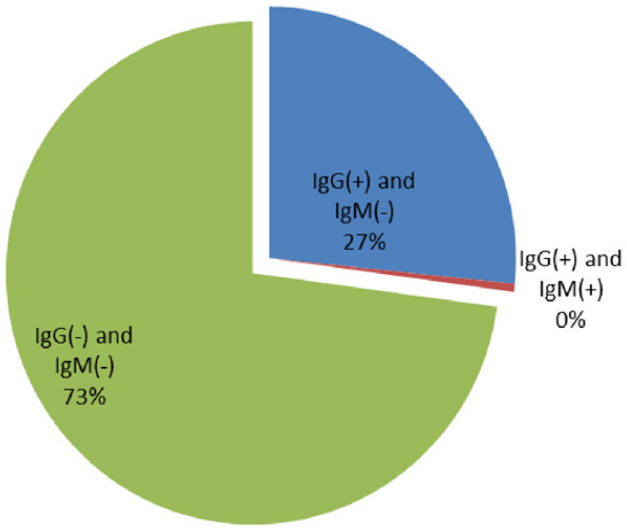
Variable	Univariate		Multivariate	
	COR (95% CI)	P value	AOR (95% CI)	P value
Age				
≤ 27	1		1	
28-33	1.66 (1.00-2.77)	0.050	1.86 (0.94-3.67)	0.074
34-39	3.07 (1.56-6.06)	0.001*	3.71 (1.52-9.06)	0.004*
≥ 40	1.05 (0.21-5.25)	0.949	0.67 (0.09-4.94)	0.697
Marital status				
Single	1			
Divorced/separated	0.50 (0.05-4.73)	0.546		
Married	1.15 (0.52-2.53)	0.735		
Occupation				
Student	1			
Housewife	1.15 (0.12-11.32)	0.908		
Peasant	0.52 (0. 04-6.34)	0.610		
Businesswomen	1.21 (0. 12-11.88)	0.871		
Employed	1.00 (0.07-13.64)	1.000		
Educational level				
None	1			
Primary school	0.85 (0.23-2.82)	0.789		
Secondary and above	1.05 (0.31-3.53)	0.935		
Gravidity				
Primigravid	1			
Multigravid	1.32 (0.76-2.29)	0.318		
Trimester				
First trimester	1			
Second trimester	0.77 (0.426-1.40)	0.393		
Third trimester	0.78 (0.423-1.42)	0.415		
Presence of domestic cat				
Yes	2.71 (1.53-4.80)	0.001*	1.90 (0.89-4.08)	0.098
No	1		1	
History of cat contact				
Yes	2.57 (0.89-7.44)	0.081		
No	1			
Eat unwashed fruits				
Yes	11.03 (6.55-18.58)	0.000*	7.39 (4.00-13.66)	0.000*
No	1		1	
Wash hands after meat preparation				
Yes	1		1	
No	11.10 (5.81-21.20)	0.000*	7.53(3.40-16.64)	<0.001*
Wash hands after household gardening				
Yes	1		1	
No	2.80 (1.69-4.50)	0.000*	0.90 (0.46-1.80)	0.765
Source of drinking water				
Tape water	1			
Well water	1.02 (0.65-1.61)	0.922		
Mineral bottled water	0.00 (0)	0.999		
Boil drinking water				
Yes	1.24 (0.76-2.03)	0.387		
No	1			
Drinking raw milk				
Yes	1.38 (0.85-2.24)	0.194	0.98 (0.51-1.90)	0.947
No	1		1	
History of consuming undercooked meat				
Yes	5.60 (3.30-9.42)	0.000*	3.75 (1.95-7.23)	<0.001*
No	1		1	

Continue

TABLE 7: Continued

Variable	Univariate		Multivariate	
	COR (95% CI)	P value	AOR (95% CI)	P value
History of consuming raw vegetable				
Yes	3.02 (1.90-4.99)	0.000*	1.99 (1.04-3.80)	0.038*
No	1		1	
Awareness				
Yes	1.02 (0.49-2.12)	0.959		
No	1			
Level of knowledge				
High	0.51 (0.13-2.07)	0.346		
Low	1			

FIGURE 2: Prevalence of *T. gondii* Infection among Pregnant Women



DISCUSSION

In the current study, the overall *T. gondii* infection rate was 27.2% among the surveyed pregnant women, thus indicating the ongoing transmission in the studied area. The overall seroprevalence was lower than 35%, 30.9%, and 44.6% reported in Dar es Salaam, Mwanza, and Kilimanjaro in Tanzania, respectively.[10,17,18] This might be due to differences in food consumption habits, occupation status, age groups, sanitary conditions, and urban setting.[5] The findings showed the majority of surveyed seropositive pregnant women had chronic infection suggesting for either past infection or acquired immunity thus cannot infect their fetus unless they are immune suppressed.[25] Less than one percent of the seropositive respondents (0.52%) were positive for IgG and IgM. This observation is in agreement with the findings reported in Ethiopia [13] but was low compared to that reported in northern Tanzania and Brazil.[18,26] Availability of the IgM antibodies during pregnancy predicts the presence of acute *T. gondii* infection that poses a potential risk of maternal-fetal transmission.[13]

The pregnant women aged 34-39 years were three times higher at risk of *T. gondii* compared to the age group <27 years. It can be interpreted that one of the three pregnant women above 34-39 years has *T. gondii* antibodies. This is similar to the findings of the study conducted in Mwanza-Tanzania, and in Ethiopia.[17,19] This association does not mean that older age is a risk factor predisposing to *T. gondii* infection but might be explained that as the age increases, the chances of being exposed is high, and anti-toxoplasma antibodies may retain at a constant level in serum for years. Thus, call for special attention to the screen for anti-toxoplasma antibodies to all older pregnant women attending antenatal services.

The cat is the only definitive host producing feces that contain millions of oocysts within a short time and play a critical role in transmitting *T. gondii*.[5,9,13] Though the history of owning a cat increases the risk for toxoplasmosis, this study showed no significant association between cat ownership and *T. gondii* infection. This was contrary to other studies reported in Burkina Faso, Egypt and Ethiopia reported a significant association between of presence of cats at home and history of cat contact to *T. gondii* infection.[19,25,27]

Eating undercooked meat had been inconsistently reported as a potential risk factor for contracting *T. gondii* infection in many parts of Africa.[5,27,28] Pregnant women with habits of eating undercooked meat had 3.7 times increased odds of *T. gondii* infection compared with their counterparts. This could be explained by the fact that pregnant women in the study area consume barbeque from animals and birds, which is an important food that might contain tachyzoites. This finding is in agreement with studies conducted elsewhere.[5,6,25,28]

An association was found between the consumption of raw vegetables and *T. gondii* infection, with more than one-third of pregnant women (35.9%) consuming raw vegetables/salads being infected. This is because raw

vegetable (salads) might contain *T. gondii* oocysts that remain infective for 12 to 24 months under favorable conditions.[29] However, our observation was contrary to the finding reported from other studies.[27,29,30] The variation of the current results with others might be due to eating habits and food preparation practices among the studied populations.

Thoroughly washing of fruits before eating is one of the important preventive measures for toxoplasmosis. About one-third (32.6%) of pregnant women were eating unwashed fruits. Pregnant women eating unwashed fruits had 7.3 times increased risk of contracting with *T. gondii* infection compared to their counterparts. Similar to the findings from Ethiopia.[19,29,31,32] Also, more than half of the surveyed pregnant women do not wash their hands with soap following raw meat preparation and household gardening to prevent them from contracting with *T. gondii* infection. The findings show that not washing hands with soap following meat preparation increases 7.5 times more risk of being infected with *T. gondii* parasites. The cysts from infected meat might be ingested during hand-to-mouth contact following contact with raw/undercooked meat. This is in line with findings from India.[30]

Regarding awareness on toxoplasmosis, the present study shown that the large majority (89.6%) of the pregnant women were unaware that there is a disease called toxoplasmosis i.e. they never heard, read or saw any information regarding toxoplasmosis.

This is due to a lack of health education about the disease when attending antenatal care. Social media was reported as one of the leading sources of toxoplasmosis, and there was a significant association between awareness and the level of education, whereby respondents with secondary and above education were more aware compared to their counterparts. Individual with secondary school and above education acquires a good ability to explore toxoplasmosis knowledge from different sources such as socio media. This finding is in agreement with other authors reported in Malaysia and Brazil.[33,34]

In the current study, more than half (57.5%) of pregnant women aware of toxoplasmosis had high knowledge. However, close to two-thirds (62.5%) of them did not know the preventive measures. In this study, pregnant women had a high level of knowledge compared to the reports from Nigeria, in which none of the pregnant women was knowledgeable.[35] However, the level of knowledge of the surveyed population was not associated with *T. gondii* infections. This disagrees with the findings from Cameroon, which reported a high prevalence (68.25 %) of the disease among knowledgeable pregnant women compared to none knowledgeable (32.24%).[30] It has been shown that prenatal toxoplasmosis prevention education programs significantly improved the knowledge of cat owners and self-reported cat hygiene behavior of cat owners.[36]

Study Limitations

The study had the following limitations, the inability to follow up on the trend regarding IgG antibody titers for at least two months to confirm congenital toxoplasmosis. The use of serological diagnosis without molecular technique could have underestimated the prevalence of *T. gondii*. In addition, obtaining retrospective information from the participants could be subjected to recall bias.

CONCLUSIONS

The current study showed a seroprevalence of *T. gondii* infection among pregnant women in Ilala Municipality in Dar es Salaam was 27.2%. The main risk factors for transmission in the study area were increasing maternal age, eating unwashed fruits, lack of handwashing following meat preparation, consumption of undercooked meat, and consumption of raw vegetables. Therefore, we recommend regular screening of toxoplasmosis among pregnant women attending antenatal care. Provision of health education to pregnant women attending antenatal care enhances awareness and knowledge on toxoplasmosis preventive measures. In addition, the burden of maternal and congenital toxoplasmosis should be established using avidity tests and molecular techniques to advise policymakers on the need to establish toxoplasmosis control programmes.

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EAHRC
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ORIGINAL ARTICLE

Facility-Based Cross-Sectional Survey on Aedes-Borne Diseases and Associated Symptoms among Febrile Patients During the 2019 Dengue Outbreak in Moshi Rural District, Tanzania

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ABSTRACT

Background: Diseases caused by Aedes-borne viruses, such as; dengue, chikungunya, and Zika are emerging and re-emerging in different parts of the world. Tanzania has experienced several dengue outbreaks since 2010. The present study aims to determine the seroprevalence and associated symptoms of dengue and chikungunya fever in the Moshi rural district during the 2019 dengue outbreak.

Methodology: A facility-based cross-sectional survey was conducted in 15 health facilities in the Moshi Rural district. A total of 397 participants with malaria-like symptoms were enrolled. Participants were screened for seropositivity towards dengue and chikungunya Immunoglobulin G and M (IgG and IgM) using ELISA-based kits.

Results: Out of 397 participants, 28 (7.1 %) and 8 (2.0%) were dengue IgM and IgG positive respectively. Chikungunya IgM positives were 34 (8.6%). The most commonly reported symptoms were; headache 189 (27.7%), joint pains 132 (19.4%) and muscle pain 106 (15.5%). Factors such as being a farmer and history of travelling to outside regions was associated with dengue IgM seropositivity ($p<.05$).

Conclusion: Aedes-borne illnesses appear to be endemic in the area, with IgG antibodies against the Chikungunya virus being more prevalent among study participants. These results provide an understanding of arboviral diseases as well as provide an early warning signal on the risk of transmission in north Tanzania. The results inform the allocation of local and national public health intervention to prevent future outbreaks.

BACKGROUND

Arboviruses are a group of viruses that are transmitted to humans mainly through bites of infected arthropods such as; mosquitoes, ticks, sand flies, or midges.[1] Diseases caused by Arboviruses include; dengue, chikungunya, zika, yellow fever, and rift-valley fever to mention a few.

These viruses cause diseases ranging from asymptomatic to life-threatening conditions such as haemorrhagic fever as well as neurological disease.[2,3] Most individuals infected with arbovirus experience mild or no symptoms at all. Typical symptoms of most arboviral diseases include; high fever, flu-like illness, headache, joint pain, muscle pain, skin rash, backache, fatal hemorrhagic complications, myalgia, and nausea.[2,4] For long, arboviral diseases have not been considered to be among the major contributors to global mortality and disability.[5] As a result of increased urbanisation, globalisation, and international travel, arboviral diseases are expanding their geographical range and affecting global public health. International travel accelerates the introduction of arbovirus into new areas.[5,6] Dengue and other arboviral diseases are endemic in many African countries, however, the extent of arboviral infections is unknown because arboviral are not considered a priority in many African countries' ministry of Health.[7,8] In a systematic review and meta-analysis study, the prevalence of dengue in Africa is reported to be 24.8% (13.8–37.8), 10.8% (3.8–20.6), and 8.4% (3.7–14.4) for Immunoglobulin G (IgG), immunoglobulin M (IgM), and for acute dengue respectively.[9] However, the burden of dengue remains largely unknown in Africa.

In Tanzania mainland and Zanzibar, there are several reports of the prevalence of arboviral diseases.[10–13] Dengue has been reported to be 9.9% (PCR) in Morogoro[14], 43.5% in Dar es Salaam (IgG)[15], 3.7% to 9.0% (IgM) in Lower Moshi[16], and 37.8% in Zanzibar (IgG)[15], while chikungunya has been reported to be 21.2% in Dar es Salaam (IgG)[15], 11.4% in Hai Kilimanjaro (IgM)[16], 23.1% (PCR) in Lower Moshi[17] and 12.2% in Zanzibar (IgG).[15] Similarly, the *Aedes aegypti* mosquito has been implicated to transmit dengue and chikungunya in Tanzania.[16,18,19] *Aedes aegypti* mosquitoes are the main vectors for Dengue

and Chikungunya virus transmissions in Kilimanjaro.[16,18] In recent years, there has been an unprecedented emergence of epidemic arboviral diseases (such as dengue) in Tanzania. The first epidemic was reported in Dar es Salaam in 2010, among travellers returning to Europe and Japan.[20] The second outbreak was recorded in 2013 involving 20 febrile patients.[21] A third outbreak occurred in 2014, with 1000 confirmed cases.[22,23] A large-scale outbreak was reported in 2019, with a total of 6,859 dengue confirmed cases.[24] Most reported cases were from regions along the coast of the Indian Ocean, Tanzania. Besides, these arboviruses are underestimated in Tanzania due to limited health care resources that do not extend to confirming the actual pathogen causing fever in patients presenting with malaria-like symptoms.

Since the outbreak began in Dar es Salaam, other regions were not subject to the same public health measures, and as a result, dengue continued to cause severe morbidity in other regions within Tanzania. Since dengue or chikungunya may increase the risk of outbreaks, it is crucial to closely monitor the prevalence or incidence of arboviruses throughout Tanzania and use this information to support public health action. This study aimed at determining the prevalence of dengue and chikungunya in Moshi rural district during the 2019 dengue outbreak. Identification of arboviral infections in the area is essential for thorough management of cases and planning for further control.

METHODS

Study Settings

The study was conducted in Moshi's rural area which includes the proposed urban extension area. The following dispensaries were included in the study; Himo, Uoni Mission, Alep Health, Chekereni, Kyomu, Uchira, Mrawi, Mabogini, Mtakuja, Mikocheni, Kahe, Kilema Pofo, Makaa Pomuwani, as well as two hospitals TPC and Upendo. The area is characterised by dense forests, savannah type of vegetation, and irrigation schemes. Altitudes range from 700 to 950 metres above sea level. The area experiences a bimodal form of rainfall with long rains occurring from March to June and short rains occurring between October and November. The area was selected based on available evidence of dengue (3.6%) and chikungunya infection (10.1%) as well as high diagnosis of malaria cases.[10]

Study Design, Sampling, and Recruitment

This was a facility-based cross-sectional study conducted in selected dispensaries. The study utilised a purposive sampling technique to get the 397 participants. The study included participants who visited a health facility for care and treatment during the 2019 dengue outbreak. The study included all patients irrespective of their age who experienced febrile illness during the data collection period (June to July 2019).

Data Collection

Community members and heads of facilities were involved before recruiting study participants. Study staff explained the purpose of the study and answered any questions in an open forum. The forum was conducted in a local language and participants were allowed to ask questions. Participants' consent to take part in the study was sought. The questionnaires were completed by the study staff who were trained in data collection. A structured Swahili questionnaire was used to collect information from the study participants. Information collected included; socio-demographic characteristics such as; age, sex, marital status, education level, occupation, housing characteristics, preventive measures, travel history, and history of fever or illness in the past 24 hours prior to questioning time.

Validity of the Questionnaire

To maximise validity, the questionnaire was pre-tested on 5 respondents before distribution as a pilot study. The aim was to examine how the participants understood and responded to the questions. After the pre-test, adjustments in phrasings were made to make the questionnaire simple to understand.

Blood Sample Collection and Serology Test

About 1 to 5 millilitre (ml) of whole blood sample was collected from suspected patients, and transported to Kilimanjaro Christian Medical University College laboratory in an ice box maintained at 2 to 8°C temperature within 24 hours. The blood samples were tested for both dengue and chikungunya antibodies using Dengue IgG/IgM Combo Rapid Test and Chikungunya IgM Combo Rapid (CTK Biotech, Inc. USA). The tests were carried out following the manufacturer's recommendations.

Statistical Analyses

Data was analysed using Statistical Package for Social Sciences 20.0 software (SPSS Inc., Chicago, USA). Descriptive statistics was summarised using frequencies and percentages for categorical variables while means and standard deviations for continuous variables, particularly respondents' age in years. The chi-square test or Fischer exact test was used to test the association between categorical variables. A *p-value* of less than 5% was considered statistically significant.

Ethical Approval

The study was approved by the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (CRERC) with certificate number 2492. Written informed consent was obtained from all participants. In the case of children under 18 years of age, a parent or legal representative provided consent on behalf of the participant. Permission to conduct this study was sought from the District Medical Officers and the heads of each health facility. Confidentiality and restricted access to collected data were adhered to throughout the study.

RESULTS

Characteristics of the Studied Population

A total of 397 participants were included in the analysis. The mean age of the population was 33.4 ± 20.1 years (range 1–80 years) and 261 (65.7%) were female. Adults aged above 15 years were more represented as compared to other age groups, 313 (78.8%). Ninety-eight participants had a history of travel within the past 2 weeks to various regions of Tanzania. Most participants had travelled to epidemic regions such as; Dar es Salaam 30 (30.6%), Tanga 17 (17.3%), Arusha 15 (15.3%), Dodoma 8 (8.2%) and Zanzibar 6 (6.1%). (Table 1)

TABLE 2: Prevalence of Dengue and Chikungunya

	Variables	n (%)
Fever	Yes	171 (43.1)
	No	226 (56.9)
Chikungunya IgM	Positive	34 (8.6)
	Negative	363 (91.4)
Dengue IgM	Positive	28 (7.1)
	Negative	369 (92.9)
Dengue IgG	Positive	8 (2.0)
	Negative	389 (98.0)

Prevalence of Dengue and Chikungunya among Patients and Common Reported Symptoms

Twenty-eight (7.1%) and 8(2.0%) participants were laboratory confirmed to be dengue IgM and IgG positive respectively. A total of 34 (8.6%) participants were confirmed to be chikungunya IgM-positive. One hundred and seventy-one (43.1%) participants had fever. (Table 2) The most commonly reported symptoms were; headache 189 (27.7%), joint pains 132 (19.4%), Muscle pain 106 (15.5%), eye pain 58 (8.5%), skin rash 52 (7.6%), cough 32 (4.7%), back pain 23 (3.4%), chest pain 16 (2.3%), dizziness, cold, nausea/vomit (both) 9 (1.3%). (Figure 1).

Factors Associated with Dengue and Chikungunya IgM

Fever was associated with chikungunya seropositivity as compared to those without fever, 25 (73.5%) $p<.01$. Headache was associated with chikungunya seropositivity as compared to those without headache 23 (67.7%), $p=.01$. Other factors such as; age, sex, occupation, travel history, joint pain, and muscle pain were not associated with chikungunya seropositivity. (Table 3) Being a farmer was associated with dengue IgM seropositivity as compared to other occupations 13(46.4%), $p=.02$. Travel history was associated with dengue IgM seropositivity, 23(82.1%), $p=.003$. (Table 3)

FIGURE 1: Commonly Reported Clinical symptoms

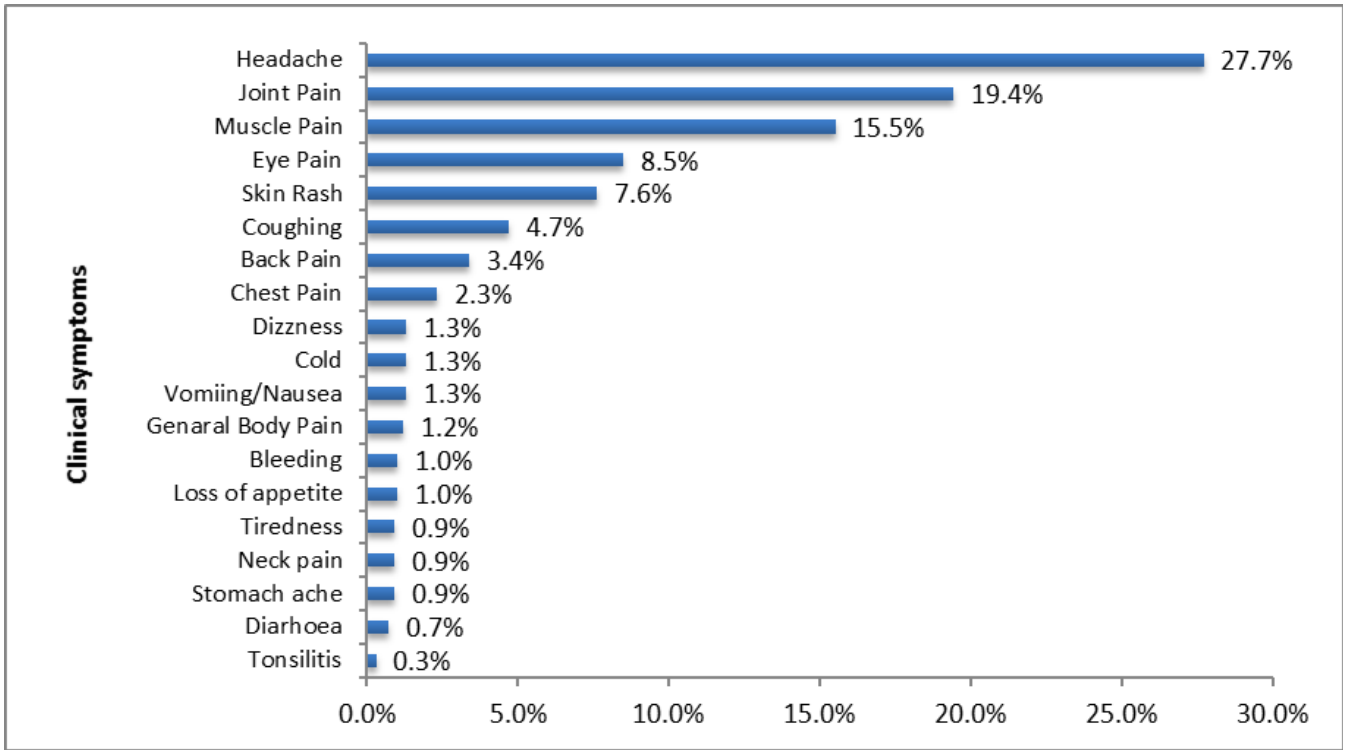

TABLE 3: Symptoms and other factors associated with Dengue IgM and Chikungunya IgM Seropositivity

Variable	Chikungunya IgM n(%) Positive	Negative	P-value	Dengue IgM n(%) Positive	Negative	P value
Age category						
≤5 Years	2 (5.9)	48 (13.2)	0.5	2 (7.1)	48 (13.0)	0.6
6 to 15 Years	4 (11.8)	45 (12.4)		3 (10.7)	46 (12.5)	
>15 Years	28 (82.4)	270 (74.4)		23 (82.1)	275 (74.5)	
Sex						
Female	26 (76.5)	235 (64.7)	0.1	20 (71.4)	241 (65.3)	0.5
Male	8 (23.5)	128 (35.3)		8 (28.6)	128 (34.7)	
Occupation						
Student/Child	5 (14.7)	85 (23.4)	0.5	5 (17.9)	85 (23.0)	0.02*
Farmer	14 (41.2)	108 (29.8)		13 (46.4)	115 (31.2)	
Employed	11 (32.4)	117 (32.2)		3 (10.7)	119 (32.2)	
Self-Employed	4 (11.8)	53 (14.6)		7 (25.0)	50 (13.6)	
Travel History						
Yes	14 (41.2)	162 (44.6)	0.6	23 (82.1)	198 (53.7)	0.003
No	20 (58.8)	201 (55.4)		5 (17.9)	171 (46.3)	
Fever						
Yes	25 (73.5)	153 (42.1)	<0.01	16 (57.1)	162 (43.9)	0.1
No	9 (26.5)	210 (57.9)		12 (42.9)	207 (56.1)	
Headache						
Yes	23 (67.7)	169 (46.6)	0.01	12 (42.9)	180 (48.8)	0.5
No	11 (32.4)	194 (53.4)		16 (57.1)	189 (51.6)	
Joint Pain						
Yes	12 (35.3)	120 (33.1)	0.7	9 (32.1)	123 (33.3)	0.5
No	22 (64.7)	243 (66.9)		19 (67.9)	246 (66.8)	
Muscle pain						
Yes	7 (20.6)	263 (72.5)	0.3	6 (21.4)	101 (27.4)	0.4
No	27 (79.4)	100 (27.5)		22 (78.6)	268 (72.6)	

*Fisher exact Test
Farmers both agriculturalist and livestock keepers

TABLE 1: Characteristics of the Studied Population (N=397)

Variables	Value n (%)
Age categories	
≤5 Years	50 (12.6)
6 to 15 Years	34 (8.6)
>15 Years	313 (78.8)
Mean age ± standard deviation 33.4 ± 20.1 years (range 1–80 years)	
Sex	
Female	261 (65.7)
Male	136 (34.3)
Occupation	
Students/Child	90 (22.7)
Farmer	122 (30.7)
Employed	128 (32.2)
Self-Employed	57 (14.4)

Continue

TABLE 1: Continued

Variables	Value n (%)
Travel History (n=98)	
Dar	30 (30.6)
Tanga	17 (17.3)
Arusha	15 (15.3)
Dodoma	8 (8.2)
Tabora	3 (3.1)
Zanzibar	6 (6.1)
Mwanza	4 (4.1)
Mbeya	2 (2.0)
Manyara	2 (2.0)
Nairobi	4 (4.1)
Pwani	3 (3.1)
Singida	4 (4.1)

DISCUSSION

The outbreak of dengue recorded in Tanzania demonstrate a potent epidemiological threat. The outbreak measures provide opportunity for combating similar vector-borne viral diseases such as; chikungunya, zika and West Nile fever since they are all transmitted by the same *Aedes aegypti* vector. This study determined the prevalence and associated symptoms of dengue and chikungunya fever during the 2019 dengue outbreak in Moshi Rural District, Tanzania. The prevalence of chikungunya IgM was 8.6%.This is relatively low when compared to results of a previous study conducted in the same area which reported a prevalence of 10.1%.[10] Despite this study having been conducted during the dengue outbreak period, findings indicate that chikungunya was also circulating during this period. The study reports the prevalence of dengue IgM to be 7.1%. This is higher than results of a previous study conducted in similar settings which reported prevalence of dengue to 3.6% among 138 febrile patients.[10] Both studies focused on health facilities located in rural areas. This implies that there is continued transmission of dengue in such area spread by mosquitoes infected with the dengue virus. Moreover, it is reported that dengue viral infections may be more prevalent than reported data suggests, and that the *Aedes aegypti* mosquito vectors appear to be increasing in different geographical settings.[25]

This study reports that the most common symptoms were; headache 27.7%, joint pains 19.4%, Muscle pain 15.5%, eye pain 8.5% and skin rash 7.6%. These commonly reported symptoms concur with results of a study that was conducted in Mexico and Cuba which reported fever, joint pain, myalgia, and skin rash as the most common symptoms among patients with arboviral infection.[26,27] Strategies to successfully control and eliminate dengue and chikungunya depend on early and accurate diagnosis. It has been reported that symptoms of these infections overlap[28], and therefore, jeopardise the clinical decision if a clinical diagnosis is done. To achieve better diagnosis, health facilities need to be equipped with tools that can rapidly and effectively detect pathogens in a patient sample. In areas like Tanzania, where dengue, chikungunya, and other pathogens like malaria are endemic, there is a need for a multiplexing assay that can detect both dengue and malaria and hence be able to differentiate dengue, chikungunya or malaria from other febrile illness.[29]

In this study, it was observed that farmers were more likely to be dengue IgG seropositive as compared to other occupation. This is due to the fact that farmers are more exposed to mosquito bites since farming is generally an out-door activity. Likewise, since Aedes mosquitoes bite animals as an alternative host, being close to animals also increase the risk of mosquito bite. This has also been reported in studies conducted in other settings that livestock keeping can influence higher number of mosquito vectors close to humans as the keeping of livestock is associated with the presence of larval habitats.[30,31] A study in Vietnam reported similar results that farmers have up to almost eight times more risk of dengue virus infection.[32]

The reported serology results (IgM and IgG) indicate endemicity of both diseases. Future studies should conduct more sensitive tests such as molecular tests that will be able to detect active circulation of the virus. Effective management and control of dengue and chikungunya must include surveillance of other diseases such as malaria and bacteria that mislead clinical diagnosis. Vector control is the cornerstone for vector-borne diseases and should be escalated in rural areas in the fight against arboviruses.[33,34]

CONCLUSION

Aedes-borne illnesses such as Chikungunya appear to be endemic in the area, with IgG antibodies against Chikungunya virus being more prevalent among study participants. These results provide an understanding of arboviral diseases as well as provide an early warning signal on the risk of transmission in north Tanzania. The results inform the allocation of local and national public health intervention to prevent future outbreaks.

Study Limitations

The study reports the prevalence of dengue during the outbreak period, however, the reported signs and symptoms may be overlapping with other febrile illness such as malaria. Future studies should consider excluding all cases positive for other pathogens causing fever or febrile illness. This study didn't report entomological and molecular data. Future studies should incorporate such data so as to clearly explain transmission patterns.

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ORIGINAL ARTICLE

Hygiene Practices, Water Supply, Sanitation, and Childhood Diarrhoea in Resource-Poor Settings of Rural Central Tanzania: A Mixed-Method Study

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ABSTRACT

Diarrhoeal diseases are associated with high morbidity and mortality, especially in children less than five years of age in many low- and middle-income countries (LMICs). This cross-sectional convergence mixed-method study explored water, sanitation and hygiene challenges as the important contributors to childhood diarrhoea in rural Tanzania. The study involved questionnaire survey (N=340), key informant interviews (KII) (n=10) and eight focus group discussions (FDG) (n=61). Prevalence of diarrhoea was 22.2% and 18.7% in Sanza and Iwondo Wards, of Manyoni and Mpwapwa Districts respectively. Improved houses (iron roof and baked brick walls) were more common in Sanza, while 80% of the houses in both wards had earth floor. Water sources in dry period and frequency of sharing water sources with animal were significantly different between wards ($P<.001$). Boiling drinking water was uncommon, practised by only 5.2% and 8.6% of the households in Sanza and Iwondo, respectively. More than 95% of the households in both wards used traditional pit latrines, and latrine sharing was more common in Iwondo than in Sanza ($P=.035$). The themes from KII and FGD were: knowledge of occurrence and causes of diarrhoea, water safety, hand-washing, availability of improved sanitation services, keeping chickens inside the house overnight, health effects associated with keeping chickens inside the house and knowledge of occurrence and causes of diarrhoea. Mixed methods analysis through merging data sets revealed poor community knowledge on the causes of childhood diarrhoea, ineffective hand washing, seasonal variation of drinking water sources and high human-chicken interactions. Prevention and control of gastrointestinal infections in resource-poor settings should promote the use of cheap and locally available resources and feasible practices in response to the existing challenges related to water and sanitation services, financial constraints, economic activities, and cultural practices.

BACKGROUND

Good hygiene practices, sanitary services, and a reliable and safe water supply are key elements in reducing the impact of gastrointestinal infection in a community. Global data indicate that in 2015, out of 159 million people depending on surface sources for drinking water, 58% were from sub-Saharan Africa.[1] Unsafe drinking water, poor sanitation and lack of hygiene contributed to about 870,000 deaths in 2016 worldwide, mainly as a result of diarrhoeal diseases, malnutrition and intestinal nematode infections.[1] In Tanzania, 13.8% of urban households and 52.2% of rural households depend on untreated water sources.[2] Despite the differences in safe water availability, the prevalence of diarrhoea in children aged between 6 and 11 months in urban communities (14.1%) differs little from that seen in rural areas (11%) of Tanzania.[2]

Poor hygiene practices can lead to contamination of food by gastrointestinal pathogens. Washing hands at critical intervals, including before eating, before feeding children, after cleaning children following defaecation, before food preparation and after using the latrine reduce the incidence of diarrhoea in children.[3] Affordability of clean water is a key factor forcing households in rural Tanzania to use highly contaminated and distant water sources that are free of charge.[4] Home water treatment through boiling or using chemicals is generally recommended, but most households do not treat drinking water.[5] Repeated gastrointestinal infections in children occur as a result of consumption of contaminated food and water and

mouthing contaminated objects and resulting in malabsorption of nutrients and undernutrition.[6]

Improvement in sanitary services and use of improved water sources are sometimes challenging to implement in rural areas of low- and middle-income countries (LMIC) mostly due to household financial reasons.[7,8] Some households continue to practise open defaecation or share one latrine between several households.[5] Children under five years of age from households lacking an improved water supply, access to sanitation facilities and sanitary disposal of faeces have a high risk of developing diarrhoea, fever and coughing.[9] House construction can impact household hygiene practices. Earth or sand floors cannot be adequately cleaned compared to cement floors and have been reported to be significantly associated with diarrhoea in children.[10] Houses and compounds shared with domestic animals and harbouring pests, such as rats, can also be the source of contamination of utensils and the household compound with animal faeces.[11,12] In Tanzania however, there are few studies conducted in rural resource-poor settings to indicate the status of the water, sanitation and hygiene. Therefore, this study aimed to identify deficiencies in hygiene practices, sanitation, availability of water services and occurrence of diarrhoea, and community knowledge of the causes of diarrhoea. Ultimately the study aimed to identify manageable areas to prioritise in devising community-based interventions to reduce childhood diarrhoea and its impact. It is envisaged that the results of the current study will assist the policy-makers in devising appropriate interventions specific to these areas by considering locally available resources.

MATERIALS AND METHODS

Study Area

The cross-sectional convergence mixed-method study was used to explored water, sanitation and hygiene challenges in Sanza and Iwondo Wards, of the Manyoni and Mpwapwa Districts, respectively. Both wards are found in the Great Rift Valley and are among areas that form the semi-arid zone of central Tanzania. The number of households in the Sanza and Iwondo Wards was 1,730 and 2,004 respectively, based on the census conducted in May 2014 in Sanza and December 2016 in Iwondo, within the project titled 'Strengthening food and nutrition security through family poultry and crop integration in Tanzania and Zambia (Nkuku4U project)[13]

These areas are characterised by low, short and often erratic rainfall of about 600 mm per annum in a unimodal pattern, from November to April.[14] Due to low and sometimes unpredictable rainfall, these areas frequently face food and water shortages and mostly depend on surface water sources which await rainfall for refilling. Keeping scavenging indigenous chickens is a common activity practised by more than half of the households throughout the year.[15] Scavenging indigenous chickens are the livestock least affected by the dry season and unpredictable climatic conditions affecting feed and water availability.[16] This study does not claim these sites to be a representative of communities in Central Tanzania rather, the study was implemented in collaboration with a larger project and the findings reflect the situation in the studied community.

Quantitative Data Collection

This study was conducted in association with a broader cluster randomised control trial study of a food and nutrition security project (Nkuku4U project).[13] The project was implemented in central Tanzania and Zambia as five-year cluster-randomised controlled trial to reduce childhood undernutrition by strengthening household nutrition through improving poultry and crop integration systems. The project collected reports of the incidence of diarrhoea in children under five years of age, but there was limited information collected on safe water supply, hygiene, sanitation and human-animal interactions. The current study was implemented to address the information gaps about water, sanitation, hygiene and human-animal interactions. These elements are important in determining the occurrence of diarrhoea in children, which in turn may have acted as a confounding factors to the Nkuku4U intervention outcomes.

The calculation of the overall sample size used by the Nkuku4U study was based on an estimated baseline stunting prevalence of 35%, aiming at achieving a 10% point reduction at the end of the project, and giving 80% power to detect this difference as being significant at the two-sided 5% level, assuming an intra-cluster correlation coefficient of 0.014.[16] The project census across the two wards was conducted by trained male and female enumerators recruited from both wards. All households within reach by foot, motorbike or vehicle were visited by trained enumerators to collect information on age and sex of household members, current ownership of village chickens and intention of chicken-keeping in the near future. A sampling frame was generated from all households with at least one child under two years of age during the census, keeping chickens or intending to keep chickens and intending to remain in the study areas for at least the next five years.

Two stage sampling was used to first enrol all eligible households with children under 12 months of age and then enrol additional households with children aged 12–24 months by random selection through a lottery draw using household identification numbers to give the required number of children. The numbers of children enrolled were 240 and 300 for Sanza and Iwondo Wards, respectively. The number of children in Iwondo were increased to offset the drop-out effect as was previously observed in the Sanza Ward. This occurred because of household members moving outside the study area, death of a child and household decisions to terminate participation. The younger child was enrolled from the households with more than one eligible child, and for twins random sampling through a lottery draw was employed to select one child.

Households participating in the current study were a subset of households participating in the larger project (Nkuku4U project), encompassing all households either currently owning chickens or owned chickens within the six months prior to questionnaire administration. A total of 340 (153 and 187 from Sanza and Iwondo, respectively) out of 532 households participating in the larger project during data collection fulfilled this criterion, and were all included in the present study. A cross-sectional questionnaire survey was conducted to 340 mothers or caregivers of participating households,

in February 2018. The questionnaire was pre-tested by first administering to a number of households from the study area, the areas of the questionnaires with ambiguities or lacking clarity were identified, and correction made before the survey commenced. The questionnaire was administered face-to-face through traditional paper and pencil interviews by trained male and female enumerators recruited from each ward. Survey questions were written in Swahili, but enumerators were encouraged to make use of the two predominant language groups (Gogo and Sukuma). Each questionnaire was close-checked by the supervisor (research team member) for completeness and correctness before being included in study. In case there was any skipped question or an ambiguity, the enumerator revisited the household alone or with supervisor depending the complexity of the deficit identified. The information collected by the questionnaire were related to childhood diarrhoea, housing, seasonal water supply, hand washing practices, water treatment, sanitary infrastructure availability and uses and human-chicken interactions.

Qualitative Data Collection

Cross-sectional FGDs and KIIs were conducted to collect qualitative data in the same wards (Sanza and Iwondo). The FGDs involved gender-disaggregated participants of the different socio-economic status. The socioeconomic status existing in communities (very rich, rich, poor and very poor) and inclusion criteria was determined through conducting a preliminary FGD comprising elders, social workers and community leaders. Establishing socioeconomic status was based on possession of properties, number of cattle owned, area of land cultivated (acres), year round food security and possession of a bank account. The established study FGD groups were Very rich male, Rich female, Poor male, Very poor female, Rich male, Very rich female, Very poor male and Poor female formed by combining the language group, socioeconomic status and gender. The current study conducted eight FGDs, one male and one female group from each of the four social groups comprising a total of 32 and 29 men and women, respectively under moderation of the lead author. Selection of participants was accomplished by the assistance of the community leaders based on stipulated inclusion criteria. Each FGD comprised seven to nine participants with a mean of eight participants, and each discussion was guided by a semi-structured questionnaire in Swahili or on-the-spot translation to indigenous languages if the need arose (appropriate translation into indigenous languages constituted part of enumerator training). Key informant interviews were conducted by the lead author on one-to-one basis with selected informants including the health workers, community workers, community leaders and traditional healer. All discussions during the FGDs and KIIs were detailed in notebooks by lead author and audio recorded by electronic tablet (Apple iPAD Model MD523X/A) operated by another research team member.

Data Analysis

Quantitative data were first entered in Microsoft Excel 2007 spreadsheets, saved as Stata files (STATA® version 14.2) and then imported to R studio software (R version 3.6.0) for analysis.[17] Proportions were used to present categorical variables and means and 95% confidence intervals were used to explore quantitative variables. The *t*-test was used to determine differences in means, and the chi-square test was used for evaluating differences in proportions for categorical variables between wards. Fisher's exact test was used for categorical variables with low expected frequencies (i.e. <5). The differences were considered significant at $p \leq 0.05$. The recorded KIIs and FGDs were translated from Swahili into English, transcribed and stored as Microsoft Word documents by lead author (Microsoft Word Office 2007). The saved translations were cross-checked against audio recordings by another research team member for correct translation and inclusion of all information. The draft code list was prepared by reading the transcript followed by the preparation of the final code list, and then transcripts were manually coded accordingly by the lead author supported by research team anthropologist using tools from the grounded theory approach.[18] Study themes were derived from data related to the research questions, then iteratively reviewed and revised through constant comparative analysis during the process of coding. The final themes that emerged were categorised into housing, water supply, hygiene, sanitation, human-animal interactions and knowledge of occurrence and causes of diarrhoea in children. The qualitative and quantitative data were linked in the analysis to aid the interpretation of the results obtained by the study. The key areas presenting the supporting and diverging results on specific areas in both quantitative and qualitative data sets were identified and interpreted to gain further understanding of the questionnaire responses and themes that emerged from the qualitative data. The study design, procedures, type of data collected, and data analysis are illustrated in Figure 1.

Ethical Considerations

Study design, protocols and research tools used by this study were approved by the Tanzanian National Institute for Medical Research (NIMR) ethics committee and the University of Sydney's Human Research Ethics Committee (approval number 2014/209). The informed consent forms were clearly read and the persons willing to participate in the study signed or thumb printed the form before commencing data collection exercise.

RESULTS

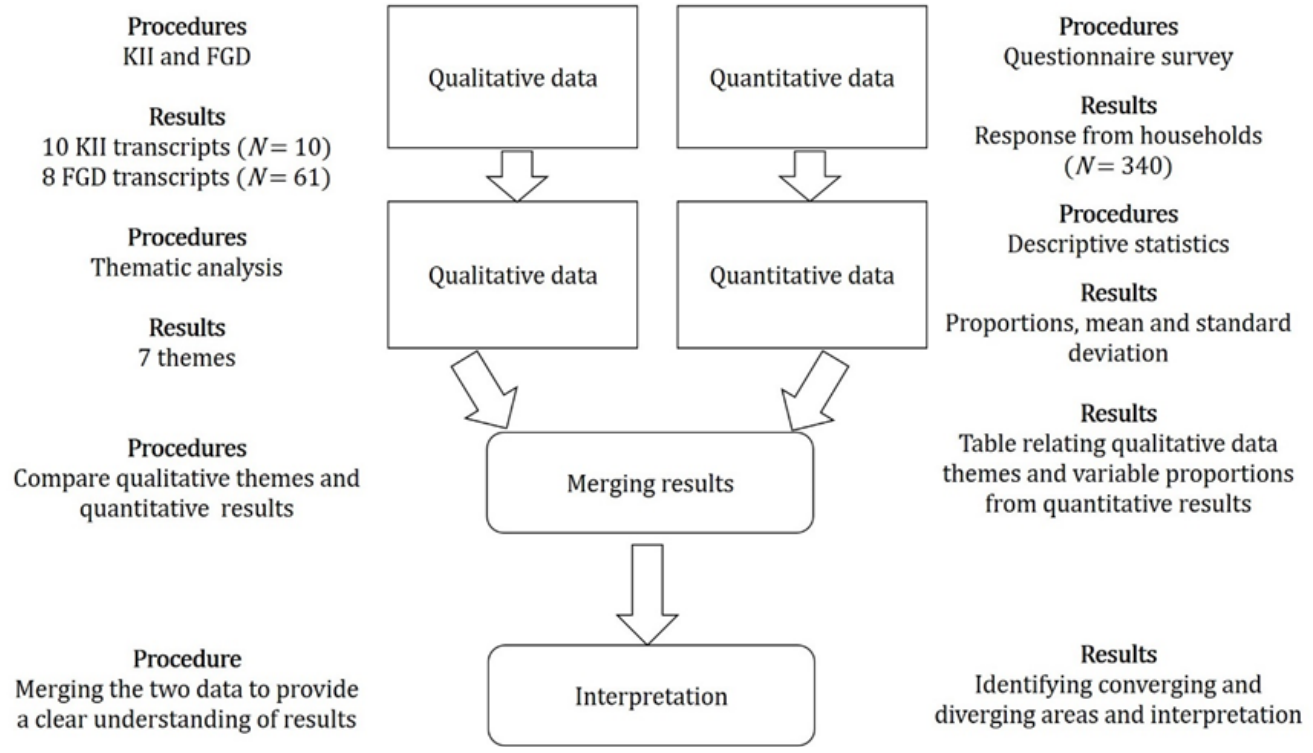
Socio-Demographic Characteristics

The mean age of respondents in Sanza and Iwondo was 33.9, (95% CI = 32.6–35.1) and 30.9 (95% CI = 29.9–32.0), respectively. The proportion of participants with schooling above primary school in Sanza (9.8%) was greater than in Iwondo (1.1%). In both wards, male-headed households were predominant, and the proportion of female-headed households in Sanza was almost twice (25.5%) that of Iwondo (13.4%) (Table 1).

Occurrence and Causes of Diarrhoea

Prevalence of children diarrhoea in the 30 days preceding the day of the questionnaire survey was 22.2% and 18.7% amongst children in Sanza and Iwondo Wards, respectively, and the difference between the wards was not significant (*P*=.50). Most of the FGD participants reported observing a high frequency of diarrhoea in

FIGURE 1: Mixed Methods Study Design of the House, Water, Hygiene, and Sanitation of Rural Households in Central Tanzania. Modified from reference[16]



children during the rainy season, and associated high incidences of diarrhoea with the use of pond water and eating fresh foods from the farm. Complementary feeding period was mentioned by the survey respondents and FGD participants as the stage most associated with high diarrhoea prevalence in children under five years of age (Table 2). The majority of households (>70%) in both wards perceived children under five years of age to be the age group most prone to diarrhoea; however, significant differences in perceptions about other age groups were seen between wards (<.001).

Among the proposed list of possible causes of diarrhoea in children under five years of age in the questionnaire survey, growth stages (44.4%) especially complementary feeding period were frequently mentioned by the respondents as the most important cause of diarrhoea (Table 2). The mixing of milk and solid foods in a child's stomach during complementary feeding was believed to be the cause of the problem as mentioned by KII and FGD participants. A small proportion of KII and FGD participants mentioned serving cold food and insufficient washing of utensils as the main reasons for an increase in diarrhoea frequency during complementary feeding, as mentioned by Health Volunteer Worker KII participant. '*Diarrhoea is mostly observed during complementary feeding because of inappropriate food preparation and handling, by feeding the children with cold food and allowing the flies and chickens to have access to children's food and the utensils*' (KII, Health Volunteer Worker) (Table 2).

Housing Condition and Cleaning

An unimproved house in the context of the study areas is one with a grass and mud roof, walls made from poles and mud or sun-dried bricks, and an earth or sand floor. Improved houses are those with an iron sheet roof, walls made from baked bricks or cement bricks, and at least a cement floor. Iron sheet roofing were more common in Sanza (41.2%) than in Iwondo (24.6%), *P*=.002, just as the number of baked earth or cement brick-walled houses was higher in Sanza (34.0%), than in Iwondo (10.7%), *P*<.001.

In both wards, 80% of the houses had earth floor, (Table 4). Earth floor was reported as a hindrance to sufficient cleaning of the house, as summarised by one FGD participant: '*The house with a cement floor is easier to clean - you can use even water and soap to mop, which cannot be done on an earth floor*' (FGD, Very poor male), (Table 5). The common types of waste encountered during cleaning the house mentioned by KII and FGD participants were faeces from children, chickens, rats, bats or owls, crop remains, food particles, small insects, dust and sand.

TABLE 1: Socio-Demographic Characteristics of the Study Population in Sanza and Iwondo Wards

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Age of respondents (years)				<.001
Mean (95% CI)	33.9 (32.6-35.1)	30.9 (29.9-31.9)	32.2 (31.4-33.0)	
Range	18-56	18-53	18-56	
Number of children under 5 years in the household				.002
Mean (95% CI)	1.7 (1.6-1.9)	1.5 (1.4-1.6)	1.6 (1.5-1.7)	
Range	1-4	1-4	1-4	
Highest level of formal education of mother (%)				<.001
Primary school	75.8	71.7	73.5	
Above primary school	9.8	1.1	5.0	
None	14.4	27.3	21.5	
Sex of head of household (%)				.005
Male	74.5	86.6	81.2	
Female	25.5	13.4	18.8	

TABLE 2: . Occurrence and Perceived Self-Reported Causes of Diarrhoea

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Households reported diarrhoea cases (s) in 30 days preceding questionnaire administration (%)				.498
Children with diarrhoea	22.2	18.7	20.3	
Children without diarrhoea	77.8	81.3	79.7	
The age group reported being most commonly experiencing diarrhoea (%)				<.001
Children under five years of age	72.6	80.8	77.1	
Children above five years of age	4.6	1.6	2.9	
Adults over 18 years of age	2.0	0.0	0.9	
No difference	17.7	6.4	11.5	
Don't know	3.3	11.2	7.6	
Growth stage frequently affected by diarrhoea, amongst children under five years of age (%)				<.001
Exclusive breastfeeding stage	16.5	15.5	15.9	
Complementary feeding stage	38.8	44.4	41.9	
Weaning stage	7.9	12.3	10.3	
All stages are the same	34.2	16.0	24.2	
Don't know	2.6	11.8	7.7	
Reported causes of diarrhoea (multiple responses possible) (%)				
Unhygienic food handling	18.9	27.8	23.8	
Drinking unsafe water	30.7	20.3	25.0	
Poor cleaning of utensils	2.6	4.8	3.8	
Unhygienic environment	32.0	25.1	28.2	
Partially cooked food	20.2	4.8	11.8	
Dirty house	0.0	3.2	1.7	
Staying with animals in the same house	2.0	4.3	3.2	
Sharing the utensils with chickens	2.6	10.7	7.1	
Eating without washing hands	12.4	26.7	20.3	
Washing hands in a communal bowl	1.3	12.3	7.4	
Not using toilet	2.0	6.4	4.4	
Growth stage	35.3	51.9	44.4	
Evil actions	0.0	5.9	3.2	
Don't know	1.3	4.3	2.9	

Water Sources, Water Treatment and Seasonal Variations in Water Access

During the dry season, the primary source of drinking water was open wells in Sanza (79.1%) and public taps in Iwondo (65.2%), and the difference in sourcing drinking water between wards was significant (P<.001) (Table 6). During the rainy season, most households in Sanza (74.5%) and Iwondo (79.1%) used a stream, river, pond or dam as their main source of drinking water. In Iwondo, tap water was frequently used in the dry season because other sources had no water and in the rainy season, surface water was mostly used as it was convenient, plentiful and free of charge. This was further confirmed by Traditional Healer KII participant: '*During the rainy season, we cut the cost of buying water by using pond water which is free and close to our house*' (KII, Traditional healer) (Table 7).

The proportion of households which shared water sources with animals increased from 15.0% in the rainy season to 61.4% in the dry season in Sanza, but decreased from 31.6% in the rainy season to 26.7% in the dry season in Iwondo. Sharing of water sources with animals differed significantly between wards in both seasons (P<.001) (Table 6). The KII and FGD participants connected increased water sources sharing with animals during the dry period with decreased number of available water sources in Sanza and reverting from using tap water to surface water during the rainy season in Iwondo.

A majority of participants in the KIIs and FGDs claimed not using water sources contaminated with animal faeces and urine because animals cannot drink directly from the wells and a few believed the contamination occurs because of the flow of urine and faeces from animals using the well for drinking water (Table 7). During the rainy season, the proportion of households which spent less than one-hour fetching water increased in both wards. On the other hand, time spent fetching water differed significantly between wards (<.001), being more in Iwondo in both seasons: in the dry season because most households source water from the public tap, which is relatively far from most households and in the rainy season. Knowledge of the methods and reasons for treating water was high among the participants in all wards. Despite the reasonable level of knowledge of water treatment revealed by FDG and KII participants, boiling of drinking water was practised by only 5.2% and 8.6% of the households in Sanza and Iwondo, respectively. A large number of participants claimed that boiling reduces palatability and others believed water used for drinking is safe, hence, that there was no need for boiling (Table 7).

Handwashing Before Meals and After Latrine Use

The proportion of questionnaire respondents reportedly using soap to wash hands before meals were 12.4% and 13.9% in Sanza and Iwondo (Table 8). These results are in agreement with the information given by KII and FGD participants in both wards, reporting to commonly washing hands without soap before meals. The proportion of households washing hands in a shared container was significantly higher in Sanza (40.5%) than in Iwondo (23.0%) (P=.001) (Table 9), however, FGD participants indicated this to be a common practice in the Barbecue selling points (i.e. street food vendors) were suggested as the areas where most people eat without washing hands. A little over half of the questionnaire respondents (51.8%) reported not washing their hands after latrine use. This was in agreement with most FGD participants. '*We occasionally wash our hands after using toilets, because we are not used to that practice*' (FGD, rich male) (Table 9). The percentage of households that reported not washing their hands after latrine use in Iwondo was almost twice (66.3%) that of Sanza (34.0%), with a significant difference between the two wards (P<.001) (Table 8). Water scarcity, failure to place water near the latrine or handwashing place and complete absence of a designated area for handwashing were the main reasons for not washing hands after toilet usage, as mentioned by a majority of the KII and FGD participants (Table 9).

Sanitation Services Availability and Use

The traditional pit latrine is commonly used in both of the study areas, by 99.5% and 96.6% of the enrolled households in Sanza and Iwondo, respectively (Table 10). High costs were mentioned by FGD participants as a major barrier to build improved latrines. Some participants did not see the justification for spending money building an improved latrine while they do not have an improved house, as summarised by a FGD participant: '*How can I use a lot of money to build the good latrine, while I am living in a poor traditional house?"* (FGD, Very poor female) (Table 11). Latrine sharing among households was more common in Iwondo (51.3%) than in Sanza (37.3%), and the difference between the wards was significant (P=.035). Temporary latrines commonly found in the areas are frequently destroyed by rain, leading the household members to use the neighbouring household's latrine.

Frequency of disposing children faeces in the latrine differs statistically significantly between wards (73.2% and 54.0% for Sanza and Iwondo, respectively) (P<.001) (Table 10), with FGD participants mentioning common alternatives for disposal being discarding faeces in the field or bushes, or covering with soil. The belief that children's faeces is harmless to human health and latrines being situated at a distance from the house was mentioned by FGD participants as reasons that discouraged disposal of children's faeces into latrines (Table 11). A small proportion of households practised open defaecation (2.6% and 2.7% in Sanza and Iwondo, respectively). This was reported to be most common amongst farmers and livestock keepers with relatively large areas for crop production and keeping animals; they stay in isolated seasonal camps and rarely build toilets.

Human-Chicken Interactions

Keeping chickens inside the house overnight was commonly practised by 81.7% and 75.4% of households in Sanza and Iwondo, respectively. (Table 12). The majority of the KII and FGD participants mentioned theft and predation as the reasons for keeping chickens inside the house (Table 13). The proportion of households reporting chickens gaining access to washed kitchen utensils was higher in Sanza than in Iwondo, and the difference was statistically significant between the wards (44.4% and 32.6%, respectively, (P=.034) (Table 12).

TABLE 3: . Themes and Selected Supporting Quotes Emerging From Key Informant Interviews (KII) and Focus Group Discussions (FGD), Describing Local Knowledge of the Occurrence and Causes of Diarrhoea and Children's Growth Stage Most Prone to Diarrhoea

Theme	Selected Quotes	Information Source	Participants
Knowledge of occurrence and causes of diarrhoea	'Diarrhoea is just like other diseases; it normally occurs without genuine cause.'	KII	Community leader 1, male
	'Diarrhoea episodes are frequently seen during the rainy season in all ages because during this period a lot of wild green vegetables are eaten, and the problem becomes more evident when the children start eating groundnuts from the farm.'	FGD	Very rich, male
	'To my understanding, the child must develop diarrhoea in every stage of growth; when they start sitting, crawling, standing and with the growth of the teeth.'	FGD	Very rich, female
	'When the child is given the food which has been kept overnight and other food.'	FGD	Rich, female
	'When the child is still breastfeeding and mother is practising sex.'	FGD	Very poor, male
	'When the mother breastfeeds the child while she is pregnant, this may result in the breastfed child developing diarrhoea because they will be suckling the baby's milk in the womb.'	FGD	Rich, male
	'Children under five are eating everything they encounter, some of which causes diarrhoea'	FGD	Very poor, male
	'Mostly observed during the rainy season because during this period most people use pond water, which results in people drinking contaminated water as when it rains all animal and human faeces are washed out towards the water sources.'	KII	Health volunteer worker 2, female
Child growth stage most prone to diarrhoea	'During complementary feeding stage, because of mixing the mother's milk with food in the child's gut.'	FGD	Very poor, female
	'Mostly observed when the child starts sitting or crawling because during this period the children eat dirt from the environments where humans and chickens are spending a day.	KII	Community assistant 1, female
	'During complementary feeding, because of inappropriate food preparation and handling, by feeding the children with cold food and allowing the flies and chickens to have access to children's food and the utensils.'	KII	Health volunteer worker 2, female
	'During weaning, itis associated with stress due to an abrupt stop from breastfeeding.'	FGD	Very rich, male
	'Mostly during crawling as the result of eating everything they encounter on the ground.'	FGD	Rich female
	'The child develops diarrhoea when they start to develop the molar teeth.'	FGD	Rich, male

TABLE 4: Types of Materials Used in the Construction of Houses of Study Participants in Each Ward, and the Overall Study Sample, Based on Questionnaire Responses

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Roof materials (%)				.002
Grass/thatch/leaves/mud	58.8	75.4	67.9	
Iron sheets	41.2	24.6	32.1	
Wall materials (%)				<.001
Grass	2.0	1.1	1.5	
Wooden poles and mud	35.3	70.5	54.4	
Sun-dried	28.8	17.6	22.7	
Baked bricks	32.7	9.6	20.0	
Wood, timber	0.0	0.5	0.3	

Continue

TABLE 4: Continued

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Cement bricks	1.3	1.1	1.2	
Floor materials (%)				.625
Unimproved floor	86.3	88.3	87.4	
Improved floor	13.7	11.7	12.6	
Presence of windows in house (%)				.371
Without windows	35.9	41.2	38.8	
With windows	64.1	58.8	61.2	

TABLE 5: Themes and Selected Supporting Quotes Emerging From Key Informant Interviews (KII) and Focus Group Discussions (FGD) describing local knowledge of the importance of cleaning the house, important features of an improved house and house cleaning practices

Theme	Selected Quotes	Information Source	Participants
Important features of an improved house and cleaning considerations	'We feel very uncomfortable to stay inside our traditional dwellings (tembe) which normally have no windows, because it is very hot inside during the night which sometimes makes us sleep outside.'	FGD	Very poor, male
	'A house with plastered walls and a cement floor looks beautiful and presentable.'	FGD	Rich, female
	'Unplastered walls can easily harbour even bedbugs, and once they are there they cannot be easily seen or killed, even with insecticide.'	FGD	Very poor, male
	'I don't see the difference between sand or soil and a cement floor. Both types serve the same purpose.'	FGD	Very rich, male
	'The house with a cement floor is easier to clean, you can use even water and soap to mop.'	FGD	Very poor, male

TABLE 6: Percentage of Seasonal Sources of Drinking Water and Treatment in Each Ward and the Overall Study Sample, Based on Questionnaire Responses

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Source of drinking water (%)				<.001
Dry season				
Stream/river/pond/dam	20.9	26.2	23.8	
Open wells	79.1	8.6	40.3	
Public tap	0.0	65.2	35.9	
Rainy season				
Stream/river/pond/dam	74.5	79.1	77.1	<.001
Open wells	24.8	0.0	11.2	
Public tap	0.7	20.9	11.8	
Source of drinking water (%)				
Dry period				<.001
Not shared with animals	38.6	73.3	57.6	
Shared with animals	61.4	26.7	42.4	

Continue

TABLE 6: Continued

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Rainy period				<.001
Not shared with animals	85.0	68.4	75.9	
Shared with animals	15.0	31.6	24.1	
Treatment of drinking water (%)		.067		
Boiling always	5.2	8.6	7.1	
Occasional boiling	3.9	9.1	6.8	
No treatment	90.9	82.4	86.2	
Time spent fetching water (%)				
Dry season				<.001
Within one hour	78.4	49.7	62.6	
More than one hour	17.7	43.3	31.8	
Unspecified	3.9	7.0	5.6	
Rainy season				<.001
Within one hour	93.5	79.6	85.9	
More than one hour	2.0	13.4	8.2	
Unspecified	4.6	7.0	5.9	

TABLE 7: Themes and Selected Supporting Quotes Emerging From Key Informant Interviews (KII) and Focus Group Discussions (FGD) Describing the Drinking Water Handling, Treatment and Water Source Sharing With Animals in the Study Area

Theme	Selected Quotes	Information	Participants
Water safety and the importance of water treatment	'In my house, the water is fetched in a big bucket and we start drinking right away.'	FGD	Very rich, male
	'When I fetch drinking water, I normally make sure the container is tightly closed all the time to ensure safety.'	FGD	Rich, female
	'We boil drinking water occasionally, not always.'	FGD	Very rich, male
	'Boiled water tastes different and does not quench the thirst.'	FGD	Rich, male
	'No need for boiling, because we are confident with the safety of our water.'	FGD	Very poor, female
Water source sharing with animals	'We do not share the same source of water with animals, rather we build traditional troughs (mrambo) beside the well then we take water from the well to fill up the trough for animals to drink.'	FGD	Very rich, male
	'Cattle, goats, sheep and donkeys are getting water from the river or temporary wells dug in the dry riverbed (korongoni), which is the same source of water for home use.'	FGD	Very rich, male
	'Animals are defaecating around the wells, therefore there is a high possibility of the faeces dropping into the wells.'	FGD	Rich, male
	'Wells cannot be protected from animal faeces and urine contamination, because during filling of the traditional trough (mrambo) there is water which splashes, resulting into backflow into the well.'	FGD	Very poor, female
	'At the small dams we have, you may find the cattle drinking and on the other side, the people are fetching water for home use. With this practice, there is a high risk of using water contaminated with animal faeces. I feel bad seeing people using such kind of water. They are using those water sources because they have no alternative.'	KII	Nurse 3, female
	'We are buying water from the public tap here, during the rainy season we cut the cost of buying water by using the pond water which is free and close to our houses, but it is used by animals as well.'	KII	Traditional healer, male

TABLE 8: Handwashing and Water Treatment Practices in Each Ward and the Overall Study Sample, Based on Questionnaire Responses

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Handwashing before meal (%)				.749
With soap	87.6	86.1	86.8	
Without soap	12.4	13.9	13.2	
Methods of handwashing (%)				.001
One by one in running water	59.5	77.0	69.1	
In a shared container of water	40.5	23.0	30.9	
Hand washing after toilet use (%)				<.001
With soap	66.0	33.7	48.2	
Without soap	34.0	66.3	51.8	

TABLE 9: Themes and Selected Supporting Quotes Emerging From Key Informant Interviews (KII) and Focus Group Discussions (FGD), Describing the Handwashing Practices During a Critical Time in the Study Area

Theme	Selected Quotes	Information Source	Participants
Hand washing practices before the meal	'Hand washing is mostly done before and after eating, but rarely before food preparation.'	KII	Community assistant, female
	'Some people do not wash hands thoroughly before eating; they just dip their hands in a bowl of water to make their fingers wet.'	FGD	Very poor, male
	'We do not use soap during washing hands because we are using clean water and we are sure that it is safe because it is the same water we are using for drinking.'	FGD	Rich, female
	'We do not use soap to wash our hands because we are not used to do so.'	FGD	Rich male
	'At the barbeque places, people who are preparing barbeques do not wash hands and do not put water for the customers to wash their hands.'	FGD	Very poor, male
	'Washing our hands before meals in the same pot, even if we are six people, is common here; a thing which is not proper because we are sharing germs on our hands, but not washing hands.'	FGD	Very poor, female
	'We do not always wash hands after toilet use and in most cases, if we do, we do it without soap.'	FGD	Rich, male
	'There is nothing we can do about leaving the toilet without washing hands because we were born and found things are done that way.'	FGD	Very poor, male
	'A large proportion of the households do not put water in the toilets for washing hands as it is a very expensive practice to maintain because we buy water here.'	FGD	Very rich, female

TABLE 10: Sanitation Facilities and Practices in Each Ward and the Overall Study Sample, Based on Questionnaire Responses

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Using latrine (%)				.759
Yes	97.4	97.3	97.4	
No	2.6	2.7	2.6	
Type of latrine used by household (%)				.094
Ventilated pit latrine	0.5	3.4	1.8	
Traditional pit latrine	99.5	96.6	98.2	
Latrine sharing among households (%)				.035
Not shared	58.1	46.2	51.5	
Shared with one or more households	37.3	51.3	48.5	
Disposal of children's faeces (%)				<.001
In a latrine	73.2	54.0	62.6	
Not in a latrine	26.8	46.0	37.4	

TABLE 11: Themes and Supporting Quotes Emerging From Focus Group Discussions (FGD), Describing the Availability and Use of Sanitation Facilities in the Study Area

Theme	Selected Quotes	Information Source	Participants
Availability of improved sanitation services	'It is very expensive to build the ventilated latrine'	FGD	Very rich, male
	'How can I use a lot of money to build the good latrine, while I am living in a poor traditional house?'	FGD	Very poor, female
Toilet sharing among households	'Several households organise themselves to build a toilet, and all households participating in the building of the toilet use that toilet.'	FGD	Very rich, male
	'Our latrines are not that strong. As a result, during the rainy season, latrines in some households get washed away by floods. This leads people to use the neighbouring latrine while waiting to build another one and not all households manage to rebuild their latrines.'	FGD	Rich, female
	'We are living in cooperation. Building a toilet for each household is a sign of segregating from each other.'	FGD	Rich, male
Open defaecation and children faeces disposal	'Open defaecation is mostly practised by the big farmers and livestock keepers because they live in isolation. When they clear the area for their activities, they do not build a toilet and end up defecating in nearby bushes.'	FGD	Very rich, male
	'We are farming away from where we are staying and on the farm, there is no latrine; therefore, when we are in the fields we help ourselves in the bushes or dig a shallow hole and cover it with soil when we are done.'	FGD	Very poor, female
	'I do not see that there is a need to dispose of the children's faeces in the toilet, which is not even close to my house, while it is just children's faeces, which have no problems like adult faeces; therefore, I normally dispose of it in the field.'	FGD	Rich, female

TABLE 12: Selected Variables Indicating Human-Chicken Interactions in Each Ward and the Overall Study Sample, Based on Questionnaire Responses

Variable	Sanza Ward (n=153)	Iwondo Ward (n=187)	Overall Study Sample (N=340)	P Value
Chicken roosting locations (%)				.187
Inside the house	81.7	75.4	78.2	
Not inside the house	18.3	24.6	21.8	
Access of chickens to pots and plates (%)				
Before washing				.264
No access	22.9	28.9	26.1	
Access	77.1	71.1	73.8	
After washing				.033
No access	55.6	67.4	62.1	
Access	44.4	32.6	37.9	
Drinking water containers				.012
No access	52.3	38.5	44.7	
Access	47.7	61.5	55.2	
Access of chicken to human faeces (%)				<.001
No access	37.9	23.5	30.0	
Access	62.1	76.5	70.0	

TABLE 13: Themes and Supporting Quotes Emerging From Key Informant Interviews (KII) and Focus Group Discussions (FGD), Describing the Public Health Risks Associated With Chicken Keeping Practices in the Study Area

Theme	Selected Quotes	Information Source	Participants
Reasons for keeping chickens inside the house overnight	'We have a chicken house but when you keep chickens outside in their houses, they are attacked by the cats.'	FGD	Very rich, male
	'We prefer to keep our chickens inside because when left outside in their house they get stolen.'	FGD	Very rich, male
	'We were born and found our parents keeping the chickens inside the house and we are continuing doing the same.'	FGD	Very poor, female
	'The number of chickens also matters. There is no way I can waste my energy to build a house for only five chickens.'	FGD	Very poor, female
Effects associated with keeping chicken inside the house overnight	'There is no problem, maybe mite infestations to humans, but I don't think it is a big problem.'	KII	Community leader
	'Chickens are carrying insects including ticks and mites which attack humans, and when you become infested by these insects you cannot sleep at night.'	FGD	Rich, female
	'Chicken is eating human faeces, especially from children who have not yet started to use the latrine, and dirt that remains on the beaks and legs can lead to contamination of the pots and transmit diseases.'	KII	Nurse 3, female
	'Keeping the chickens in the house overnight makes the house smell bad, because of chicken faeces.'	FGD	Rich, male

TABLE 14: Mixed Method Analysis of the Qualitative and Quantitative Data Related to the Key Areas Explored

Key Areas	Qualitative Findings KII (n=10) and FGD (n=61)	Quantitative Findings Questionnaire Surveys (N = 340)	Mixed Method Interpretation
Community knowledge on the causes of diarrhoea in children	• 'To my understanding, the child must develop diarrhoea in every stage of growth; when they start sitting, crawling, standing and with the growth of the teeth.' • 'Children under five are eating everything they encounter, some of which causes diarrhoea.'	Causes of diarrhoea (multiple responses possible): • Growth stages (44.4%) • Unhygienic environment (28.2%) • Drinking unsafe water (25%) • Unhygienic food handling (23.8%) • Eating without washing hands (20.3%) • Partially cooked food (11.8%) • Chickens access to the utensils (7.1%)	Both data sets indicate mosr participants relate particular growth stages with the occurrence of diarrhoea in children. Other important causes, including open defaecation and a dirty house environment, were not mentioned (qualitative data) or rarely selected (quantitative data). Both data sets indicate a knowledge gap on the causes of diarrhoea.
Child growth stage in relation to high incidence of diarrhoea	• 'During complementary feeding stage, because of mixing the mother's milk with food in child's gut.' • 'Mostly observed when the child starts sitting or crawling because during this period the children eat dirties from the environments where humans and chickens are spending a day.'	Growth stage was reported to be most affected by diarrhoea, amongst children under five years of age: • Exclusive breastfeeding period (15.9%) • Complementary feeding (41.9%) • Weaning period (10.3%) • All stages are the same (24.2%)	Both data sets are in agreement that diarrhoea mostly occurs during the complementary feeding stage, despite some incorrect reasons reasons for the increase in the frequency of occurrence of diarrhoea during this period provided by FGD and KII participants.
Relationship between seasons and occurrence of diarrhoea	'Mostly observed during the rainy season because during this period most people use pond water, which results in people drinking contaminated water as when it rains all animal and human faeces are washed out towards the water sources.' 'Diarrhoea episodes are frequently seen during the rainy season in all ages because during this period a lot of wild green vegetables are eaten, and the problem becomes more evident when the children start eating groundnuts from the farm.'	Use of stream, river, pond or dam as the source of drinking water: • Dry season (77.1%) • The rainy season (81.3) Households spending less than 1 hour to fetch water: • Dry season (62.6%) • The rainy season (85.9%)	Both data sets show shifting to streams, rivers, ponds or dams as the main source of water in the rainy season. Changes in the time spent fetching water between seasons indicate an increase in surface water use in the rainy season. Relating diarrhoea with the type of food further indicated a knowledge discrepancy in the causes of diarrhoea.
Use of water contaminated with animal faeces and urine	'We do not share the same source of water with animals, rather we build traditional troughs (mrambo) beside the well then we take water from the well to fill up the trough for animals to drink.' 'At the small dams we have here, you may find the cattle drinking, and on the other side, the people are fetching water for home use.'	Households using drinking water sources shared by animals: • Dry period (42.4%) • Rainy period (24.1%)	Both data sets are in agreement that there is a significant proportion of households using surface water contaminated with animal faeces. The mismatch seen in qualitative data on the the level of understanding of how the water can be contaminated with animal faeces, as seen in these two quotes in qualitative data, may results in under-reporting of the households using water contaminated with animal faeces in quantitative data,

Continue

TABLE 14: Contnued

Key Areas	Qualitative Findings KII (n=10) and FGD (n=61)	Quantitative Findings Questionnaire Surveys (N = 340)	Mixed Method Interpretation
			may results in under-reporting of the households using water contaminated with animal faeces in quantitative data, hence the latter should be interpreted with caution.
Handwashing practices	'We do not use soap because we are using clean water and we are sure that it is safe as it is the same water we are drinking.' 'Washing our hands before meals in the same pot, even if we are six people, is common here; a thing which is not proper because we are sharing germs on our hands, but not washing hands.'	Households washing hands with soap: • Before meals (13.2%) • After using the toilet (48.2%) • Methods of hand-washing: • One by one in running water (69.1%) • In a shared container (30.9%)	Both data sets indicate rare use of soap during handwashing before meals and after latrine use as well as ineffective handwashing. Qualitative data indicate that some people are aware of the health effects of these practices, despite continuing to do otherwise.
Effect of chicken gaining access to kitchen utensils	'Chickens are eating human faeces, especially from children, who have not yet started to use latrines, and enter every part of the house which predisposes the pots and food to human faecal contamination.'	Access of chickens to: • Unwashed cooking and serving utensils (73.8%) • Washed cooking and serving utensils (37.9%) • Water containers (61.5%) • Human faeces (70.0%)	Both data sets complement each other by showing the existence of the access of the chickens to kitchen utensils and access of the chickens to human faeces which both indicates the possibility of using the utensils contaminated by human faeces.

The difference in the number of chickens drinking water from household water containers was significant between wards, being more commonly reported in Iwondo (61.5%) than in Sanza (47.7%) (P=.012). Unimproved houses for household members, keeping chickens under scavenging production systems, and keeping chickens in the house overnight were mentioned by KII and FGD participants as the main reasons for chickens drinking water from household containers. Chickens gaining access to human faeces was reported in 62.1% and 76.5% of the households in Sanza and Iwondo, respectively, and differed significantly between wards (P<.001). Human infestation with external parasites (e.g. mites, fleas, lice and ticks), smell of chicken faeces in houses, and contamination of the house and kitchen pots with human faeces carried in by scavenging chickens were the health problems mentioned as more likely to occur under such human-chicken interactions (Table 13).

Data Triangulation

The quantitative and qualitative datasets were merged and compared to identify the areas of convergence, divergence and mutual complementarity. This enabled a deeper understanding of the messages communicated by the available data. Both sets of data showed a clear gap in knowledge of the causes of diarrhoea, which was considered as normal event occurring during the growth stages of children under five years of age. Questionnaire survey data showed a marked shift from using public tap water during the dry period to relying on streams, rivers, ponds or dams during the rainy season, especially in Iwondo. This shift was well explained by FGD participants, who indicated more households using water from open sources during the rainy season because it was free of charge, available and convenient.

Information on sharing of water sources with animals was gathered in the questionnaire survey to capture the proportion of households using water potentially contaminated by animal faeces and urine. Some FGD participants considered water from the well to be shared only if animals were able to drink directly from the well, not from troughs built beside the well. Therefore, the number of households using water potentially contaminated with urine and faeces from animals may be underrepresented in the questionnaire survey, as contamination can occur even if animals are drinking from areas adjacent to the well. More detailed information on data triangulation is presented in Table 14.

DISCUSSION

This study provides an insight into the housing, water supply, hygiene and sanitation challenges that households face in resource-poor settings in rural communities of central Tanzania. Despite differences in gender, and socio-economic status, there was very little variation in the responses given by the participants from different FGD groups. For example, in Iwondo Ward, all groups claimed to use water from streams, rivers, ponds or dams during the rainy period for financial reasons, regardless of their economic status. Although there were significant differences in many of the variables tested between wards, the prevalence of diarrhoea in children was not significantly different, which may be an indication of these areas not sharing common determinants of diarrhoea and/or the potential for other important variables, not included in this study, to drive prevalence. Nonetheless, both categories of the data indicate the common drinking water sources, hygiene practices, existing type and use of sanitation facilities and animal-human interactions which may be contributing factors to the observed prevalence of diarrhoea in each ward.

Among important variables that differed significantly between wards, Sanza had a higher percentage of households washing hands in a shared container of water before meals, sharing sources of water with animals during the dry season and allowing chickens to access washed utensils. On the other hand, Iwondo had a higher percentage of multiple households sharing latrines, not disposing of children's faeces in the toilet, spending more time fetching water in both seasons, not washing hands with soap after toilet use, and allowing chickens to gain access to human faeces. All of these practices could increase the risk of developing diarrhoea, which may explain the similar reported prevalence of diarrhoea cases. Additionally, there was a shared seasonal pattern of sourcing drinking water, whereby in both wards the percentage of the households accessing water from a stream, river, pond or dam increased by more than three times in the rainy season, compared to that observed during the dry season. There was a significant difference in the perception of the age groups more prone to diarrhoea between wards, but this could have been due to failure to recognise age-specific predisposition to diarrhoea in some households in both wards. In general, commentary reporting increased diarrhoea episodes under five years of age was observed frequently among KII and FGD participants. This supports the general observation produced in the questionnaire data that children under five are seen to be more prone to developing diarrhoea in this area.

Although the differences in house roofing and wall materials were statistically significant, these did not appear to greatly influence the frequency of diarrhoea in these two wards. The majority of houses in the study areas were made up of a soil roof and walls made from mud and wooden poles. This type of house has small windows and is sometimes windowless, which may affect the effectiveness of house cleaning if there is no enough light admitted to make dirt easily visible to the cleaner. Additionally, sunlight has been reported to have bactericidal effects which may help to reduce the microbial community built up and survival on the house floor and walls.[19,20] Unimproved floors (without cement) and walls (without plaster) were mentioned by many participants as one of the obstacles to effective house cleaning, as mopping with water and soap is not possible on such surfaces. Unimproved floors are difficult to clean and are more likely to contain high microbial populations than cement floors, as reported in one study conducted in Peru.[21] Chicken, rat, bat and owl faeces and chicken feathers were commonly encountered in the houses and many of these animals have been implicated in harbouring pathogens of zoonotic importance.[22,23,24]

Availability of free surface water during the rainy season was the reason for a large proportion of the households shifting from using tap water to surface water, especially in Iwondo, where tap water is frequently used by a

relatively large proportion of the households in the dry season. The Tanzania National Water Policy of 2002 directs the establishment of user-pays water schemes, operated and maintained by communities.[25] One borehole and two windmills in the Sanza Ward were not working during the data collection period, because community members could not afford to pay for the service, which is the source of funds for repair and buying fuel (personal communications from the Sanza Village Executive Officer). Unless current financial conditions improved in rural areas, the effective use of upgraded sources of water for improving community health will remain hard to achieve. Communities will continue to turn to unimproved water sources for financial reasons.[7] The proportion may differ between seasons, but both wards use water potentially contaminated by animal faeces through the sharing of water sources with animals and using rain water run-off. This predisposes community members to pathogens associated with animal faeces.[26] Home water chlorination and boiling can be promoted to mitigate these effects, as it has been reported to be effective in the control of diarrhoeal diseases in children in Ethiopia and Nepal, respectively.[27,28] However, some people in the study areas and elsewhere consider boiled water unpalatable for drinking.[8]

Effective handwashing can be achieved by cleaning under the fingernails and rubbing the fingertips with soap under running water.[29] The use of soap during handwashing was rare in the study areas and washing hands in shared containers of water were practised by a reasonable proportion of households, which may be contributing to the prevalence of diarrhoea observed. Most households do not ensure availability of water for handwashing in the latrine and do not have designated areas for handwashing. This forces household members to carry water with them when visiting the toilet or to collect water from the house for handwashing after toilet usage. The latter practice may predispose utensils and water within the house to human-faecal contamination. Encouraging behavioural change based on emotional drivers through hygiene promotion campaigns may be appropriate in these areas, as it has been more effective in advancing behaviours of handwashing with soap than a cognitive approach.[30,31] Training of stakeholders on hygienic food handling at ready-to-eat food selling points, especially the barbecue areas, and instituting by-laws related to food hygiene may build good 'hygiene habits' among the community during their outdoor activities.

Keeping chickens inside the house overnight, as is commonly done in the study areas as a precaution against theft and predation, results in external parasite bites on humans. This not only causes a significant nuisance, but can be associated with allergic conditions, for example, flea bite dermatitis, and can transmit zoonotic diseases.[32] Host-associated genetic markers in rural Bangladesh indicated an occurrence of avian faecal markers more frequently in the soil, hand rinses and stored water from households owning birds.[11] However, the participants in the current study were more concerned about the role of chickens in contaminating utensils with human faeces, than direct contamination with chicken faeces. The current study indicates access to human faeces being common amongst scavenging chickens, due to poor disposal of faeces and poorly constructed latrines which are easily accessed by chickens. Placing utensils in large containers with lids may solve the problem, as this deters not only chickens but also rats, bats and other animals and insects from gaining access to utensils. The knowledge of the community on the health effects associated with living with chickens under the same roof was centred mainly on the direct effects of ectoparasite transmission, while the effects of potential diseases transmission due to avian faecal contaminations were not known. Animals are potential sources and transmitters of gastrointestinal infections and their role should be well addressed in water, sanitation and hygiene intervention programs implemented in extensive livestock-keeping communities.[33]

More than 90% of the households in both wards use unimproved latrines and nearly half of all households share latrines. As reported in other studies,[34,8] high construction costs were pointed out by most KII and FGD participants in the study areas as the factor that prevented access to improved latrines. Children's faeces were perceived by many participants of FGD and KII as being free from pathogens causing human diseases. Hence, proper disposal was not considered necessary, predisposing the house compounds to human-faecal contaminations. Latrine sharing, improper disposal of children's faeces, open defaecation and dirty latrines have been reported in different countries as risk factors for developing mild and severe diarrhoea in children.[35] Among others, children growth stages was mostly mentioned by the questionnaire respondents as the main cause of diarrhoea in children in the current study. This belief needs to be addressed through greater awareness of the risk factors associated with diarrhoea among community members.[36] Despite their importance, the practices of inadequate cleaning of utensils, not using latrines, washing hands in a communal basin, and access of chickens to the kitchen utensils were rarely mentioned as the possible causes of diarrhoea. In Sanza, the occurrence of diarrhoea was reported to be related to eating fresh foods that are plentiful during the rainy season; however, this is more likely due to the use of surface water, which is abundant during this period but should not be perceived to have a causal relationship. The current study like another study[37] indicates increase in diarrhoea cases in children during the complementary feeding stage. Complementary feeding involves the provision of other food and liquids along with breastfeeding, normally between 6 to 24 months of age, when breast milk alone is no longer adequate to meet the nutritional requirements of infants, however, for some reasons, complementary feeding is sometimes introduced before 6 months of age. Apart from consuming contaminated food, this is a stage when children are sitting and crawling. This results in mouthing potential contaminants, including animal faeces from the soil, as well as soil itself, predisposing them to gastrointestinal infections.[38,39] Children from households practising free-range as the means of raising animals are more likely to acquire animal-derived gastrointestinal infections, due to the contamination of compounds with animal faeces.[40] However, evidence exists showing that childhood contact with livestock can be associated with improved human immune system function, particularly around allergic responses.[41] In addition, livestock ownership was

associated with animal source food consumption and increased expenditure on human healthcare, leading the authors to conclude that interventions that improve general animal health may have the greatest impact on human health through increasing household wealth.[41]

The Tanzanian Government's Vision 2025 promises to increase access to improved sanitation services to 95% of the population, fighting against water, sanitation and hygiene-associated diseases that are estimated to absorb 70% of the total health budget.[42] Furthermore, the Tanzanian Government, along with 192 other nations, has committed to achieving Sustainable Development Goals 6, focuses on achieving equal access to safe, sufficient and affordable drinking water and sufficient sanitation and hygiene services by 2030.[43] Deaths of under five years of age attributed to diarrhoea in Tanzania decreased from 14% in 2000 to 8% in 2016 i.e. a decrease of 24,466 to 9,441 of total deaths in children under five years of age.[44]

Improved water supply, sanitation and hygiene services form an integral component in the control of diarrhoea in children and have contributed largely to the achievements recorded during this period. Despite these achievements, access to important services which are vital for diarrhoea control, have not been equitably distributed between rural and urban areas. This is contrary to the 2030 Agenda for sustainable development, which advocates reducing inequality and leaving 'no-one' behind.[45] This is evident in Tanzania, whereby in 2016 the proportion of rural households in the Tanzania mainland depending on unimproved sources of drinking water was almost four times higher than that in urban areas.[2] Provision of improved water sources under a user-pay community program, designed to ensure sustainability (as directed in National Water Policy of 2002), may not work in many rural areas as significant numbers of households tend to turn to unimproved water sources during the rainy period. This tendency is due to convenience and financial constraints as documented by this study, which is in agreement with another study conducted in other rural areas of Tanzania.[7] Until living standards are improved in rural areas, households will have difficulty affording user-pay fees system. Alternatives, including government subsidies, should be taken into consideration to reduce the service access gap between rural and urban areas.

CONCLUSION

The barriers to the use of safe water in resource-poor settings are not limited only to availability, but also to affordability, convenience and prioritisation of financial resources. Based on findings of the current study, the immediate manageable areas of control of childhood diarrhoea should be directed at water treatment at household level and preventing recontamination, reduction of children-chicken interaction and chicken access to kitchen pots to prevent chicken-faecal contamination, proper human faeces disposal to reduce possibility of chickens transferring human faeces to kitchen pots and children, and educating community on the causes of childhood diarrhoea. Educating the communities on the multifaceted nature of risk factors associated with childhood diarrhoea is an important step towards better use of available resources to control childhood diarrhoeal diseases.

It is recommended that the Government of Tanzania develop area-specific water policies as the user-pays for service system used countrywide has not been successful in many rural areas.

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ORIGINAL ARTICLE

Iron Deficiency and Iron Deficiency Anemia Among Children 3 to 59 Months of Age in Kinondoni Municipal, Dar es Salaam: A Facility-Based Cross-Sectional Study

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ABSTRACT

Background: Iron deficiency with subsequent iron deficiency anemia is the most common micronutrient disorder in children below 5 years of age worldwide. The developing countries bear more weight on the problem as the result of multifactorial factors including but not limited to recurrent infections such as malaria, helminths infestation, and inadequate food security. However, its magnitude in children living in Kinondoni Municipal in Dar es salaam is not well understood. Therefore, the aim of this study was to determine the prevalence of anemia and how it is contributed by the presence of iron deficiency among children between 3-59months of age in the above-mentioned setting.

Methods: A facility-based cross-section study was conducted among children 3-59months attending Reproductive and Child Health Services at Kairuki, Sinza Hospital, and Kambangwa dispensary. Children who met the criteria, their basic social demographic information, complete blood count and differentials as well as blood ferritin levels were collected to assess the level of anemia, erythrocytic indices, and iron deficiency. Data were analyzed using the Statistical Package of Social Sciences (SPSS version 22). The magnitude of anemia and iron deficiencies were presented in percentages, and the relationship between hemoglobin and blood ferritin was assessed using Spearman's correlation test for two continuous variables. The p-value of less or equal to 0.05 was considered statistically significant.

Results: A total of 350 children were recruited for the study, 255 Children (72.9%) were anemic. Children below 24 months of age were more anemic compared to the older age group (X2 = 50, p <0.001). Furthermore, anemia was significantly associated with low ferritin levels (X2 = 65, p <0.001). Iron deficiency was found in 156 (44.6%) participants while iron deficiency anemia (low MCV, low ferritin, and low hemoglobin) was found in 138 (39.4%) participants. However, among 255 participants with anemia, 147(65.3%) had iron deficiency. There was a significant positive correlation between hemoglobin and blood ferritin levels (Spearman's correlation coefficient = 0.6; p<0.01.

Conclusion: Prevalence of anemia was high among children and was highly associated with younger age and iron deficiency. To overcome this problem, appropriate interventions such as massive promotion of breastfeeding, appropriate complementary feeding, and ensuring food security are warranted.

INTRODUCTION

Anemia is defined as a quantitative or qualitative deficiency of hemoglobin, or reduction in the number or volume of red blood cells with subsequent reduction in oxygen carrying capacity of blood to meet the body's physiological needs. For children below 5 years of age, it is defined as hemoglobin below 11g/dl.[1] According to the 2019 World Health Organization (WHO) data on anemia, globally up to 39.8% of children under-fives were anemic with the African region recording up to 60.2% of children of the same age with anemia.[2] The most common causes of anemia in low resources settings are nutritional deficiency (iron, vitamin B12, and folic acid); infections such as malaria, hemoglobinopathies, and hookworm infestation.[3,4] Iron deficiency anemia is the most common type of anemia in children, especially in developing countries where nutritional deficiency and hookworm infestation are rampant.[3–5] The diagnosis of iron deficiency anemia is made in combination of hemoglobin level being below 11g/dl and blood ferritin less than 12µg/L while isolated iron deficiency is confirmed when blood ferritin is less than 12µg/L with normal hemoglobin. [1] Iron is an instrumental element in red blood cell formation (erythropoiesis). It is an essential component of hemoglobin and oxygen-carrying molecule in the red blood cells by supplying ferrous ions for heme ring formation that links four polypeptide chains in the hemoglobin structure.[6] Moreover, iron plays a vital role in enzymatic functions during energy metabolism, neurotransmission, soft tissue formation,

and immune system modulation.[7,8]

Children are more vulnerable to iron deficiency and iron-deficiency anemia due to the high demand for growth and development. Chronic deprivation of iron leads to iron depletion with subsequent low hemoglobin levels (anemia). Iron deficiency has detrimental effect on children such as poor weight gain; language, behavior, psychomotor skills, and cognitive impairment.[9,10] It has been proved that prolonged iron deficiency in early life, may lead to permanent disability especially in cognitive ability which cannot be re-corrected by iron supplementation.[7,8] If not well addressed, it will continue to be a stumbling block to children's wellbeing and survival. Unfortunately, the signs and symptoms of iron deficiency in children are not specific and start gradually over time which makes early detection and management a challenge; hence, prevention is the best intervention.

To prevent iron deficiency and iron-deficiency anemia in children, it is recommended to exclusively breastfeed for the first 4-6 months and then initiate infant feeding using iron-containing complementary foods such as green leafy vegetables, legumes, meat, poultry, and seafoods.[11] Children below 5-years of age should take around 10gm/day as a daily dietary requirement for metabolic demand and body iron stores.[11] In settings where the prevalence of anemia is to equal or more than 40%, WHO recommends daily iron supplementation in children aged 24-59 months as a preventive strategy. [12]

Despite the public health burden of iron deficiency and iron-deficiency anaemia worldwide, there is limited data on the magnitude in different settings in Tanzania. Therefore, this study aimed to determine the magnitude of iron deficiency and iron deficiency anemia among children 3 to 59 months of age in Kinondoni municipal in Dar es Salaam. Findings from this study may provide more insight on the problem in urban areas such as Dar es Salaam and inform on more appropriate mitigation strategies.

METHODS

Study Area

This study was conducted in Kinondoni Municipality in Dar es Salaam metropolitan city. Kinondoni is among 5 administrative municipals on 321 square meters of land. In the 2012 national census, Kinondoni had around 930,000 inhabitants with a steady population growth rate of 5% per annum.[13] Being in the urban area, the economic activities range from being employed in formal and informal sectors, and large to small scale businesses.

Study Sites

This study was conducted at Kambangwa Dispensary, Kairuki and Sinza Hospitals which are in Kinondoni Municipal. Participants were healthy children between 3-59 months who were attending Reproductive and Child Health (RCH) clinics. These facilities were randomly selected from the list of health care facilities in Kinondoni Municipal.

Study Design

This was cross-sectional descriptive study among children 3-59 months of age attending RCH clinics for six months from May 2016 to November 2016.

Sample Size Determination

The sample size was calculated using Cochran's formula for cross-sectional studies[14] by using a selected critical value at 95% confidence interval (1.96), desired level of precision of 0.05, and an estimated prevalence of iron deficiency anemia at 35% from Tanzania Demographic and Health Survey 2010.[15] Therefore, a total of al 350 children were recruited into our study.

Inclusion and Exclusion Criteria

Children attending Reproductive and Child Health Clinic (RCH) at Kairuki, Kambangwa, and Sinza Hospitals aged between 3 months and 59 months were eligible for participation. Children with a known genetic or congenital condition that can cause anaemia such as active haemorrhage; bleeding disorders or hemoglobinopathies; history of blood transfusion and/or surgery within three months before screening date; those with febrile illness, known history of chronic infections and other inflammatory conditions were excluded from the study.

Ethical Issues

Ethical clearance was obtained from the Hubert Kairuki Memorial University (HKMU) Research Ethics Committee before this study was conducted. The permission to conduct the study was obtained from Kinondoni Municipal health administration authorities as well as from respective hospitals. An informed consent was obtained from the parents/caretakers of participants before enrolment. Confidentiality was ensured for all individuals who participated in the study. No personal identifiers were used in the data collection tools. To minimize the risks, blood collection was performed by experienced personnel using routine procedures and observing aseptic techniques. Children below 3 months of age were excluded to minimize pain and discomfort because their visits coincide with injectable vaccinations. Participants who were found to be anaemic were managed according to Tanzania National guidelines.[16]

Recruitment Procedures

All non-ill children who were attending at Reproductive and Child Health Clinic (RCH) at Kairuki, Kambangwa (Mwananyamala), and Sinza Hospitals during the day of an interview were eligible to participate in this study. Participants were screened for eligibility, and those who met the inclusion criteria, the informed consent was sought from their parents/caregivers to participate in the study. For those whose parents consented, they were recruited sequentially until the required sample size was obtained.

Data collection

Socio-Demographic Information

Sociodemographic information was collected from the participants and their parents/caretakers using a pre-designed Case Report Form (CRF). Information regarding patients' age, sex, residence, birth weight, natal history of prematurity, breastfeeding practices, age at introduction of other types of food, and types of complementary food in the first year of life, dietary recall were obtained from parents/guardians. Additional information that was taken includes marital status, family history of Sickle cell disease, and history of chronic illnesses g. HIV/AIDS.

Parents/guardians' occupation, information about family size, and education level of the mother were also collected. Moreover, a clinical examination of the participants was performed including anthropometric measurements which were conducted as per WHO standard guidelines.[17]

Anthropometric Measurements

The children under 24 months of age were weighed with non-clothes at the enrolment site using a 25kg hanging Salter weigh scale (SECA, Hamburg, German), while those above 24 months of age were weighed while standing on the Salter digital floor scale. Height was measured using a standard wooden stadiometer. Height for children above 24 months was taken while standing with bare feet while the length for those below 24 months was measured while lying flat on the measuring board with an assistant supporting the legs on the measuring board to ensure that the child was lying flat. Measurements were recorded to the nearest 0.1cm. The nutrition status of participants was determined as mild, moderate, or severe according to the z-score of the weight for height on the WHO reference charts for age and sex.[18]

Full Blood Count and Blood Ferritin Determination

Two milliliters of venous blood sample from each study participant were collected and transported for analysis at Hubert Kairuki Memorial University Clinical Research Laboratory in line with the International Council for Standardization in Hematology (ICSH) using the point of care standard operating procedures explained elsewhere.[19] A complete Blood Count (CBC) was performed using an automated hematology analyzer (Beckman-Coulter, Model Act 10, Brea, CA, USA,). Anemia status was determined by hemoglobin values, and severity of anemia was determined using WHO standards for children whereby normal values should be ≥11g/dl, mild anemia 10-10.9g/dl, moderate anemia 7-7.9g/dl, and severe anemia <7g/dl[2] . Serum ferritin levels as a marker of iron deficiency were determined using the electrochemiluminescence method[20] (Maglumi -62000, Biomedical Engineering Co. Ltd, Shenzhen, China). Blood ferritin levels less than 12µg/L were classified as low (iron deficiency) while those with anemia, MCV less than 80fl on CBC, and ferritin level less than 12µg/L were classified as iron deficiency anemia.

Data Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA). Association between independent variables and presence of anemia was assessed using the Chi-square test or Fisher's exact test whenever appropriate. The relationship between blood hemoglobin and ferritin levels was determined using Spearman's test. A cut-off point for statistical significance of analyses (*p*-value) was set at less or equal to 0.05.

RESULTS

A total of 350 children were enrolled in the study. Males were 193 (55.1%) while females were 157 (44.9%). The mean age for participants was 17.3±11 months. Other Baseline characteristics of participants and parents are indicated in Table 1.

Prevalence of Anemia

Anemia was present in most participants, whereby 255 participants (72.9%) were anemic. Severe anemia was present in 2 (0.6%) of the study participants, while the rest had either mild or moderate anemia (Table 2). Children under 24 months were more affected whereby 218 out of 267 (81.6%) children below 24 months were anemic; $X^2 = 50$, $p < .001$ (Table 3). Moreover, anemia was associated with low ferritin levels; $\mathbf{X^2} = 65$, $p < 0.001$ (Table 3). Furthermore, among 225 participants with anemia, 147 (65.3%) had iron deficiency (Table 4).

Iron Deficiency and Iron-Deficiency Anemia

Iron deficiency was found in 156 (44.6%) participants (Table 3) while iron deficiency anemia (low MCV, low ferritin and low hemoglobin) was found in 138 (39.4%) of participants (Table 4). However, among 255 participants with anemia, 147 (65.3%) had iron deficiency. There was a significant positive correlation between hemoglobin and blood ferritin levels (Spearman's correlation coefficient = 0.6; $p < .01$ (Figure 1).

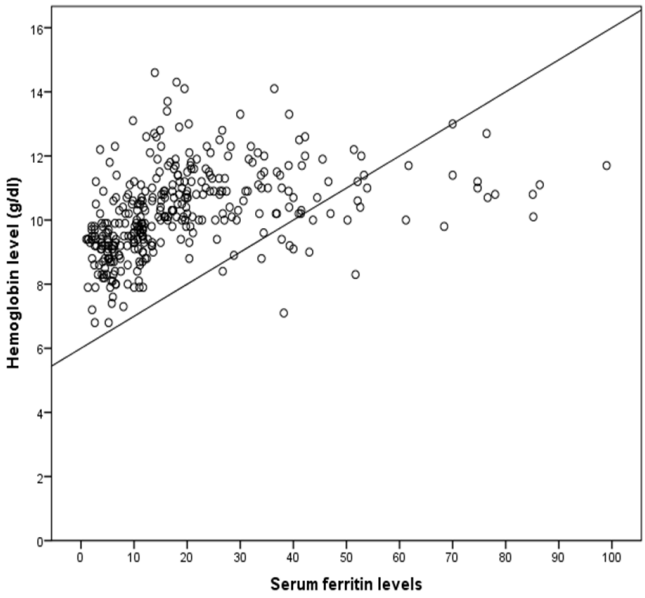
TABLE 2: Distribution of Anemia among Study Participants

Anemia status	Frequency	Percent
Normal (≥ 11gm/dl)	95	27.1
Mild anemia (10-10.9g/dl)	101	28.9
Moderate anemia (7-9.9)	152	43.4
Severe anemia (≤7g/dl)	2	0.6
Total	350	100.0

TABLE 1: Baseline Characteristics of Study Participants (N=350)

Variable	Number (n)	Percentage (%)
Sex		
Male	193	55.1
Female	157	44.9
Age group (months)		
<24 months	268	76.6
≥ 24 months	82	23.4
Gestation age at delivery period (weeks)		
<37 weeks	12	3.4
≥37 weeks	338	96.6
Nutritional status		
Normal	285	81.4
Mild malnutrition	55	18.7
Moderate malnutrition	10	8.9
Birth order of the child		
1st t child	168	48
2nd child	106	30.3
3rd child and above	76	21.7
Parental care		
Both parents	309	88.2
Single parent	41	11.8
Number of persons/households		
<6 people	320	91.4
6+people	30	8.6
Age when complementary feeding was initiated		
Still on exclusive breastfeeding	184	52.6
Less than 6 months	158	45.1
At 6 months or more	8	2.3

FIGURE 1: tionship Between Hemoglobin and Blood Ferritin Levels



Spearman's Correlation Coefficient=0.6, p<.01

TABLE 4: Morphological Classification of Anemia in Relation to Ferritin Levels

MCV status	Ferritin level status	Is the child anemic? (Hb<11g/dl)		Total
		Yes	No	
Low (<80fl)	Serum ferritin level status			
	Low	138	8	146
	Normal	101	63	164
	Total	239	71	310
Normal (≥80fl)	Serum ferritin level status			
	Low	9	1	10
	Normal	7	23	30
	Total	16	24	40
Total	Serum ferritin level status			
	Low	147	9	156
	Normal	108	86	194
	Total	255	95	350

TABLE 3: Factors Associated with Anemia Among Children Aged 3 to 59 months

Variable	Is the child anemic? (Hb<11g/dl) Yes n (%)	No n (%)	Total	Chi-Square test	P value
Age groups					
Less than 24 months	218 (81.6)	49 (18.4)	267 (100)	50	<.001
24 to 59 months	37 (44.6)	46 (55.4)	83 (100)		
Total	255 (72.9)	95 (27.1)	350 (100)		
Sex					
Female	109 (69.4)	48 (30.6)	157 (100)	1.69	.11
Male	146 (75.6)	47 (24.4)	193 (100)		
Total	255 (72.9)	95 (27.1)	350 (100)		
Birth weight					
Low birth weight (<2.5kg)	23 (74.2)	8 (25.8)	31 (100)	0.03	.86
Normal Birth weight (≥ 2.5kg)	232 (72.7)	87 (27.3)	319 (100)		
Total	255 (72.9)	95 (27.1)	350 (100)		
Gestation age at birth					
Preterm (<37 weeks)	9 (75)	3 (25)	12 (100)	0.29	.58
Term (≥37 weeks)	246 (72.8)	93 (27.2)	333 (100)		
Total	225 (79.2)	95 (27.1)	350 (100)		
Mother's age (years)					
≤ 20	24 (72.7)	9 (27.3)	33 (100)	0.47	.99
21-30	152 (73.1)	56 (26.9)	208 (100)		
31-40	72 (72.7)	27 (27.3)	88 (100)		
≥41	7 (70)	3 (30)	10 (100)		
Total	225 (79.2)	95 (27.1)	350 (100)		
Blood ferritin level					
Low (<12µg/L)	147 (94.2)	9 (5.8)	156 (100)	65	<.001
Normal (≥12µg/L)	108 (55.7)	86 (44.3)	194 (100)		
Total	255 (72.9)	95 (27.1)	350 (100)		
Age when complementary feeding started					
Exclusive breastfeeding	130 (70.7)	54 (29.3)	184 (100)	1.65	.44
Less than 6 months	120 (75.9)	38 (24.1)	158 (100)		
Six months or more	5 (62.5)	3 (37.5)	8 (100)		
Total	255 (72.9)	95 (27.1)	350 (100)		
Mother's level of education					
No formal education	12 (66.7)	6 (33.3)	18 (100)	3.8	.44
Primary education	7 (58.3)	5 (41.7)	12 (100)		
Secondary education	8 (72.7)	3 (27.3)	11 (100)		
Certificate to diploma	153 (76.2)	51 (23.8)	214 (100)		
Graduate/master/PHD	65 (68.4)	30 (31.6)	95 (100)		
Total	255 (72.9)	95 (27.1)	350 (100)		
Mother's source of income					
Employed in formal sector	17 (63)	10 (37)	27 (100)	2.7	.43
Housewife	137 (75.7)	44 (24.3)	181(100)		
Pet trader	100 (71.4)	40 (28.6)	140 (100)		
Others	1 (50)	1 (50)	2 (100)		
Total	255 (72.9)	95 (27.1)	355 (100)		

DISCUSSION

In this study, the prevalence of anemia was 72.9% which is relatively higher compared to the results from Tanzania Demographic Health Survey (TDHS) 2015-16, and a study by Kessy. J et al in Kilimanjaro, which showed an overall prevalence of 56% and 55.8%, respectively.[15,21,22] However, the findings in the Dar es Salaam cluster in TDHS, and another hospital-based study in Mwanza were similar to our findings. [22,23] Furthermore, the prevalence in this study was similar to other studies conducted in other Low and Middle-Income Countries (LMIC) which share similar socioeconomic characteristics.[4,25,26] However, the prevalence from our study is higher than the global estimates (39.7%).[27] This could be explained by previous studies findings which have implicated high prevalence of anemia in developing countries with poor dietary intake of iron containing foods such as green vegetables and meat; low socioeconomic status and infections such as malaria, recurrent diarrhea, and helminths infestations.[3,24,28]

We found that the magnitude of anemia and iron deficiency was relatively higher (81.6%) in the young

group below 24 months compared to the older age group. This finding is similar to the findings from the TDHS 2015-16 which was estimated to be at 81%.[22] Also, other studies done in Zanzibar, Mwanza, and Kilimanjaro revealed the same trend.[21,24] Furthermore, most of the children who were anemic (94.2%) had iron deficiency anemia evidenced by microcytic hypochromic anemia with low serum ferritin levels. Further analysis showed that the hemoglobin levels were highly correlated with serum ferritin levels which suggests that most of anemia cases in children are contributed by iron deficiency.

These findings imply that there could be a lot of children under five who are anemic, and mostly likely as the result of nutritional deficiency of iron. This could be contributing to poor physical and cognitive development, with subsequent failure to attain their full potential. Vulnerability in the younger age group could be the result of rapid growth with subsequent high demand of daily intake of iron compounded by early weaning and inappropriate complementary feeding practices.[29,30] It is recommended that infants should be exclusively breastfed for 4- 6 months for optimal growth and prevention of micronutrients deficiency including iron.[31,32] However, in Tanzania, it is estimated that only 50-60% of infants are exclusively breastfed during their first 6months of age[29,33–35] ,which may be the significant contributor of the observed iron deficiency anemia in the younger age group in our study and other previous studies. Additionally, those living in urban area like in Dar es Salaam where our study was conducted are more likely not to breastfeed their children exclusively for the recommended period which may subject them to increased risk of iron deficiency anemia.[29,36]

The practice of mixed feeding before the recommended time such as use of cow's milk which is the most common substitute in low resources setting is associated with gut inflammation with subsequent malabsorption of nutrients including iron.[21,28] Furthermore, other mostly used complementary solid foods in children worldwide do not contain adequate iron to meet the physiological demand of rapidly growing infants.[37] WHO recommends that the composition of complementary foods should be similar to that of the breastmilk for optimal growth and development.[38] However, this may not be practical in developing countries with limited resources and research. Iron fortified foods and meat are the most recommended complementary source of iron.[37] However, these foods are expensive and not readily available in low resource setting.

This study had some limitations; most of the information was provided by parents, and some of the details could have been forgotten leading to recall bias. Moreover, previous studies have shown that recurrent infections in young children such as malaria, diarrhea and helminths infestations are associated with anemia and iron deficiency[4,24], however, we were limited in resources to screen for these illnesses. Furthermore, it is well known that low birth weight and prematurity delivery are associated with iron deficiency and anemia especially starting from six months of age, however, this was not evident in our study. This could have been resulted from not having enough participants who were born prematurely or with low birth weight, as we could not match participants by their birth weight. Nonetheless, we still believe that our findings show a big picture of the problem, which needs to be analyzed further and to provide appropriate solutions.

CONCLUSION

In conclusion, anemia is highly prevalent among children under five years of age in Kinondoni Municipal in Dar es salaam, and iron deficiency could be the main contributor. This could be an important bottleneck for them to survive and thrive. Henceforth, more implementation research should be conducted to evaluate effective preventive strategies such as promotion of exclusive breastfeeding for the first 4 to 6 months, continued breastfeeding up to at least 24 months, intensification of community education on appropriate complementary feeding practices, and ensured food security. Additionally, there is a need to conduct research on iron content in locally available foods and develop a local content specific dietary guideline for appropriate complimentary feeding.

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ORIGINAL ARTICLE

Point Prevalence Survey and Patterns of Antibiotic Use at Kirinyaga County Hospitals, Kenya

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ABSTRACT

Background: Antibiotics are useful in treating and managing infections in outpatient and inpatient care settings. However, irrational antibiotic use can lead to improper patient care, antimicrobial resistance, wastage of resources and sometimes even death. The pattern of antibiotic use varies from one medical practitioner to another, infection, patient, wards, country and region. The study was a conducted as a baseline to describe the prevalence and patterns of antibiotic use in Kirinyaga County hospitals.

Methodology: The study was a point prevalence survey of antibiotics use among patients admitted to four hospitals in Kirinyaga county and the study utilised the World Health Organization methodology for point prevalence survey of antibiotics in hospitals. Data were abstracted from patients' files of patients who consented using a pretested tool. The data was exported to MS Excel for cleaning and analysed descriptively

Results: The prevalence of antibiotic use in the four hospitals in Kirinyaga county was 44.0% (95%CI 38.6-49.5%). Penicillins were the most prescribed antibiotic class at 29.1%, followed by cephalosporins at 23.0%. Ceftriaxone and metronidazole were the highest prescribed at 22.0% and 19.8%, respectively. Antibiotics were mainly prescribed for community-acquired infections at 58.2%, followed by surgical prophylaxis at 26.0%. Most patients (52.5%) received two antibiotics, predominantly benzylpenicillin and gentamicin, at 40.3%. The majority, 63.0%, of all antibiotics were administered parenterally. There was poor documentation of administration of medicines on the treatment sheet.

Conclusion: There was a relatively high prevalence of antibiotic use, all prescribed empirically. Community acquired infections were the most common indication for antibiotics. There was extensive use World Health Organization "watch" category of antibiotics without microbiological tests. There is a need for antibiotic stewardship program to ensure judicial use of antibiotics.

BACKGROUND

Antibiotics are essential in treating and managing most infections in routine patient care. They are used in treating and preventing microbial infections in both inpatient and outpatient settings. Their use varies across continents, countries, hospitals and even wards in the same hospital.[1,2] A global point prevalence survey showed that 1 out of 2 and 1 out of 3 hospitalised patients received antibiotics in Africa, Asia and Europe respectively.[3]

Medical wards had the least consumption at 27.4%, while transplant surgical wards had the highest at 77.0%.[1] The most prescribed class of antibiotics in the world is a combination of penicillins with B lactamase inhibitors followed by third-generation cephalosporins and fluoroquinolones.[1] A 2018 study by the World Health Organization (WHO) reports penicillins as the most commonly used group of antibiotics in 65 countries.[2] Lower respiratory infections, especially pneumonia, accounted for the highest antibiotic prescriptions.[1]

Numerous studies conducted in Kenya have documented the prevalence of antibiotics use in hospitals found in Nakuru County (54.7%), Nyanza region (67.7%), Kiambu County (62%) and at 3 tertiary hospitals (46%).[4-7] There is need to document more antibiotics prevalence studies in other hospitals in Kenya.

Antibiotic resistance is a mounting danger to humankind as discovering new molecules, especially those against gram-negative bacteria is slow and scarce.[8] This is projected to be severe in low and middle-income countries, which are burdened by a high number of infectious diseases and low access to alternative antibiotics due to high costs.[9] Some factors contributing to antibiotic resistance are; inappropriate and irrational antibiotic use in hospitals, self-medications, failure to complete therapy and substandard antibiotics.[9-11] Irrational use of antibiotics is wasteful and harmful due to the increased risk of adverse effects.[12] Antimicrobial stewardship is crucial in curbing antimicrobial resistance.[13-15] The prevalence and patterns of antibiotic use in Kirinyaga County hospitals have not been documented before

The study was conducted as a baseline to describe the prevalence and patterns of antibiotic use prior to the establishment of the antimicrobial stewardship program.The study was essential to identify areas where antimicrobial stewardship strategies can be applied and to minimise inappropriate antibiotic use.

MATERIALS AND METHODS

Study Setting and Design

The study was conducted at 4 Kirinyaga County public hospitals' inpatient departments in October 2021. The hospitals have a total bed capacity of 359, located in peri-urban centres, and offer varying clinical services for outpatient and inpatients.

The largest hospital was the county referral hospital (typical district hospital) with general medical wards, maternity, orthopaedic, surgical, psychiatry, paediatrics and obstetric wards. The other 3 Sub-county hospitals refer their patients to the referral hospital for further/ specialised management. The sub-county hospitals have maternity and medical wards only. All the hospitals were classified as primary hospitalsi n terms of the scope of services offered.[16]

The study design was a multicentre point prevalence survey of antibiotic use and utilised the World Health Organization (WHO) methodology for point prevalence survey of antibiotic use in Hospitals.[16] The study population consisted of inpatients in the study hospitals.

Inclusion and Exclusion Criteria

Patients admitted at or before 8AM on the day of the survey were included. Neonates born before 8AM and admitted to the maternity ward were also included. Patients admitted in the day surgery and renal dialysis ward were excluded. Patients admitted after 8 AM on the day of the survey and discharged patients awaiting transportation or relatives of admitted patients were also excluded. Antibiotics administered orally or parentally were included. Topical ophthalmic antibiotics and antibiotics initiated after 8 AM or discontinued before 8AM were excluded.

Sample Size

All patients who met the inclusion criteria were included in the study. The study employed universal sampling method because the bed capacity at all the 4 hospitals was less than 500. The WHO methodology for point prevalence survey for hospitals recommends the inclusion of all eligible patients for hospitals with less than 500 inpatient beds.[16]

Data Collection

Trained pharmacists collected the data from the 4 hospitals for a period of 2 weeks in October 2021. Data was collected at both ward and patient level. At the ward level, the type of ward, number of patients in the ward, number of eligible and included patients were collected from the ward admission register. At the patient level, informed consent was sought first before inclusion. For patients more than 10 and less than 18 years of age, informed assent from them and informed consent from their parents/guardians was sought. For the patients who consented, data such as age, sex, date of admission, diagnoses, history of antibiotic use and antibiotics used was abstracted from patient files. Data on antimicrobial type, dose, duration of treatment, route of administration, indication for antibiotic use, culture and sensitivity tests was also collected. Data was collected using pretested standardised forms. To ensure confidentiality, all consent forms were stored in a lockable cabinet while data collected was stored in a password protected computer only accessible to the investigators.

Data Analysis

The data collected was exported to Excel for data cleaning and analysis. The data was analysed descriptively. Descriptive statistics such as proportions and frequencies was done and results expressed with a 95% Confidence Intervals.

Ethical Considerations

Ethical approval for this study was sought from Kenyatta National Hospital /University of Nairobi Ethics Review Committee (reference number KNH-ERC/A/110). Approval to collect data in the 4 hospitals was sought from the County Director of Health, Kirinyaga County

RESULTS

Patient Characteristics

A total of 341 patients were approached for consent, of which 332(97.4%) patients gave their consent to be part of the study, only 2.6% (9/341) did not provide consent. A total of 332 records were reviewed from the 4 hospitals during the study. The majority 58.4% (194) were from the county referral hospital (Hospital A). (Table 1) Females represented 66.4% (217) of the patients. Adults represented the majority of those admitted in the wards accounting for 63%, followed by neonates at 14.8%, children aged 1 to 5 years at 12.6%, 6 to 17 years at 6.0 %, and lastly, infants at 3.9%. Most of the admissions occurred in the maternity wards at 41.5% (138/332), followed by the medical wards at 38.3%.

Majority (87.7%, 291/332) of the patients did not have a history of admission in the last 90 days before the study, 8.4% had a prior admission history, with 3.9% whose history had not been recorded. Only 11.8% (39/332) were transferred in from other hospitals. The patients had spent an average of 7 days in the ward before the survey date, ranging from <1 to 62 days (IQR 1-7). Only 15.7% (52/332) of the patients had undergone surgery while admitted, with 73%(38/52) being invasive. Of those surveyed, 1.8%(6/332) were on anti-tuberculosis treatment while 5.4% (18/332) were HIV positive. More than half of the patients had catheters inserted at the time of the survey (51.5%, 171/332), with the peripheral cannula leading at 96.5% (165/171). Only 1.8%(3/171) of those with catheters had urinary and peripheral catheters simultaneously.

Previous Use of Antibiotics

Patients were considered to have previously used an antibiotic if they had used an antibiotic which had been stopped during the current admission. Almost a quarter (23.2%, 77/332) of patients had used antibiotics which had already been stopped at the time of the survey. Most (48.1%, 37/77) had used at least 2 antibiotics during admission, followed by one antibiotic at 39%. Only 1.3%

had used more than 5 antibiotics that had already been stopped during admission.

Antibiotic Use

The prevalence of antibiotic use among the admitted patients was 44.0%, 146/332(95%CI, 38.6-49.5) Hospital B had the highest prevalence of antibiotic use at 54.8%, followed by hospital A at 42.3%. (Table 1) A total of 227 antibiotics were prescribed for 146 patients representing an antibiotic prescribing ratio of 1.55. Hospital A and B had the highest antibiotic prescribing ratio at 1.57, followed by Hospital C at 1.5 and hospital D at 1.

The Anatomical Therapeutic Chemical (ATC) classification of antibiotics and frequency of use were as shown in Table 2. The most commonly prescribed antibiotics class was penicillins at 29.1%, followed by cephalosporins at 23.0%, nitroimidazole antimicrobials at 19.8% and aminoglycosides at 15.4%. Among the penicillins, benzylpenicillin was the most prescribed at 53.3% followed by flucloxacillin at 30.3%. Ceftriaxone was the most prescribed cephalosporin at 96.2%, while gentamycin was the most prescribed aminoglycoside. The 5 most commonly prescribed antibiotics were ceftriaxone at 22%, metronidazole at 19.8%, benzylpenicillin and gentamycin at 15.4% and flucloxacillin at 8.8%. The majority of the patients, 52.7%(77/146) were on 2 antibiotics followed by one antibiotic at 45.9 %. The most common combinations were benzylpenicillin and gentamycin at 40.3%, followed by ceftriaxone and metronidazole combination at 18.2 %.

The Majority of the antibiotics prescribed were in the WHO access group. However, ceftriaxone, the most prescribed antibiotic, is in the watch group. Most of the antibiotics (63.4 %, 144/227) prescribed were administered parenterally, followed by oral at 35.7%. All the antibiotic prescriptions had their frequency of use indicated but 16.3%(37/227) were missing a stop or review date. It was not possible to assess missed doses due to poor documentation.

Common Diagnoses

The most common diagnosis for an antibiotic prescription were obstetrics and gynaecology issues at 22.6% (33/146) followed by pneumonia at 20.7%. The obstetrics and gynaecology issues included caesarean sections, incomplete abortions and postpartum haemorrhage. Other common diagnoses were septic wounds at 4.8%, fractures, upper respiratory infections and convulsive disorders at 4.4%.

Indications, Presence of Catheter and Microbiological Tests

Community-acquired infections were the most common indication at 58.2% (85/146) followed by surgical prophylaxis at 26% (38/146) and medical prophylaxis at 11% (16/146). Almost all (98.1%53/54) of those on antibiotic prophylaxis were on the antibiotics for more than a day. All the hospitals surveyed did not have the capacity to do culture and sensitivity tests prior to use of antibiotics. None of the patients had culture and sensitivity tests ordered or documented at all hospitals under review.

DISCUSSION

The prevalence of antibiotic use across the 4 hospitals was at 44% (95% CI, 38.6-49.5).These results are close to results of a study conducted at 3 tertiary hospitals in Kenya (46%) and Uganda 45% (95% CI, 30-57%).[7,17] The prevalence of antibiotic use reported in this study is slightly lower to that reported in other studies conducted in a referral hospital in Kenya (54.7%), Ghana (51%), 4 African countries (59%) and a referral hospital in Kiambu county (62%).[4,5,7,17] The prevalence of antibiotic use in this study was higher than what was recorded in hospitals in South Africa (31% and 38.5%)and lower than what was recorded in hospitals in Botswana (70.6%) and Pakistan (77.6%).[18-21]

In all hospitals, nearly half of the inpatients (41.6%) were admitted in the maternity.[14] This is due to the fact that Hospital B, C & D offered mainly maternity services and minimal specialised care. Similar results were observed in a survey conducted in a tertiary hospital in Ghana, where most patients were from the obstetrics unit.[22] The high number of patients in the obstetrics unit also explains why most of those on antibiotics (54%) had diagnoses related to obstetrics and gynaecology and mostly as prophylaxis for surgery. This is similar to studies conducted in African hospitals where surgical prophylaxis for obstetrics and gynaecology was a common indication across all countries at between 12 to 18%.[17] Community-acquired infections accounted for the highest indication for antibiotic use at 58.2%, followed by surgical prophylaxis at 26%. This was comparable to results from several studies, such as the global Point Prevalence Survey (PPS) study in 4 African countries, a hospital in Ghana (40.1%), hospitals in Botswana (61.7%) and at a referral hospital in Kenya 54.2%.[4,17,18,22]

Penicillins were the most prescribed class of antibiotics at 29.1%, followed by cephalosporins at 23%. In Ghana, a study conducted in a tertiary hospital reported similar results at 24.9% and 23.7% for penicillins and cephalosporins, respectively.[22] Penicillins were also reported as the most prescribed class in other studies conducted in hospitals in Botswana, in African hospitals in the global PPS, and in a tertiary hospital in Kenya.[4,5,17,18] Ceftriaxone was the most used antibiotic at 22%, followed by metronidazole at 19.8%. This finding is similar to the findings in the global PPS in 3 countries; Uganda (24%), Zambia (21%) and Tanzania (32%).[22] Ceftriaxone was the most prescribed antibiotic at 2 referral hospitals in Kenya at 29% and 31%.[7] Metronidazole was more popular at Ghanaian hospitals at 12% as reported in the global PPS, at a tertiary hospital and in surgical units in hospitals in Ghana.[17,22,23]

The most prescribed class of antibiotics (penicillins) belonged to the WHO "access" category of antibiotics, similar to the global PPS study in 4 African countries.[17] However, there was a concern since the most prescribed antibiotic (ceftriaxone) is in the Watch group of antibiotics with high susceptibility to the development of antimicrobial resistance.[24] The risk of antimicrobial resistance with overuse of ceftriaxone is further compounded by lack of microbiological tests such as culture and sensitivity tests to monitor it at the 4 study hospitals. The WHO recommends that the use of "watch" category of antibiotics should be monitored and prioritised

TABLE 1: Demographic, Patient Characteristics and Antibiotic Prevalence

	Admissions N=332 n (%)	Number on antibiotics	Prevalence of antibiotic use (95%CI)
Hospital			
Hospital A	194 (58.4)	82	42.3(35.2-49.6)
Hospital B	93 (28.1)	51	54.8(44.2-65.2)
Hospital C	26 (7.8)	9	34.6(17.2-55.7)
Hospital D	19 (5.7)	5	26.3(9.1-51.2)
Gender			
Female	217(65.4)	85	39.2(32.6-46)
Male	115(34.6)	61	53(43.5-62.4)
Age group			
Adult > 18 years	209 (63.0)	89	42.6(35.8-49.6)
Child (6-17 years)	20 (6.0)	11	55(31.5-76.9)
Child (1-5 years)	41(2.3)	35	85.4(70.8-94.4)
Infant (1-11 months)	13 (3.9)	6	46.2(19.2-74.9)
Neonate <28 days	49(14.8)	5	10.2(3.4-22.2)
Wards			
Adult medical ward	41(12.4)	18	41.9(24.5-60.9)
Adult surgical ward	31 (9.3)	13	43.9(28.4-60.3)
Adult mixed (surgical/medical) ward	15 (4.5)	9	60(32.3-83.7)
Gynaecological ward	10(3.0)	3	30(6.7-65.2)
Maternity ward	138 (41.6)	41	29.7(22.2-38.1)
Neonatal medical ward	18(5.4)	4	22.2(6.4-47.6)
Paediatric medical ward	28(8.4)	24	85.7(67.3-96)
Paediatric surgical ward	10 (3.0)	6	60(26.2-87.8)
Mixed ward(adult/paediatric) medical ward	40(12.1)	27	67.5(50.9-81.4)
Mixed ward(adult/paediatric) surgical ward	1(0.3)	1	100

TABLE 2: Anatomical, Therapeutic and Chemical (ATC) and WHO Access, Watch, and Reserve (AWaRe) classification of antibiotics and their frequency

Antibiotic	ATC CODE	Frequency of use (%)	WHO AWaRe Classification
Penicillins		**66(29.1)**	
Amoxicillin	J01CA04	7(3.1)	Access
Amoxicillin /Clavulanic acid	J01CR02	2(0.9)	Access
Ampicillin/Cloxacillin	J01CA51	1(0.4)	Access
Benzathine penicillin	J01CE08	1(0.4)	Access
BenzylPenicillin	J01CE01	35(15.4)	Access
Flucloxacillin	J01CF05	20(8.8)	Access
Cephalosporins		**52(23)**	
Ceftriaxone	J01DD04	50(22)	Watch
Ceftazidime	J01DD02	2(0.9)	Watch
Nitroimidazoles		**45(19.8)**	
Metronidazole(IV)	J01XD01	45(19.8)	Access
Aminoglycosides		**35(15.4)**	
Gentamycin	J01GB03	35(15.4)	Access
Fluoroquinolones		**11(4.8)**	
Ciprofloxacin	J01MA02	10(4.4)	Watch
Levofloxacin	J01MA12	1(0.4)	Watch

Continue

TABLE 2: Continued

Antibiotic	ATC CODE	Frequency of use (%)	WHO AWaRe Classification
Tetracyclines		**7.(3.1)**	
Doxycycline	J01AA02	7.(3.1)	Access
Macrolides		**2(0.8)**	
Azithromycin	J01FA10	1(0.4)	Watch
Erythromycin	J01FA01	1(0.4)	Watch
Others		**9(3.9)**	
Clindamycin	J01FF01	6(2.6)	Access
Cotrimoxazole	J01EE01	2(0.9)	Access
Rifaximin	A07AA11	1(0.4)	Watch
Total		**227**	

in antimicrobial stewardship programs.[24] Empiric therapy was the usual practice, and thus there is a need to improve laboratory support to guide targeted antibiotic use through culture and sensitivity tests. None of the antibiotics prescribed belonged to the "reserve" group of antibiotics. This finding is similar to a study in 4 African countries.[17] This is also in line with the Kenya Essential Medicine List which restricts their use to tertiary hospitals in Kenya.[25]

The prescribing ratio in the 4 hospitals was 1.55, lower than what was reported by a study conducted in hospitals in Ghana (2.0) and higher than what was reported in a study conducted in Botswana hospitals (1.38).[18,22] Most patients (52.7%) had a combination of 2 antibiotics, similar to studies conducted in hospitals in African countries and surgical units in hospitals in Ghana.[17,23] The use of 2 antibiotics expands the spectrum of activity, perhaps compensating for the lack of microbiological tests to check the sensitivity of antibiotics. There was high use of parenteral antibiotics at 63%, similar to studies conducted in Ghana and Botswana respectively.[22,23]

On quality prescribing, all antibiotic prescriptions had the frequency indicated. Surprisingly, about 1 in every 5 antibiotics did not have a review date or stop date, contrary to studies conducted in Uganda and Tanzania, where 99 % of the prescriptions had a stop or review date and could fuel antimicrobial resistance.[17]

CONCLUSION

There was a relatively high prevalence of antibiotics use at Kirinyaga hospitals at 44% (95% CI 38.6-49.5). The use of antibiotics in the hospital was wholly empirical. There was extensive use of antibiotics in the WHO watch group category without microbiological tests support. There is a need for laboratory support to improve targeted prescription of antibiotics and establish an antimicrobial stewardship program. An in-depth study is required to establish the appropriateness of antibiotic use and compliance to clinical guidelines in the 4 study hospitals.

Study Limitations

The study had several limitations. There were low admission rates in Hospital C & D at the time of the study. Therefore, the prevalence of antibiotic use may have been under estimated and thus, the results of this study may not be generalisable to facilities at the same level. Poor documentation of medication use was noted in all hospitals, some updated the treatment sheets, while others documented drug administration in the nurse's flow sheet (Kardex). Even with cross checking different documents (Treatment sheet and Kardex), it was not possible to assess if there were missed doses which was part of the study questions. However, the choice of antibiotics across the 4 hospitals was relatively similar. With a non-existent antimicrobial stewardship program in the hospitals, the study forms a baseline to improve antibiotic use in the hospitals.

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ORIGINAL ARTICLE

Self-Medication Practice with Antimalarials and Associated Factors Among Undergraduate Health Science Students in North Western - Tanzania: A Cross-Sectional Study

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ABSTRACT

Background: Self-medication is a growing public health concern in developing and developed countries.
Objective: This study was designed to assess the prevalence of self-medication practice among undergraduate health science students and to determine its concomitant factors.
Methods: This study was conducted in May 2021 among undergraduate health science students studying at the Catholic University of Health and Allied Sciences (CUHAS) in Mwanza, Tanzania. An analytical cross-sectional design was used in this study. Semi-structured questionnaires were used to collect information on the sociodemographic characteristics of respondents and to assess their anti-malarial self-medication practice.
Results: A total of 340 participants were recruited. The prevalence of self-medication with antimalarials was 55.9%. Among 190 students who ever used antimalarials without a prescription; the majority 143 (75.3%) obtained antimalarials from community drug outlets, and 116 (61.0%) used artemether-lumefantrine. The majority reported the emergence of acute illness (ie, no time to attend health facilities) 82 (43.2%) to be the major reason for self-medication. Students aged 25 years and above were more likely to use antimalarials without a prescription compared to students aged between 18 and 21 years, (aOR=2.99 (95% CI 1.24-0.7.22). Compared to first-year students, third-year (aOR=0.18 (95% CI 0.07-0.41), fourth-year (aOR=0.32 (95% CI 0.13-0.79), and fifth-year students (aOR=0.16 (95% CI 0.04-0.64) were significantly less likely to take antimalarials without a prescription.
Conclusion: The study found a high prevalence of self-medication with antimalarials among undergraduate health science students, emphasizing the need for strategies to promote the rational use of antimalarials. It is recommended to improve access to healthcare facilities and educate students about the risks associated with self-medication to reduce its prevalence.

BACKGROUND

Malaria is still one of the major public health threats across the world.[1] Notwithstanding substantial improvements in reducing malaria prevalence, *Plasmodium falciparum* malaria caused approximately an estimated 619 000 malaria deaths globally in 2021, the majority of which were in sub-Saharan Africa.[2] Four countries accounted for just over half of all malaria deaths globally in 2021: Nigeria (31%), the Democratic Republic of the Congo (13%), Niger (4%), and Tanzania (4%).[2] Common antimalarial drugs used in Tanzania include artemether-lumefantrine, sulphadoxine/pyrimethamine, piperaquine/(dihydro)artemisinin, and amodiaquine/artesunate.[3] Self-medication with anti-malaria drugs is a common practice in countries where the malaria burden is high.[4] This is due to a variety of reasons, including limited access to healthcare facilities, high healthcare costs, and inadequate knowledge about the appropriate use of antimalarial drugs.[5]

Self-medication is defined as the use of medications to treat self-identified disorders or symptoms, or the intermittent or continuous use of a prescribed medicine for chronic or recurrent diseases or symptoms without seeking medical guidance.[6] Self-medication is a serious public health concern, both in developing and developed countries.[7] Antimalarials are not permitted for self-medication and must only be obtained from drug outlets with a doctor's prescription.[8] Self-medication with antimalarials can lead to serious consequences such as delay in diagnosis of illness, drug resistance, increase of co-morbidities, and, in some cases, death.[9,10]

Studies have shown that the prevalence of self-medication with antimalarials differs across the globe, and is more prevalent in low- and middle-income countries.[11] The prevalence of self-medication with antimalarial drugs in sub-Saharan Africa varies widely among countries and populations. Previous studies done in the Congo, Ghana, and Cameroon reported a prevalence of self-medication between 41.0% and 57.8%.[12–14] In the general population, self-medication with antimalarials is a phenomenon common in Tanzania.[15] In a study conducted in Saudi Arabia, the prevalence of self-medication among university students was 98.2%.[16] However, data on self-medication practice with antimalarials among undergraduate health science students, particularly in low- and middle-income countries, are scarce.

Studies on the prevalence and factors associated with antimalarial self-medication among undergraduate health science students in Tanzania are necessary to help with the planning of interventions to improve the use of these medicines. To the best of our knowledge, no study has yet been undertaken in Tanzania that has reported antimalarial self-medication among university students. Thus, this study was conducted in order to determine the prevalence of self-medication with antimalarials among undergraduate students at the Catholic University of Health and Allied Sciences (CUHAS) in Mwanza, Tanzania, as well as to assess the sociodemographic factors associated with self-medication with these medications.

METHODS

Study Design and Setting

The study employed an analytical cross-sectional design. It was conducted in CUHAS in May 2021. The university is located at Bugando Hill, within the Bugando Medical Centre (BMC). premises in north western, Mwanza, Tanzania, the setting with stable high malaria transmission.[17] Its core business is training, research, and consultancy services. CUHAS train health professionals in the fields of medicine, pharmacy, medical laboratory sciences, nursing, radiology and public health through diploma, bachelor, masters and PhD programmes. Undergraduate students from the following courses were the study population: Doctor of Medicine (MD), Bachelor of Science in Nursing (BSN), Bachelor of Pharmacy (BP) and Bachelor of Medical Laboratory Sciences (BMLS).

Sampling and Data Collection Procedure

The Taro Yamane formula (ie, $n=N/(1+N(e)^2)$) was used to obtain a minimum sample size of 332 because this study was conducted in a finite population with a known population size.[18] The Taro Yamane formula is a statistical sampling formula used to determine the sample size required of a finite population to achieve a certain level of accuracy and confidence in the survey results. The formula takes into account the population size, desired level of precision, and level of confidence. These parameters have the ability to influence the accuracy and reliability of the survey results.[19] The total number of undergraduates was 1968 and the acceptable sampling error was 0.05. All the 1,968 students were invited through their class representatives to participate in the study and, from these, 340 students volunteered and consented in writing by signing a consent form to participate through the completion of a survey questionnaire.

Semi-structured questionnaires consisting of both open-ended and closed-ended questions were used to collect information on the sociodemographic characteristics of respondents and to assess their anti-malarial self-medication practice. The self-administered questionnaire was set in English, printed, and pretested. The self-administered questionnaires were completed in the presence of the research assistants. Self-administered questionnaires were used to minimize interviewers' bias in the way questions were asked, and more importantly because all respondents were literate. Based on the reports from previous studies,[20–22] the following information was included in the questionnaire: social demographic characteristics (gender, age, marital status, course of study, and year of study), practice of self-medication (if one has ever used antimalarials for self-medication in his or her lifetime, source of supply of antimalarials, health outcome after self-medication, if one had stopped treatment without finishing the course), common types of antimalarials used, and reasons for practicing self-medication.

The questionnaires was assessed for content validity by experts in the School of Public Health at CUHAS.

Data Analysis

Data were analyzed using STATA version 14. Categorical data were presented in frequencies (percentages) and with the aid of charts and tables. Quantitative variables were presented as median (Interquartile range). Differences between social demographic characteristics between students who had practiced self-medication and those who had not were examined using

Chi-square or Fisher exact tests where appropriate. The trend analysis was conducted to examine the difference between self-medication practice and the year of study. Multivariable logistic analysis was used to identify social demographic factors associated with self-medication among the students. All independent variables were included in the model. *P* values of less than .05 were considered statistically significant. The strength of the association was also measured by odds ratio (OR) and adjusted odds ratio (aOR) with corresponding 95% confidence interval (CI).

Ethical Consideration

This study was approved by The Catholic University of Health and Allied Sciences and Bugando Medical Centre's Joint Ethics and Research Review Committee (IRB approval No: UEC/1821/2021). Participants' verbal and written consent to participate in the study were sought and obtained before the questionnaires were administered. During the data collection, personal identifiers such as name and phone numbers of the participants were not recorded to ensure confidentiality. Rather, numbers were assigned for coding purpose. The collected information was coded and kept confidential, only to be accessed by the principal investigator.

RESULTS

Socio Demographic Information of Respondents

A total of 340 students were enrolled in the study, of whom the

most (n=182, 53.5%) were female, and age category 22 to 24 years had more respondents (n=166, 48.8%) than the rest. The median (interquartile range) age of participants was 22 (21 to 24) years. The predominant course was MD (n=142, 41.8%), while second-year students were 89 (26.2%) (Table 1).

Practice of Self-Medication with Antimalarials

One hundred ninety (55.9%) of the respondents admitted to having ever used antimalarials without a prescription. Among them, the majority (n=143, 75.3%) obtained antimalarials from community drug outlets and almost half of them (n=98, 51.6%) recovered. Furthermore, about one quarter (n=45, 23.7%) of those who admitted to having self-medicated did not finish the treatment course (Table 2).

Out of 190 undergraduate students that practiced self-medication with antimalarial, majority 116 (61.0%) used artemether-lumefantrine. Others used sulphadoxine/pyrimethamine 36 (19.0%), piperaquine/(dihydro) artemisinin 30 (15.8%) and amodiaquine/artesunate 8 (4.2%) for malaria treatment without the doctor's prescription (Figure 1).

The reason most reported (n=82, 43.2%) for self-medication was the emergence of acute illness (ie, no time to attend health facilities). Other reasons included too much time taken at health facilities (ie, long queues at facility) (n=46, 24.2%), accessibility of drug outlets (n=34, 17.9%), high treatment cost at health facility (n=22, 11.6%) and out of stock of medicines in health facilities (n=6, 3.2%). (Figure 2).

Demographic Factors Associated with Self-Medication with Antimalarials

There was no statistically significant association with self-medication practice for gender (X^2=3.635; P>.05), and age group (X^2=1.268; P>.05) of undergraduate students. However, self-medication practice statistically significantly differed among the courses (X^2=9.420; P<.024) and year of study (X^2=18.450; P<.001). The prevalence of self-medication was higher among BSN (n=46, 66.7%) and first year students (n=57, 68.7%) compared to other courses. With P value=.004, the declining trend in the rates of self-medication with increase in year of study was statistically significant (Table 3).

Having controlled for confounders, multivariable logistic regression analysis revealed that self-medication practice was significantly independently associated with age and the year of study. Students aged 25 years and above were more likely to use antimalarials without a prescription compared to those aged 18 to 21 years, (aOR=2.99; 95% CI, 1.24 to 0.7.22; P<.015). Compared to first year students, the third-year students (aOR=0.18; 95% CI, 0.07 to 0.41; P<.001), fourth year students (aOR=0.32; 95% CI, 0.13 to 0.79; P<.013), and fifth year students (aOR=0.16; 95% CI, 0.04 to 0.64; P<.034) were significantly less likely to take antimalarials without a prescription, (Table 4).

TABLE 1: Sociodemographic Characteristics of Participants (N=340)

Variable	Frequency	Percentage (%)
Gender		
Female	182	53.5
Male	158	46.5
Age (Years)		
18-21	103	30.3
22-24	166	48.8
25 and above	71	20.9
Median age years (Interquartile range)	22 (21 - 24)	
Course of study		
MD	142	41.8
BP	85	25.0
BSN	69	20.3
BMLS	44	13.0
Year of study		
First	83	24.4
Second	89	26.2
Third	80	23.5
Fourth	70	20.6
Fifth	18	5.3

Abbreviations: MD, Doctor of Medicine; BSN, Bachelor of Science in Nursing; BP, Bachelor of Pharmacy; BMLS, Bachelor of Medical Laboratory Sciences.

TABLE 2: Practice of Self-Medication with Antimalarials

Variable	Frequency	Percentage (%)
Ever used antimalarials for self-medication (n=340)		
Yes	190	55.9
No	150	44.1
Source of supply of antimalarials (n=190)		
Drug outlets	143	75.3
Family members	25	13.2
Leftovers from previous treatment	12	6.2
Friends	10	5.3
Health outcome after self-medication (n=190)		
Recovered	98	51.6
Improved	71	37.4
Did not improve	21	11.0
Stopped treatment without finishing the course (n=190)		
Yes	145	76.3
No	45	23.7

TABLE 3: Association Between Sociodemographic Characteristics and Self-Medication Practice

Sociodemographic Characteristics	Self-Medication (n=190)	No Self-Medication (n=150)	P Value	Chi-Square Value
Gender				
Female	93 (51.1)	89 (48.9)	.057	3.635
Male	97 (61.4)	61 (38.6)		
Age (Years)				
18-21	59 (57.3)	44 (42.7)	.531	1.268
22-24	88 (53.0)	78 (47.0)		
25 and above	43 (60.6)	28 (39.4)		
Course of study				
MD	67 (47.8)	75 (52.8)	.024	9.420
BP	48 (56.5)	37 (43.5)		
BSN	46 (66.7)	23 (33.3)		
BMLS	29 (65.9)	15 (34.1)		
Year of study[a]				
First	57 (68.7)	26 (31.3)	.001	18.450
Second	55 (61.8)	34 (38.2)		
Third	31 (38.7)	49 (61.3)		
Fourth	40 (57.1)	30 (42.9)		
Fifth	7 (38.9)	11 (61.1)		

[a]In the trend analysis, P=.004
Abbreviations: MD, Doctor of Medicine; BSN, Bachelor of Science in Nursing; BP, Bachelor of Pharmacy; BMLS, Bachelor of Medical Laboratory Sciences.

TABLE 4: Univariate and Multivariable Analysis of the Association Between Sociodemographic Variables and Self-Medication

Sociodemographic characteristics	OR (95% CI)	P-value	aOR (95% CI)	P-value
Gender				
Female	1		1	
Male	1.52 (0.99-2.34)	.057	1.32 (0.82-2.11)	.243
Age (Years)				
18-21	1		1	
22-24	0.84 (0.51-1.38)	.494	1.80 (0.92-3.55)	.088
25 and above	1.15 (0.61-2.12)	.666	2.99 (1.24-7.22)	.015
Course of study				
BMLS	1		1	
BP	0.67 (0.31-1.43)	.301	0.67 (0.30-1.51)	.330
BSN	1.03 (0.46-2.30)	.934	1.12 (0.47-2.64)	.802
MD	0.46 (0.23-0.96)	.032	0.49 (0.22-1.05)	.065
Year of study				
First	1		1	
Second	0.73 (0.39-1.39)	.345	0.58 (0.29-1.17)	.128
Third	0.28 (0.15-0.55)	<.001	0.18 (0.07-0.41)	<.001
Fourth	0.61 (0.31-1.18)	.141	0.32 (0.13-0.79)	.013
Fifth	0.29 (0.10-0.83)	.022	0.16 (0.04-0.64)	.034

Abbreviations: MD, Doctor of Medicine; BSN, Bachelor of Science in Nursing; BP, Bachelor of Pharmacy; BMLS, Bachelor of Medical Laboratory Sciences; OR, Crude Odds Ratio; aOR, Adjusted Odds Ratio.

FIGURE 1: Commonly used antimalarials used for self-medication (n=190)

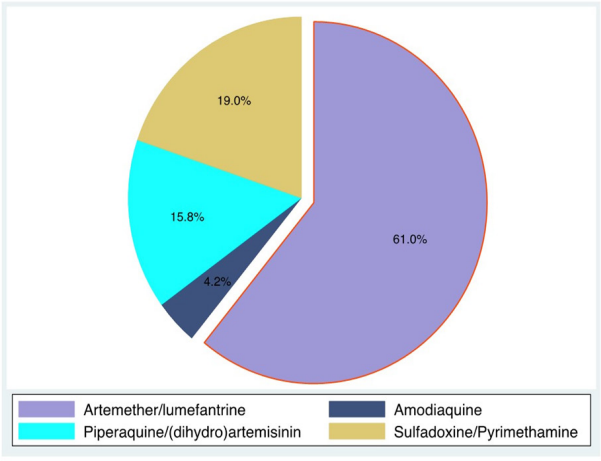
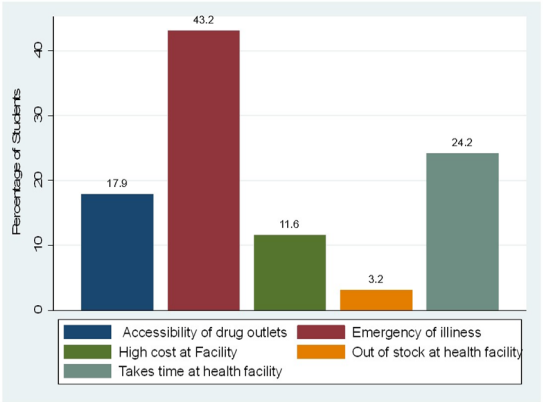


FIGURE 2: Reasons for Practicing Self-Medication (n=190)



DISCUSSION

Self-medication is a widespread practice across the globe.[23] Self-medication is still popular, and it is more prevalent among adolescents and more common among university students.[24] This study examined the prevalence of antimalarial self-medication and related factors among undergraduate health science students.

In the present study, more than half (55.9%) of the sampled undergraduate health science students reported practicing self-medication which compares with the

findings reported in Ethiopia.[25] However, a lower prevalence was reported in two previous Nigerian studies[21,22]. On the other hand, in a cross-sectional study conducted in Gondar Town, North Western Ethiopia, the private Health Sciences students reported a higher prevalence compared to the current study.[7] Variations in socioeconomic status, sample size, study setting, sampling procedure, and law enforcement could possibly explain these differences among the studies. The setting of the current study was CUHAS, a health science university, and it essentially investigated if the students had ever practiced self-medication with antimalarials in their lifetime, contrary to other studies. Additionally, this study investigated self-medication specifically with antimalarials only. Moreover, the higher prevalence of self-medication among the students at CUHAS could be attributed to the medical knowledge that they had acquired from their courses, which somehow but not surprisingly predisposed them to the antimalarial self-medication practice. Reports have been published that knowledge of disease or treatment is one of the determinants of self-medication.[26]

The present study findings on common antimalarials used for self-medication are in agreement with results from a study conducted in community settings in Kenya and Tanzania.[15,27] However, sulfadoxine/pyrimethamine was the first-line medicine for the treatment of uncomplicated malaria until it was replaced by artemether/lumefantrine in 2006.[28,29] Nevertheless, sulfadoxine/pyrimethamine remains the medicine of choice for intermittent preventive treatment for malaria in pregnancy.[30] The current study revealed that some of self-medicating students did not comply with antimalarial treatment protocol. About a quarter did not finish the treatment courses. Inappropriate use of antimalarials may increase the risks of developing malaria parasite resistance.[11] Adherence to antimalarial therapy is a key public health practice in attaining effective implementation of malaria case management strategy and prevention of antimalarial resistance.[31] Furthermore, self-medication with antimalarials can lead to drug wastage, hence becoming a potential for drug shortage, failure to treat the actual cause of fever (non-malarial febrile illnesses) and causing unnecessary undesired side effects.

While the present study showed that self-medication was higher than what was reported in some other studies, between year of study and between age group comparisons displayed significant variation in self-medication. For instance, compared to first year students, the present study revealed that third year to fifth-year students were less likely to take antimalarials without prescription. This could be attributed to the knowledge that third- to fifth-year students had acquired in the course of their study that self-medication is not a good practice. Conversely, and surprisingly, older students (ie, those 25 years and above) were more likely to take antimalarials without a prescription compared to younger ones. Probably, as reported elsewhere,[32] cumulative illness events could be the cause of the reported older students' increased rate of self-medication. Consistent with previous studies, the major dispenser of antimalarials without prescription was community drug outlets.[22,26] In low and middle income countries, community drug outlets and pharmaceutical personnel are the most accessible health facilities and healthcare providers to the members of the community including university students.[33]

According to previous research, factors that significantly influence self-medication include gender, age, economic status, level of education, past successful use, and severity of illness.[34,35] Women are more likely to self-medicate than men; older individuals may have more experience with self-medication and may be more likely to do so; individuals with limited access to healthcare may be more likely to self-medicate due to financial constraints; individuals with lower levels of education may have less knowledge about the potential risks of self-medication; and past successful use of medication can also influence an individual's decision to self-medicate, as they may believe that the same medication will be effective for a new illness or condition. Last but not least, people may feel the need to self-medicate if they perceive their symptoms as urgent.[34–37] Previous studies have reported lifestyle, readily available drugs, drugs stored at home, increased medical consultation costs, time-consuming clinical processes, a lack of nearby healthcare facilities, and extensive advertising as some of the leading reasons for university students' self-medication-seeking behavior.[36,37] This study revealed that the most common factor that led to self-medication among university students was emergency of illness (no time to attend health facilities). Health facility charges contribute greatly to self-medication as one tries to cut down medical costs by avoiding the high consultation fees of physicians. [38] In this study the high treatment costs at health facilities contributed less in self-medication, possibly because, according to the university policy, every student should be covered by health insurance. These and other findings suggest that efforts should be made by the government to improve access to healthcare facilities that provide prompt and effective treatment for malaria to discourage the practice of self-medication. Future studies should be conducted to investigate, through a qualitative methodology, whether students in health science universities understand the consequences of self-medication and inappropriate use of antimalarials and if they do, why do they still practice it.

CONCLUSION

The study found a high prevalence of self-medication with antimalarials among undergraduate health science students, emphasizing the need for strategies to promote the rational use of antimalarials. It is recommended to improve access to healthcare facilities and educate students about the risks associated with self-medication to reduce its prevalence. We recommend that policymakers ensure that all community drug outlets dispense antimalarials only to clients who have a prescription and that the drug use regulations are strictly abided by.

Study Limitations and Strengths

The strength of the study was the good sample size, and it is the first study to assess self-medication practice with antimalarial among university students in Tanzania. There are some limitations to this study. First, this study was conducted in only one health-sciences University. Thus, its results cannot be generalized to a larger population of health science universities in the country. Additionally, a cross-sectional study design was used; hence, causal

relationships between variables cannot be established. Furthermore, there is a possibility of recall bias, over and under reporting, since the analyses were based on self-reports. Another limitation that could affect the reported prevalence is the use of a convenience sampling procedure. Nevertheless, the results shed some light on what is happening with regard to utilization of antimalarial drugs among undergraduate students, thereby forming the basis for a wider study on the subject to be conducted in order to rectify the problem.

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ORIGINAL ARTICLE

Contamination of Automated Teller Machines Surfaces with Multi-drug Resistance Gram-negative Bacteria in Dar es Salaam, Tanzania

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ABSTRACT

Background: In Tanzania, little is known about the proportion of Multi-drug resistance (MDR) Gram-negative bacteria contamination on Automated Teller Machine (ATMs) surfaces. The study aimed to determine the proportion of MDR Gram-negative bacteria contamination on ATMs surfaces, antimicrobial resistance patterns as well as associated factors.

Methodology: A cross-sectional study was conducted between January and March -2021 in Dar es Salaam, involving 298 ATMs. Cultures were performed on Mac-Conkey agar while antimicrobial susceptibility was done using the Kirby Bauer disc diffusion method with *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 used as controls. Data analysis was done using STATA version 15.1. Chi-square and Modified Poisson regression was used to assess factors associated with MDR contamination. Data was presented as prevalence ratio (PR) and 95% Confidence Interval. A *p-value* of <.05 was considered statistically significant.

Results: More than half (55.4%) of ATMs in Dar es Salaam are contaminated with Gram negative bacteria, mostly by *Klebsiella pneumoniae* 18.5% (31/168). The highest level of resistance was observed against ampicillin (68.9%). About one-third (34.5%) of the isolates were MDR. About 35.7% were Extended-Spectrum Beta-Lactamases (ESBL) producers while 19.6% were quinolone/ fluoroquinolones-resistance. Risk factors for contamination of ATMs included highly populated location such as; Ubungo (PR adjusted = 3.62, 95%CI = 1.58-8.30, *P=.002*), Kigamboni (PR adjusted = 2.78, 95%CI = 1.20-6.42, *P=.017*), and Temeke (PR adjusted = 2.75, 95%CI = 1.04-3.72, *P=.023*), and less frequent cleaned ATMs (PR adjusted = 1.98, 95%CI = 1.04-3.73, *P=.04*)

Conclusions: More than half of ATMs in Dar es Salaam are contaminated with Gram-negative and one-third of them with MDR bacteria, especially those located in highly populated areas and those that are less frequently cleaned. This calls for interventional measures regarding public awareness of ATMs as potential vehicles for the transmission of infectious agents.

BACKGROUND

Automated Teller Machines (ATMs), regarded as mini-banks are important devices in the banking sector. ATMs make banking convinient and serve thousands of customers daily.[1] As helpful as ATM machines are, a number of studies across the world have identified them as a source of infections to the users.[2] Bacteriological examinations carried out on ATMs have reported that they are contaminated with various microorganisms,[3–6] associated with both community-acquired and hospital acquired infections.[7] Microbes bear the potentials for survival on dry fomites like ATM machine key pads. They have different physiological resting stages and thus are capable of surviving or hibernating due to low water activity. Some Gram-negative bacteria can remain active up to month on their resting surfaces.[8] *Klebsiella pneumoniae, Escherichia coli, Enterobacter species* as well as non-lactose fermenting bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter species* have been identified as major cause of multi-drug resistant bacterial infections.[9,10] This group of bacteria developed resistance to a wide range of antibiotics, including third generation cephalosporins, carbapenems, and fluoroquinolones, which are the best antibiotics currently available for treating multi-drug resistant bacteria.[11]

Most of these bacteria are on the World Health Organization (WHO) priority list of pathogens that poses substantial threat to morbidity and mortality worldwide.[11] These bacteria are resistant to extended-spectrum beta-lactamase (ESBL)- producing bacteria and quinolone/ fluoroquinolone-resistant bacteria and have been linked to a number of environments,[12,13]

including ATMs,[14–16] and thus posing a serious public health threat.[17-19] ATMs, despite being used by people of various backgrounds, lack constant and frequent monitoring of hygienic measures. Some are not provided with disinfectants and have no instructions for clients. This raises their potential to be vehicles for the transmission of micro-organisms, which cause infections that are difficult to treat as substantiated in numerous studies conducted in Europe, Asia and Africa.[3,20–24]

In Tanzania, little is known about the presence of pathogenic bacteria on ATMs surfaces, however, there is literature indicating the existence of pathogenic bacteria including ESBLs and quinolone resistance bacteria in the community.[25–29] This study aimed to determine the proportion of MDR Gram-negative bacteria contamination on ATMs surfaces and antimicrobial resistance patterns as well as associated factors in Dar es Salaam, Tanzania. Surveillance of AMR and MDR pathogens is one of the strategic objectives in the Tanzania National action plan on antimicrobial resistance.[30] In its part, this study provide data on the burden of MDR Gram-negative bacteria contamination on ATMs in Dar es Salaam.

Data emanating from this study will sensitise both owners and users of these machines, of their potential to transmit pathogens. The study will provide evidence for better management of ATMs to curb transmission of pathogens. Our hypothesis is that ATMs are essential to our social life, localised in city centres, trade areas, and around hospitals and are used by hundreds of people of varying socio-economic levels and hygienic status are potential vehicles of microbial pathogens, including MDR bacteria.

MATERIAL AND METHODS

Study Area

This was a cross-sectional study, carried out in Dar es Salaam Tanzania from January to March 2021. Dar es Salaam is the most populated city in Tanzania, with approximately more than 7 million people.[31] The use of ATMs is significantly high since Dar es Salaam is a commercial city. In 2019, Dar es Salaam had 290 bank branches, which constituted 30.3% of all bank branches in the country and the use of ATMs was reported to be 6.4 ATMs per 100,000 adults.[32,33] (Figure 1).

Sample Size Estimation

The sampling frame included ATMs of the 3 largest banks in Dar es Salaam. A list of the ATMs was obtained from respective banks, which totalled to 432. The sample size was calculated using Kish Leslie formula (1965),[34] a prevalence of 21.4% (*E. coli* bacteria isolated on ATMs surfaces)[35] and margin error of 5% was used. The minimum required sample size was 258 ATMs which was raised to 298 ATMs so as to increase the power of the study. The proportion of ATMs of a specific bank included in the sample size (298) depended on the proportion of the given bank's number of ATMs that contributed to the sample frame. Thus, banks with high number of ATMs in the sample frame contributed higher number of ATMs in the sample size. Simple random technique was used to select the ATMs that were included in the sample size (298). Samples were distributed as follows: First bank (I) 121 out of 176 ATMs, second bank (II) 119 out of 173 ATMs, and third Bank (III) 58 out of 83 ATMs.

Information regarding frequency of ATM cleaning and disinfection, availability of hand-washing and cleaning facilities, and location of the ATM (Remote ATMs versus Branch ATMs) was collected using a structured observation checklist.

Sample Collection and Transportation

A Sterile swab[36] was moistened in sterile saline and moved several times over the surfaces of the most-used keys on the ATM keypad/screen in an aseptic procedure and placed into a nutrient broth media.[37] The collected samples were transported to the National Public Health Laboratory (NPHL) in cooler boxes packed with ice packs at temperatures ranging between 2 to 8^{0}C degrees for processing within 4 hours of collection.

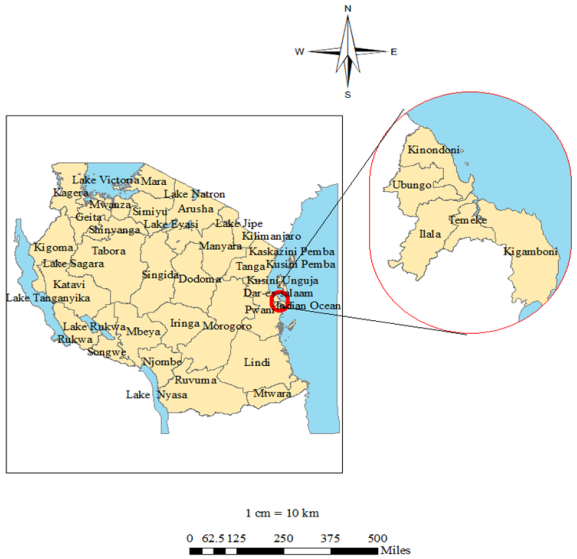
Sample Processing and Bacteria Isolation

Samples in nutrients broth were incubated at 37°C for 18 to 24 hours before culture. The culture was performed on Mac-Conkey (MCA) agar[37] with crystal violet and bile salt. Cultured plates were incubated aerobically at 37°C. Plate growths were noticed after 18 to 24 hours incubation, the isolates were then sub-cultured on fresh media plates until pure isolates were observed.

Bacteria Identification

Isolated bacteria were identified by performing gram stain and standard biochemical tests, which included; oxidase, urease, Indole, Citrate test, and Triple Sugar Iron (TSI).[38] The TSI test is designed to differentiate among the different groups or genera of the Enterobacteriales, which are all Gram-negative bacilli, based on fermentation of glucose and lactose or sucrose and hydrogen sulphide production. For identification of Gram-negative bacteria with ambiguity, API 20 E systemm was used as per manufacture instructions.[38]

FIGURE 1: Map Showing Districts in Dar es Salaam City, Tanzania



Antibiotic Susceptibility Testing

Identified gram-negative bacteria were subjected to antibiotic sensitivity test using the Kirby Bauer diffusion disk method on Muller Hinton agar[37] to determine their susceptibility patterns against selected antimicrobial agents, as described by Clinical Laboratory Standards Institute (CLSI), 2020.[39] Antimicrobial classes used were; Aminoglycoside, Fluoroquinolone, Quinolones, Tetracycline, third generation cephalosporin, carbapenem, penicillins, chloramphenicol, cotrimoxazole and cephalosporin (cefotaxime/clavulanic acid-30/10µg). Bacteria that showed resistance or decreased susceptibility (intermediate) to any of the third generation cephalosporins were selected for phenotypic ESBL confirmation. The potential ESBL-producing gram-negative bacteria were screened by cefotaxime (30 µg) and were confirmed by the combination disk method. Cefotaxime (30 µg) and the combination disc cefotaxime plus clavulanic acid (30 µg+ 10 µg) were placed 25 mm apart. An increase of ≥ 5 mm in the zone of inhibition for cefotaxime plus clavulanic acid compared to cefotaxime alone was confirmed as an ESBL producer.[40] Bacteria showing resistance against ciprofloxacin and nalidixic acid were regarded as quinolones/fluoroquinolones-resistant.[41] Bacteria that showed resistance to at least one antimicrobial in 3 or more antibiotic classes was regarded as MDR.[42]

Quality Assurance

Culture media used for isolation and identification of organisms were controlled using standard organisms *E. coli* ATCC 25922 strains. For ESBL producing gram-negative bacteria, ESBL producing *K. pneumonia* ATCC 700603 and non-ESBL producing *E. coli* ATCC 25922 were used as positive and negative controls.

Data Analysis

Data management and analysis was done by using STATA version 15.1. Frequencies and proportions of bacteria isolated and their antibiograms were determined. A Chi-square test was used to determine the univariate association of factors that are associated with MDR contamination on ATM surfaces. Variables with $P<.25$ were subjected to multivariate analysis. Since the proportion of MDR was greater than 15%, Modified Poisson regression was used to determine independent predictors of ATM surface contamination. Results from modified Poisson regression analysis were presented as Prevalence Ratio (PR) and 95% Confidence Interval. A *p-value* of <.05 was considered statistically significant.

Ethical considerations

Ethical approval for this study was obtained from Muhimbili University of Allied Sciences (MUHAS) Senate Research and Publications Committee (Ref. No. DA.282/298/01.C/).

RESULTS

The Proportion of Gram-Negative Bacteria Recovered from ATM Surfaces

A total of 298 ATMs from 3 largest banks in 5 districts of Dar es salaam city namely; Kinondoni, Ubungo, Ilala, Temeke and Kigamboni were screened. The proportion of contaminated ATMs across the districts ranged from 35.7 % to 75% (Figure 2).

Of the 298 swabs collected from ATM surfaces (screen/key-pads), 55.5% (n=165/298) showed microbial growth, and 168 bacteria were isolated. The distribution of bacteria recovered from ATM surfaces is shown in Table 1. *Klebsiella pneumoniae* 18.5% (n=31/168) was the predominant isolate followed by *Acinetobacter* spp 12.5% (n=21/168) and *E. coli* 10.1% (n=17/168), while *Proteus* and *Providencia species* were the least recovered, each accounting for 0.6% (n=1/168) of the isolates.

Antimicrobial Resistance Pattern of Isolates Recovered from ATM Surfaces

Bacteria were particularly resistant against ampicillin (68.9%), followed by cefotaxime (26.8%), and least resistant against gentamicin (1.3%). *K. pneumoniae*, *Acinetobacter species*, *E. coli* and *P. aeruginosa*, showed high, moderate and low levels of resistance ranging from 3.2% to 87.1%. (Table 2).

TABLE 1: The Pattern of Gram-Negative Bacteria Recovered from ATM Surfaces in Dar es Salaam, Tanzania

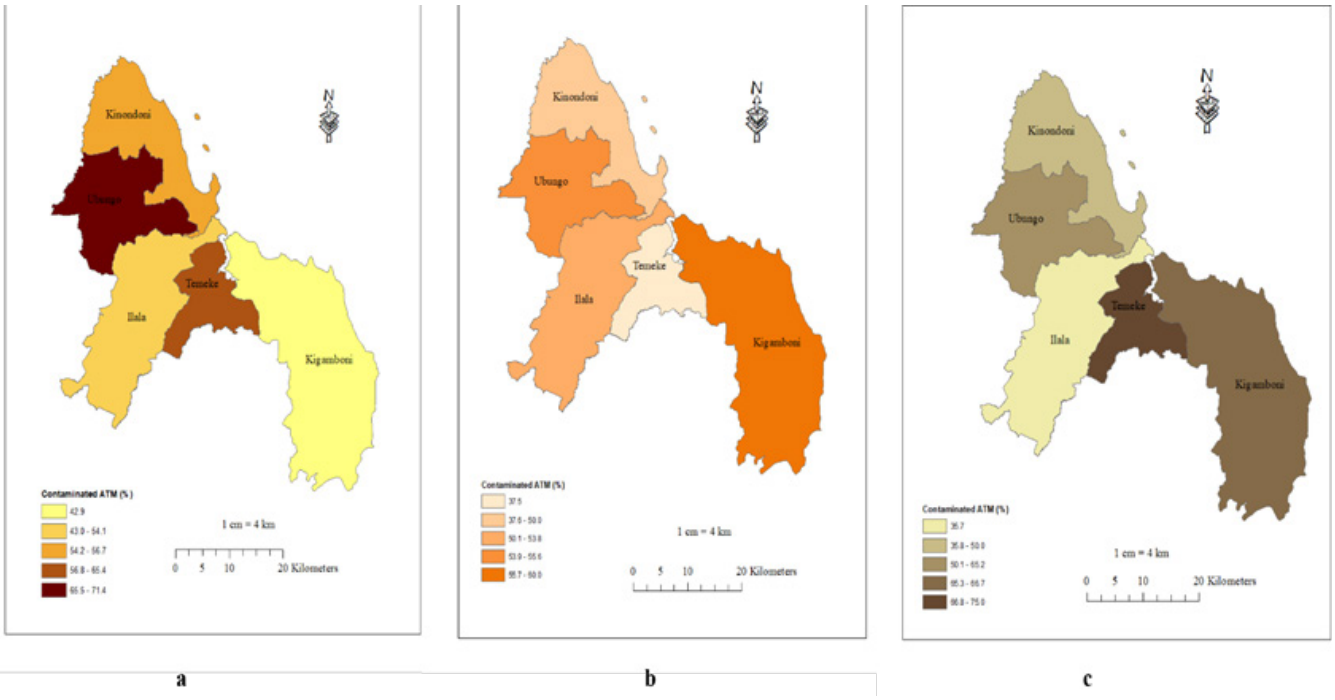
Organism	# isolates	Percent
Klebsiella pneumoniae	31	18.5
Acinetobacter spp	21	12.5
Escherichia coli	17	10.1
Pseudomonas aeruginosa	14	8.3
Enterobacter aerogenes	13	7.7
Shigella spp	13	7.7
Enterobacter spp	12	7.1
Serratia spp	11	6.6
Klebsiella oxytoca	9	5.4
Salmonella spp	8	4.8
Citrobacter spp	7	4.2
Pseudomonas spp	4	2.4
Yersinia spp	4	2.4
Morganella spp	2	1.2
Proteus spp	1	0.6
Providencia spp	1	0.6
Total	**168**	**100**

The Proportion of Multi-Drug Resistance Gram-Negative Bacteria from ATM Surfaces

Out of 168 isolates, 35.1% (n=59/168) were MDR against 3 to 7 classes of tested drugs. *Salmonella spp* had the highest proportion of MDR isolates 62.5% (n=5/8) compare to other Gram-negative bacteria, which ranged between 9.1% and 50% (Table 3).

From the most frequently isolated bacteria, the common resistant pattern observed were Cephalosporin's/Penicillin's/Phenicals, Cephalosporin's/Sulphonamides/Fluoroquinolone/ Penicillin's/ Quinolones and Sulphonamides/ Penicillin's/ Quinolones. One isolate each from *E. coli*, *P. aeruginosa*, *and Acinetobacter species*, were resistant to 6 and up to 7 classes of antibiotics (Table 4).

FIGURE 2: Proportion of contaminated ATM among three banks in each Districts of Dar es Salaam city, Tanzania



Key: a- bank (I), b-bank (II), c-bank (III)

TABLE 3: Proportion of MDR Isolates Recovered from ATM Surfaces in Dar es Salaam, Tanzania

Bacteria name	#Isolates	#MDR Isolates	%MDR Isolates
Klebsiella pneumoniae	31	7	22.58
Acinetobacter spp	21	10	47.62
Escherichia coli	17	8	47.06
Pseudomonas aeruginosa	14	7	50.00
Enterobacter aerogenes	13	6	46.15
Shigella spp	13	5	38.46
Enterobacter spp	12	4	33.33
Serratia spp	11	1	9.09
Klebsiella oxytoca	9	3	33.33
Salmonella spp	8	5	62.50
Citrobacter spp	7	1	14.29
Pseudomonas spp	4	2	50.00
Yersinia spp	4	0	-
Morganella spp	2	0	-
Proteus spp	1	0	-
Providencia spp	1	0	-
Total	168	59	35.12

TABLE 4: Multi-Drug Resistance Pattern among most Frequently Isolated Gram-Negative Bacteria Recovered from ATM Surfaces in Dar es Salaam, Tanzania

Organism	Resistant Profile	# Resistant classes	# Isolates
Acinetobacter spp	CEPH3, FQ, QUIN	3	2
	FOLATE, PEN, QUIN	3	3
	CEPH3, PEN, PHEN	3	1
	CEPH3, PEN, PHEN, QUIN	4	1
	CEPH3, FOLATE, FQ, PHEN, QUIN	5	1
	AG, CEPH3, FOLATE, PEN, PHEN	5	1
	CARB, CEPH3, FOLATE, FQ, PEN, QUIN	6	1
E coli	FOLATE, PEN, QUIN	3	1
	FOLATE, PEN, PHEN	3	2
	CEPH3, PEN, PHEN	3	1
	FOLATE, FQ, PEN, QUIN	4	1
	CEPH3, FOLATE, FQ, PEN, QUIN	5	2
	CARB, CEPH3, FOLATE, FQ, PEN, QUIN	6	1
Klebsiella oxytoca	FOLATE, FQ, PEN, QUIN	4	2
	CARB, CEPH3, FOLATE, PEN, PHEN, QUIN	6	1
Klebsiella pneumoniae	CEPH3, FOLATE, PEN	3	2
	FOLATE, FQ, PEN	3	1
	CEPH3, PEN, PHEN, QUIN	4	1
	CARB, CEPH3, PEN, QUIN	4	1
	CEPH3, FOLATE, FQ, PEN, QUIN	5	2
Pseudomonas aeruginosa	CEPH3, FQ, QUIN	3	1
	FOLATE, PEN, PHEN	3	1
	CEPH3, PEN, PHEN	3	2
	CEPH3, FQ, PEN	3	1
	CEPH3, FOLATE, PEN, PHEN	4	1
	CARB, CEPH3, FOLATE, FQ, PEN, PHEN, QUIN	7	1

Key: QUIN, quinolones; PHEN, phenicol's; AG, aminoglycosides; PEN, penicillin's; FQ, Fluoroquinolone; FOLATE, sulphonamides; CEPH3, cephalosporin's; CARB, carbapenems

TABLE 5: Proportion of ESBL Producers Isolated from ATM Surface in Dar es Salaam, Tanzania

Organism	#Isolates	# ESBLs producers	% ESBLs
E.coli	7	4	57.14
P. aeruginosa	8	0	-
K. Oxytoca	2	1	50.00
K. pneumoniae	11	5	45.45
Total	**28**	**10**	**35.71**

Key: ESBL- Extended spectrum beta-lactamase; #, number of; Spp- Species; % Percentage

Isolation Frequency of ESBL-Producing Gram-Negative from ATM Surfaces

A total of 28 Gram-negative bacteria isolates, (*K. pneumoniae, E. coli, K. oxytoca and P. aeruginosa*) that showed resistance or decreased susceptibility (intermediate) to any one of the third generation cephalosporin's were screened for ESBL production, and 35.7% (n=10/28) were positive for the test. Among screened isolates, the proportion of ESBL producers was highest among *E. coli* isolates 57.1 % (n=4/7) (Table 5).

Quinolone-Resistant Gram-Negative Bacteria Recovered from ATM Surfaces

Out of 168 isolates tested, 19.6% (n=33/168) were found

TABLE 2: Antibiotic resistance pattern among the 168 isolates recovered from ATM surfaces in Dar es Salaam, Tanzania

Organism	#Isolates	AMP	CIP	MEM	CTX	SXT	GEN	CHL	NAL	DOX
K. pneumoniae	31	87.1	3.2	0	32.3	16.1	0	3.2	0	6.5
Acinetobacter spp.	21	28.6	4.8	4.8	14.3	38.1	4.8	9.5	47.6	0
Escherichia coli	17	70.6	5.9	0	23.5	41.2	0	11.8	17.6	11.8
P. aeruginosa	14	78.6	0	0	42.9	21.4	0	28.6	7.1	14.3
Enterobacter spp.	13	61.5	15.4	7.7	15.4	30.8	7.7	7.7	30.8	7.7
Shigella spp.	13	38.5	15.4	7.7	38.5	15.4	0	7.7	38.5	0
E. aerogenes	12	83.3	8.3	8.3	58.3	16.7	8.3	8.3	16.7	8.3
Serratia spp.	11	81.8	0	0	36.4	0	0	0	0	0
Klebsiella oxytoca	9	88.9	0	0	11.1	44.4	0	11.1	22.2	0
Salmonella spp.	8	62.5	12.5	12.5	62.5	12.5	0	0	37.5	25
Citrobacter spp.	7	71.4	14.3	0	42.9	0	0	14.3	14.3	28.6
Pseudomonas spp.	4	100	0	0	25	25	0	0	0	25
Yersinia spp.	4	50	0	0	25	0	0	0	0	0
Morganella spp.	2	0	0	0	0	0	0	0	0	0
Proteus spp.	1	100	0	0	0	0	0	0	0	0
Providencia spp.	1	100	0	0	0	0	0	0	0	0
Total	**168**	**68.9**	**4.9**	**2.6**	**26.8**	**16.4**	**1.3**	**6.4**	**14.7**	**7.9**

Key: CIP, ciprofloxacin; CHL, chloramphenicol; NAL, nalidixic acid; GEN, gentamycin; AMP, ampicillin; DOX, doxycycline; SXT, trimethoprim/sulfamethoxazole; CTX, cefotaxime; MEM, meropenem; ESBL, Extended spectrum beta-lactamase;#,number of; Spp, Species; %R, Percentage resistance

TABLE 7: Comparison of resistance levels between ESBL vs non-ESBL producers, and Quinolone's resistance versus non-Quinolone's resistance among gram-negative bacteria recovered from ATMs surfaces in Dar es Salaam, Tanzania

Drug	ESBL producers (n=10) %R (n)	Non-ESBL producers (n=18) %R(n)	P-Value	Quinolone's resistance (n=33) %R(n)	Non-quinolone resistant (135) %R(n)	P-Value
STX	50(5)	22.2(4)	0.23	51.5(17)	14.8(20)	<.001
MEM	30(3)	0(0)	0.04	27.3(9)	3.7(5)	<.001
DOX	20(2)	5.6(1)	0.24	5.4(4)	4.4(6)	.38
GEN	0(0)	0(0)	1.00	0.0(0)	2.2(3)	1.00
CHL	10(1)	11.1(2)	1.00	9.1(3)	8.15(11)	1.00

Key: CHL, chloramphenicol; GEN, gentamycin; AMP, ampicillin; DOX, doxycycline; SXT, trimethoprim/sulfamethoxazole; MEM, Meropenem; ESBL, Extended spectrum beta-lactamase, %R, percentage resistance; (n), number of isolates

TABLE 6: Proportion of Quinolone/ Fluoroquinolone Resistant Bacteria Recovered from ATM Surfaces in Dar es Salaam, Tanzania

Organism	#Isolates	# FQ/QUIN resistant	%FQ/QUIN resistant
Klebsiella pneumoniae	31	8	25.81
Acinetobacter spp	21	0	-
Escherichia coli	17	5	29.41
Pseudomonas aeruginosa	14	3	21.43
Enterobacter aerogenes	13	1	7.69
Shigella spp	13	4	30.77
Enterobacter spp	12	5	41.67
Serratia spp	11	0	-
Klebsiella oxytoca	9	0	-
Salmonella spp	8	0	-
Citrobacter spp	7	2	28.57
Pseudomonas spp	4	0	-
Yersinia spp	4	4	100.00
Morganella spp	2	1	50.00
Proteus spp	1	0	-
Providencia spp	1	0	-
Total	168	33	19.64

Key: FQ- quinolone, QUIN-fluoroquinolone, #-number of, %-Percentage

to be quinolone/fluoroquinolones -resistant. *Yersinia species* were observed to be more resistant to quinolones 100% (4/4) than the other Isolated Gram-negative bacteria which ranged from 7.7% to 50 % (Table 6).

Antibiotic Resistance Levels among ESBL and Quinolone Resistance Isolates

ESBL producing bacteria were more significantly resistant to meropenem (*P=.04*), while quinolone/ fluoroquinolone resistant isolates were more significantly resistant to trimethoprim/sulfamethoxazole (*P <.001*), and meropenem (*P<.001*). (Table 7) Additionally, out of 10 isolates that were ESBLs producers, almost 50% (n=5/10) of those isolates were also resistant to quinolone/fluoroquinolone.

Factors Associated with MDR Bacteria Contamination on ATM Surfaces

Table 8 show independent predictors of ATM surface contamination. ATM surface contamination were more likely significantly associated with ATMs located in Ubungo ($PR_{adjusted}$ = 3.62, 95%CI = 1.58-8.30, *P=.002*), Kigamboni ($PR_{adjusted}$ = 2.78, 95%CI = 1.20-6.42, *P=.017*), and Temeke ($PR_{adjusted}$ = 2.75, 95%CI = 1.04-3.72, *P=.023*) compared to those located at Ilala municipal. On the other hand, ATMs with less frequency of cleaning were significantly associated with an increased likely hood of MDR bacteria contamination compared to those cleaned at least once a day ($PR_{adjusted}$ = 1.98, 95%CI = 1.04-3.73, *P=.04*). There was a decreased risk of MDR bacteria contamination on remote ATMs, though the decrease was not statistically significant ($PR_{adjusted}$ = 0.79, 95%CI = 0.43-1.46, *P=.46*).

DISCUSSION

This study revealed that more than half of ATMs in Dar es Salam were contaminated with gram-negative bacteria and one-third of these bacteria were MDR against 3 to 7 classes of common antibiotics used in hospital settings. ATMs located in Ubungo, Kigamboni and Temeke as well as less cleaned ATMs were observed as risk factors for MDR bacteria contamination.

The current study found 55.4% of ATMs contaminated with Gram-negative bacteria. This finding is lower than what was reported in a study conducted in India where 95.7% of ATMs were found to be contaminated with such bacteria.[43] This variation is probably contributed by the fact that the current study took place in the middle of the COVID-19 pandemic, where the use of hand sanitisers was high. Nonetheless, this poses a public health risk given the fact that half of the machines inspected were contaminated with pathogenic bacteria, including multi-drug resistant bacteria.

In this study, *K. pneumoniae* was the most frequent isolate, followed by *Acinetobacter* sp and *E. coli*. These results conform to observation reported in a study conducted in India where *K. pneumoniae* was the most isolated bacteria from ATM surfaces.[44] However, this finding is contrary to the findings of a study conducted in west Iran[45] where *E.coli* was the predominant isolate followed by *Klebsiella spp*. Collectively, numerous studies report the predominance of *K. pneumoniae* and *E. coli* as the most significant gram-negative bacteria in contamination of ATM surfaces.[44-46] These microbes are members of Enterobacteriaceae.

The current study revealed that the risk of contamination was higher in less cleaned ATMs. This observation conforms to a study that showed that cleaning and disinfection reduce microbial contamination by 94.1%.[47] The risk of contamination of ATMs with MDR bacteria was also significantly associated with the location of the ATM. The risk was high in densely populated areas namely; Ubungo, Kigamboni, and Temeke. This observation is in keeping with a study conducted in Nigeria where ATMs from the Abakaliki metropolis had higher isolation of bacteria compared to ATMs in low populated Afikpo town.[16] Collectively, these findings support the need for maintaining strict hygienic measures on frequently touched public surfaces especially in overcrowded areas. This is supported by multiple studies.[30,48,49]

Regarding AMR pattern, isolates recovered from this study showed high levels of resistance against ampicillin, moderate levels of resistance against, cefotaxime (CTX), trimethoprim/sulfamethoxazole (SXT) and nalidixic acid (NAL), and low level against meropenem (MEM) and gentamicin (GEN). An estimated one-third of all isolates were MDR, with some exhibiting resistance to more than 6 different classes of antibiotics and could be classified as pan-drug resistant (PDR).[50] Notably, most MDR combinations included penicillin, tetracycline, and ciprofloxacin, which is in keeping with several studies conducted in Dar es Salaam, showing high resistance to such antibiotics.[51,52] Resistance to these antibiotics can be explained by the fact that there is irrational use of antibiotics in the community, as most antibiotics are relatively cheap and can be obtained over the counter without a prescription,[53] which fuels the occurrence of the resistance.[54] Furthermore, this study showed that *Salmonella* species had high to moderate levels of resistance against CTX and MEM respectively. This observation supports other studies' findings, where the emergence of ESBL- producing *Salmonella spp* and carbapenem resistance have been reported in the community.[55,56] An increase in resistance to *Salmonella spp* especially to meropenem (MEM) is alarming, as there are few options available to treat extensive drug-resistance (XDR) Typhoid. This is high time to take an important step to study the resistance pattern of *salmonella spp* to detect new stains timely.

Our study showed that among isolates screened for ESBL, 35.7% were ESBL producers. Compared with non-ESBL producers, ESBL-producing bacteria had insignificant resistance to trimethoprim/sulfamethoxazole, chloramphenicol; gentamycin, and doxycycline except meropenem.

On the other hand, 19.6% of isolates were quinolone/fluoroquinolones-resistant whereby quinolone/fluoroquinolones resistance isolates were more significantly resistant to trimethoprim/sulfamethoxazole, and meropenem except for gentamicin, doxycycline, and chloramphenicol when compare to non-quinolone/fluoroquinolones resistance. These findings are in contrary to a study in Dar es Salaam[57] which shows that ESBL producers and quinolone resistant isolates were more significantly resistant to all other tested antibiotics including, gentamicin, meropenem, chloramphenicol, doxycycline, and trimethoprim/sulfamethoxazole. This variation is presumably because the current study used samples from inanimate surfaces while the other study used poultry and pig, whose farming has been associated with intense use of antibiotics.[58] *Yersinia spp* were more resistance to quinolones/flouriquinolones than other Gram-negative bacteria, this is similar to a study conducted in china which showed that *Yersinia spp* isolated from animal feaces, raw/cooked livestock, poultry meat, and frozen food had high resistance to quinolones.[59,60] Nonetheless, 50% of ESBL producers were also resistant to quinolones/ fluoroquinolones, indicating and supporting shared mechanisms of resistance.[61] These findings are important since beta-lactams and quinolones/fluoroquinolones are the cornerstones for treatment of majority of infections occurring in humans and animals[62,63] and resistance to them has severe consequences on public health and animal production.[64,65]

CONCLUSION

More than half of ATMs in Dar es Salaam are contaminated with Gram-negative bacteria, with one-third of these bacteria exhibiting multi-drug resistance against the tested antibiotics. Contamination occurred especially in ATMs that were not regularly cleaned and those located in densely populated areas, calling for interventional measures such as regular disinfection of the machines and clients' precautionary measures, such as hand sanitation. The owners of the ATMs need to ensure constant application of hygienic measures, including the provision of sanitisers, and constant monitoring of compliance.

Study Limitations

The current study provides important preliminary information about the proportion of Gram-negative MDR bacteria contamination on ATM surfaces, as well as associated factors. However, the study has several limitations. Users' hand hygiene practices were not observed, which could have provided evidence of the association of hand hygiene practices with contamination of ATMs with MDR bacteria. Secondly, the preparation of the sanitisers, their composition, and expiry dates could not be verified since such information was not available.

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EAHRC
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ORIGINAL ARTICLE

Establishment of Haematological Reference Values for Healthy Individuals Attending Ruhengeri Referral Hospital in Rwanda

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ABSTRACT

Background: The Laboratory investigations are very important for reaching to definitive diagnosis of diseases orientation and thus enabling optimal patient management based on informed diagnoses. Decision, and these are very difficult to obtain in the absence of reference values. In many cases, laboratory diagnosis such as haematological analyses are dependent on pre-defined locally established reference values.

Objective of the study: The objective of this study was to describe ranges of haematological reference values for healthy individuals attending Ruhengeri referral Hospital in Rwanda

Methodology: The cross sectional study was carried out in Northern Province at Ruhengeri referral hospital from July 2018 to September 2018. Participants were 252 healthy individuals aged less than one year to 68 years. From each participant, 4mL of blood samples were collected using K3 EDTA containers, and then analysed by Sysmex XS-500i automated haematology analyser.

Results: Haemoglobin levels varied with age and sex. The level decreased with the increasing age, and males had high haemoglobin level than females (15.69g/dL versus 14.46g/dL). Minimum mean values of haematological parameters for study participants were slightly on the high side with narrow confidence intervals compared to the Manufactured Sysmex values.

Conclusion: The findings may be used to define normal haematological values for Rwandan population and help physicians to better define haematological abnormalities in patients

INTRODUCTION

The concept of reference values was conceived by a group of Scandinavian scientists in the 1970s, and then developed by many works of French and Spanish Societies as well as the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC-LM) and the National Committee for Clinical Laboratory Standards (NCCLS) in the United States during the 1980s.[1]

According to the study done in Iran appropriate local reference values for haematological parameters are essential for screening, follow up, interpreting laboratory data and detecting haematological abnormalities.[2] Haematological parameters may be affected by individual factors such as age, sex and lifestyle, and ecological factors such as ethnic background, climate, exposure to pathogens and altitude. They vary not only between individuals but also between populations.[3]

Study conducted at Ethiopia have shown that the normal ranges of red blood cell counts (RBCs), haemoglobin (Hb) concentration, haematocrit, mean cell volume (MCV), total white cell count (WBC), platelet counts are known to vary with age, sex, dietary patterns, ethnic origin, genetic and environmental factors.[4]

Moreover, the reference values, which were established by the studies conducted in different geographic locations, may not reflect the normalcy of the population in question and therefore, it is always desirable to identify the region specific reference intervals.[5] In some of African countries, laboratories uses reference values obtained from the literature or inserts accompanying the reagent kits or instrument manuals.[6] The few small studies conducted in African population syndicate differences in normal values compared with those for populations in industrialized countries.[7] Lower values for haemoglobin, red blood cells, haematocrit, mean corpuscular volumes, platelets and neutrophils and higher monocytes and eosinophils levels were reported in African population compared to similar population in Western parts.[8]

Many studies have revealed that reference values vary with several parameters such as ethnic origin, genetics, gender, altitude and environmental factors[9]. It is important to recognize that reference values may differ between different laboratories. It is therefore important to be careful when interpreting patients' results. Thus, unsuitable reference values of haematological profile might elevate the risk of

either unessential further investigations or failure to determine underlying disease.[10]

Laboratory reference intervals for healthy populations have not been established in most African countries. Currently many countries in sub-Saharan Africa including Rwanda uses the reference intervals derived from populations in Europe and North America.[3] This type of study is essential to establish haematological reference values for Rwanda population. Using local laboratory haematological reference values may help to improve diagnosis and treatment of individuals with haematological disorders.

METHODS

Ruhengeri Referral Hospital Demographic information of participants

Ruhengeri is served as a public District Referral Hospital for many years, and is located in Musanze District of North Province in Rwanda. Since 2013, the facility serves as the National Referral Hospital and receive referred patients from its neighbouring hospitals and health facilities in northwest Rwanda. The hospital provides services to 406,557 people who live in Musanze District, and to 386,078 people from surrounding districts.

Study Design

Across sectional study was conducted at Ruhengeri referral hospital with the aims of establishing haematological reference parameters for healthy individuals.

Population and Sample Size Estimation

A total of 350 individuals were screened from July to September 2018 at Ruhengeri referral hospital. Out of 350 screened individuals, 252 health individuals selected and 98 excluded because of their disease conditions that would alter haematological values

Inclusion Criteria

All healthy individuals aged 1 to 68 years who visited Ruhengeri Referral Hospital during data collection period (July to September 2018) were eligible for the study.

Exclusion Criteria

Individuals using anticoagulants, those with history of significant blood loss, blood donation, surgical operation within three months, those with disease conditions that would alter haematological values, those with pre malaria symptoms were excluded.

Sample Collection

Study participants' particulars were recorded and registered in haematology register books. Blood sample was collected after cleaning the venepuncture site with pad soaked in 70% isopropyl alcohol. About 4ml of venous blood sample were collected in K3 EDTA containers, then Sysmex XS-500i automated haematology analyser was used for analysing haematological parameters after running daily control according to the manufacturer's instructions.

Data Analysis

Data was analysed using Microsoft Excel sheet (2013) and Statistical Package for Social Sciences (SPSS) computer program (version 22). The percentile range (2.5%-97.5 %) was used to determine the higher and lower values of normal ranges.

Ethical Consideration

Ethical clearance was obtained from Ruhengeri referral hospital ethics committee with reference number:1089/HDR/HRR/2018, Informed consent was used from participants before collection of samples. The specimens and information were collected from the individuals under privacy and confidentiality and were not used for any purposes rather than this study. Each individual included in this study was given his/her tests results

RESULTS AND DISCUSSION

Social Demographic Characteristics of Study Population

Out of 252 study participants, 23.6% were children under the age of 18 years, of which 27.2% were females. For adults, significantly high proportion of participants were males (Table 1).

TABLE 1: Sex and Age of Study Participants

	Under 18 years (%)	18+ years (%)	All (%)
Males	25 (19.7)	102 (80.3)	127 (50.4)
Female	34 (27.2)	91 (72.8)	125 (49.6)
Total	59 (23.6)	193 (76.6)	252 (100.0)

Haematological Reference Ranges

Table 2 summarized mean reference values for haematological parameters age group and sex. Independent student's t-test was used to assess the differences between age groups and sex.

Haematological reference parameters for children below 18 years and adults aged 18 to 68 years are depicted in Table 2. As expected, haemoglobin levels varied with age and sex. The level decreased with the increasing age, and males had high haemoglobin level than females (15.69g/dL versus 14.46g/dL).

The sex difference in haemoglobin level is a well-established fact that has been reported in other studies[11,12]

Comparison of Study Participants' Ranges of Normal Haematological References with System Values

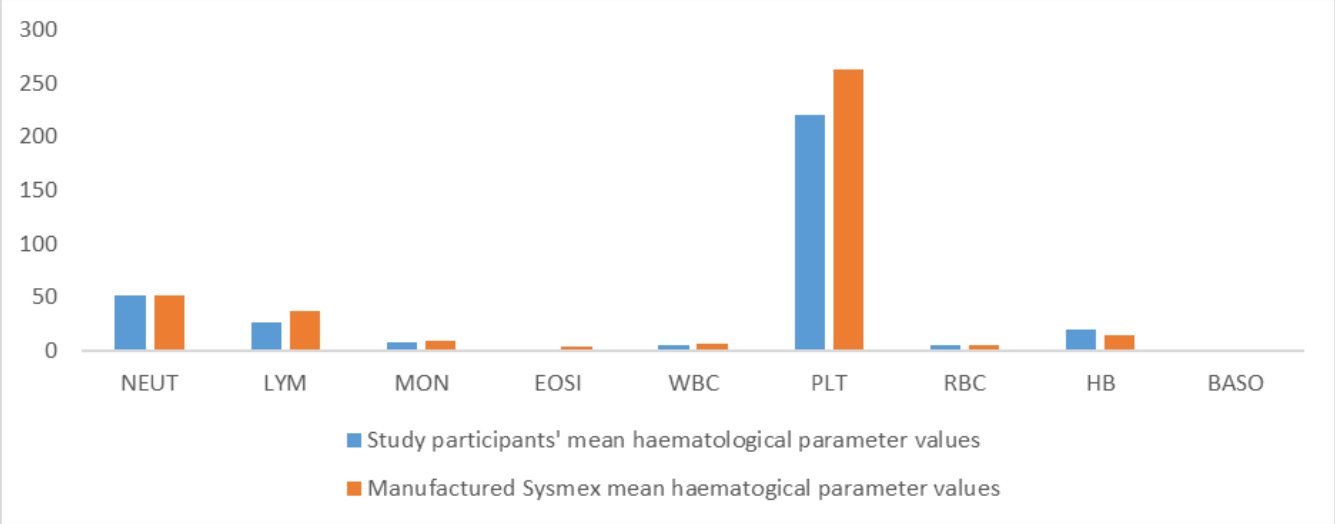
Study participants' normal ranges had narrow intervals compared to the manufactured Sysmex values (Table 3). haematological values among healthy individuals attending Ruhengeri referral hospital are lower on some parameters than sysmex reference values, obtained values of monocytes, white blood cells, platelets, neutrophil, eosinophils and lymphocytes on both genders are lower compared to sysmex values, the means of basophils are same as sysmex reference values while haemoglobin and red blood cells are higher (Figure 1)

Studies from other African countries have reported high mean Hb, PLT, RBC and WBC compared to the findings of the current study.[13,14] The reason for the lower values in this study might be due to variation of the standardization

TABLE 3: Comparison of Study Participants' Ranges of Normal Haematological References with Sysmex Values

	Study participants' normal haematological parameter ranges		Manufactured Sysmex Values	
	Males	Females	Females	Males
WBC	4.53-7.25	4.77-7.57	3.98-10.04	4.23-9.09
RBC	4.83-5.85	4.52-5.31	3.94-5.22	4.63-6.08
HB	14.50-16.88	13.541-15.38	11.2-15.7	13.7-17.5
PLT	213.01-323.59	211.05-322.00	182-369	163-337
NEUT	43.00-59.96	43.67-59.29	34-71.1	34-67.9
LYM	30.19-46.05	31.56-45.66	19.3-51.7	21.8-53.1
MON	6.36-9.85	6.21-9.63	4.7-12.5	5.3-12.2
EOSI	0.49-3.07	0.50-3.01	0.7-5.8	0.8-7
BASO	0.17-0.53	0.16-0.48	0.1-1.2	0.2-1.2

FIGURE 1: Comparison of Haematological Parameter Values



of conditions under which measurements were made. The significant difference between male and female may be due to biological and physiological factors such as the influence of the hormone androgen on erythropoiesis and due to menstrual blood loss in females.

When compared children's haematological parameter values in the current study with previous one,[15] neutrophil values were lower, while WBC, PLT, RBC, Hb, mono, and lymph values were higher. These variations could be attributed to differences in geographical locations, climate, dietary habits, and environmental factors or ethnic.[16]

Variables such as the technique, timing of sample collection, storage of specimens and posture of subjects, though if standardized may be of less effect could also contribute to these observable variations.[17] The findings of this study were similar to those obtained in Gondar Norwest of Ethiopia.[8] With exception of Hb level, males' haematological parameter values reported in the current study were slightly similar to values reported in a study conducted in Sudan.[18]

On the contrary, PLT and WBC values were higher in women, the difference was significant as compared to men just as reported in other studies, and men have high values of haemoglobin and red blood cells compared to females.[19] The reason for these differences may be due to the variations in hormone types and concentrations in the different sexes and the effect of erythropoietin release in response to regular menstruation cross-stimulating megakaryopoiesis. However, the platelet counts are lower when compared to the US derived values and other African studies.[20] The reason for these lower values is still unclear and may require additional studies but may be due to the diet, genetic factors or other environmental or genetic factors.[21]

TABLE 2: Haematological Reference Ranges

	Children aged 0 to 17 years (N=59)						Adults aged 18 to 68 years (N=193)		
	0 day to 7day (n=14)	8 day to 5 months (n=7)	6 month to 2 yrs (n=6)	3 yrs to 6 yrs (n=6)	7 yrs to 17 yrs (n=26)	P value	Males (n=102)	Females (n=91)	P values
WBC	11.37±3.83	11.34±6.46	10.74±2.25	7.97±2.69	6.74±1.74	0.00	5.89±1.36	6.17±1.39	0.15
RBC	5.34±1.04	4.91±0.46	5.30±0.45	4.91±0.37	5.04±0.53	0.05	5.34±0.51	4.92±0.39	0.00
HB	18.49±3.47	15.29±2.36	14.85±2.67	13.38±0.43	14.43±1.37	0.003	15.69±1.18	14.46±0.92	0.00
PLT	231.64±59.13	267.29±90.75	338.67±71.99	262.83±62.53	288.65±57.60	0.35	268.3±55.29	266.53±55.47	0.82
NEUT	53.31±10.64	49.59±5.75	56.02±15.98	45.52±10.14	50.51±8.06	0.83	51.48±8.476	51.48±7.81	0.99
LYMPH	33.73±10.79	36.24±9.66	31.72±16.03	41.1±10.59	38.92±7.87	0.12	38.12±7.92	38.61±7.05	0.65
MON	9.06±1.86	8.16±1.58	7.47±1.29	7.53±3.96	8.46±1.96	0.39	8.11±1.74	7.92±1.71	0.43
EOSI	1.65±1.78	0.96±0.97	1.85±2.84	2.96±2.47	1.94±1.59	0.67	1.78±1.29	1.76±1.25	0.89
BASO	0.45±0.35	0.6±0.72	0.38±0.50	0.52±0.65	0.34±0.28	0.09	0.35±0.18	0.32±0.16	0.20

CONCLUSION

The findings may be used to define normal haematological values for Rwandan population and help physicians to better define haematological abnormalities in patients. The reference values obtained in this study are recommended to be used in the medical practice. Another study with representative sample may be conducted to determine effect of geographical location on haematological reference parameters in the population of Rwanda.

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EAHRC
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ORIGINAL ARTICLE

Evaluation of the Performance of Copper Sulphate and Hemocue Methods for Haemoglobin Estimation Among Blood Donors in Dar Es Salaam, Tanzania

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ABSTRACT

Background: The National Blood Transfusion Service (NBTS) in Tanzania uses the Copper Sulphate ($CuSO_4$) gravimetric method to estimate hemoglobin (Hb) in blood donors. However, this and other point-of-care methods, including HemoCue, may provide false results. Therefore, this study aimed to evaluate the performance of $CuSO_4$ and HemoCue methods for Hb estimation compared with automated haematology analyzer (AHA).

Methods: The cross-sectional study was conducted among (N=204) blood donors in Dar es Salaam. Capillary blood samples were obtained for Hb estimation by $CuSO_4$ and HemoCue methods, 3 mls of venous blood were also collected for Hb quantification by AHA (gold standard), HemoCue and $CuSO_4$ gravimetric method. Data were analyzed by Epi info 7.2.2.6, statistical significance was defined at a P value of <0.05, and kappa agreement was calculated.

Results: The median age of the study participants was 30 years (IQR: 20-39). The proportion of false eligible donors was 19.6%, and false deferral donors were 2.9% by the $CuSO_4$ gravimetric method. The specificity, sensitivity, positive and negative predictive values, and Kappa agreement for $CuSO_4$ were 28.6%, 95.9%, 78.0%, 72.7%, and 0.1, respectively. In contrast, the specificity, sensitivity, positive and negative predictive values, and Kappa agreement for HemoCue were 62.5%, 98.6%, 87.4%, 94.6%, and 0.63, respectively.

Conclusion: Our study revealed that the performance of the $CuSO_4$ gravimetric method in Tanzania is relatively poor, with a high proportion of false eligible donors than the HemoCue method. These findings warrant further studies to evaluate the quality control measures for $CuSO_4$ gravimetric method and explore alternative point-of-care methods for Hb estimation among blood donors in similar resource limited-settings.

BACKGROUND

Haemoglobin (Hb) screening is mandatory to safeguard donors' health and ensure adequate blood supply to recipients. Hb screening, when performed appropriately, correctly identifies eligible donors who qualify to donate blood based on the set criteria.[1-3] Eligible blood donors should have a Hb concentration of more than 12.5 g/dL because one loses 0.7 g/dL to 1.5 g/dL of Hb following donation.[4] While the automated haematology analyzer (AHA) is the gold-standard test for quantifying Hb among blood donors, resource-limited settings use various point-of-care tests such as the Copper sulphate ($CuSO_4$) gravimetric and HemoCue methods to estimate Hb. However, the point of care methods may inappropriately designate an eligible donor as a deferral (not eligible) donor. Therefore, the methods used to estimate Hb should accurately detect the threshold of 12.5 g/dL.

In Tanzania and other resource-limited setting, many blood donation centers use the $CuSO_4$ gravimetric method to estimate Hb in blood donors. This method is known to be easy to perform, quick and cost effective. However, the method is known to be affected by high serum protein, high leucocyte count, and high ambient temperature, while waste disposal of the solution used is a considred a biohazard, and in some countries, it is regarded an environmental toxin; and most importantly the method cannot quantify the exact amount of Hb.[4] Moreover, there is a lack of a generally accepted quality control for the method and the fact that it cannot quantify the exact amount of Hb, therefore, it is not feasible to detect an abnormally low or high Hb level.[5-7] Hence, it may potentially provide false results, leading to donation-induced iron deficiency anemia[8-11] and the loss of blood donors.[12-14]

The proposed mechanism for this shortcoming is that

$CuSO_4 5H_2O$, when poorly reconstituted, can precipitate Hb, other proteins, and leukocytes.[10] Due to these shortcomings blood transfusion centers in other developed countries have shifted towards other point-of-care methods of Hb estimation such as HemoCue.[15-17] Despite these shortcomings, the CuSO4 gravimetric method is the recommended method for pre-donation Hb screening in resource-limited settings.[18]

There is a paucity of information regarding the performance of the $CuSO_4$ gravimetric method in Tanzania compared with other available point-of-care Hb estimation methods, such as the HemoCue, in estimating the Hb threshold among blood donors. Therefore, the present study evaluated the performance of $CuSO_4$ and HemoCue methods for estimating Hb among blood donors in Dar es Salaam, Tanzania.

METHODS

The study design and setting

This was a cross-sectional study conducted within a period of 3 months from January to March 2019. The study was conducted in three blood donation centers, namely Muhimbili National Hospital (MNH), Eastern Zone Blood Transfusion Centre (EZBTC), and Temeke blood transfusion satellite site in Dar es Salaam, Tanzania. According to the Tanzania National Blood Transfusion Service (NBTS) annual report, the selected sites contributed 90.2% of blood donated in Dar es Salaam region from January to December 2017.

Study Population, Sample Size, and Sampling Procedure

The study population was clients above 18 years of age who visited the centers for routine blood donation. The sample size was estimated using the formula for testing the sensitivity (or specificity) adopted from Hajian-Tilaki *et al.* 2014.[19] The largest sample size was 183 and was selected as the minimum number of participants required in this study, corresponding to $CuSO_4$ gravimetric method sensitivity of 98.4 %.[20] In addition, the estimate included a 10% non-response rate for a final target sample size of 204 participants.

The number of study participants for each blood donation center was selected according to probability proportional to size (PPS) sampling, whereby at MNH (n=80), EZBTC (n=64), and Temeke (n=60). We used systematic sampling to select the interval between blood donors. The time of data collection was 40 days with minimum sample size of 10 participants expected per site per day. Hence we used a formula k=population (400)/sample size(204)= 1.96 ≈ 2. Therefore, every second participant was sampled from the blood donors during the working hours until the estimated sample size was obtained.

Sample Collection Procedure

Capillary blood samples were obtained from prospective blood donors by lancing a fingertip on the index or middle finger of left hand using a dry sterile lancet after disinfecting with ethanol and massaging the finger to facilitate blood flow of a seated prospective blood donor. The first drop of blood was wiped off, while the second and third drops were collected into a capillary tube for testing using $CuSO_4$ gravimetric method and microcuvette for the HemoCue method in alternating order.[21, 22] Ethylene Diamine Tetra- Acetic acid (EDTA) anticoagulant test tubes were labelled with two identifications that of the blood donation centre and the study identification. Then three milliliters (3 ml) of venous blood were collected aseptically from each study participant into the EDTA tubes and transported to Muhimbili National Hospital at a temperature of 2^0 C to 8^0 C in a cool box with ice packs by a trained laboratory research assistant. All the samples were transported everyday within 2 hours of being collected along with the study sample laboratory request form, sample manifest and sample tracking form. The venous samples were analyzed for Hb by using an automated haematology analyzer (Abbott Cell-Dyn 3700, MN, USA), HemoCue and CuSO4 gravimetric methods.

Sample Processing

Collected capillary blood samples were directly measured onsite for Hb by using $CuSO_4$ gravimetric and HemoCue methods.[21, 22] The 3 mL venous samples collected into each EDTA tube, was gently mixed 3-5 times then 0.5 mL was aspirated for Hb estimation by HemoCue and CuSO4 gravimetric methods and the remaining volume was used for Hb testing by using an automated haematology analyzer.

Quality control (QC)

Copper sulphate pentahydrate ($CuSO_4 5H_2O$) preparation was done following the World Health Organization (WHO) Standard Operating Procedures (SOPs) to ensure quality of the $CuSO_4$ solution.[23] Briefly 170 gram of crystalline $CuSO_4$ powder was dissolved in 1000 mL distilled water to make a stock solution of $CuSO_4 5H_2O$. Then mixed well to ensure that the copper sulphate has dissolved. Then 51 ml stock solution was added into 49 mL distilled water to make a working solution. The specific gravity (1.053) was checked using a hydrometer, if 1.053 gravity was not obtained, it was adjusted by either using stock solution or distilled water.[23]

The calibration of the HemoCue was verified by a control cuvette each day before the first measurement as recommended by the manufacturer. In short, QC was performed daily as recommended by the manufacturer and the liquid QC testing was conducted prior to clients sample testing. This QC test ensures the accuracy of the HemoCue analyzer. The liquid QC comes in two levels: R&D GLU/HGB Control Level 1 (low) and R&D GLU/HGB Control Level 2 (high). In addition, the HemoCue analyzer has an Internal Electronic Quality Control (EQC) that is performed automatically each time the device is turned on. This test verifies the performance of the optronic unit of the analyzer. This test is performed eight hourly when the analyzer remains powered on. An automated Full Blood Picture (FBP) machine (Cell dyne 3700 analyzer) calibration and control were performed each day as recommended by the manufacturer. The QC for Abbott Cell-Dyn 3700, has an in-built internal quality control system and is conducted before running any patient samples after the verification that the background counts displayed are within the acceptable ranges as per manufacturer's instruction.

Data Analysis

Data were entered, cleaned, and stored in Microsoft (MS) Excel version 2019, and control of data quality was

conducted through the review of data collection tools. Then the data were exported into Epi Info version 7.2.2.6 for statistical analysis. The data set copy backup was made for any occasion that may need backup during data analysis.

The categorical variables were presented in frequency and proportions, whereas normally distributed continuous variables were presented as means with Standard deviations (SD), and those not normally distributed were presented as medians with interquartile ranges (IQR). The performance of both $CuSO_4.5H_2O$ and HemoCue was estimated by calculating the sensitivity, specificity, positive and negative predictive values, and kappa agreement with results from automated haematology analyzer as reference or gold-standard method.[20, 24, 25]

We defined sensitivity as the percentage of donors with Hb values below the cut-off of 12.5 g/dl (failed) identified by the test out of all donors with venous Hb values below the cut-off by the gold-standard test.[4] We calculated specificity as the percentage of donors with Hb value above the cut-off of 12.5 g/dl (those passed) identified by the test out of all donors with venous blood Hb above the cut-off value by gold-standard test.[4] Positive Predictive Value (PPV) was defined as the probability for a donor to have a Hb value below cut-off (failed/deferral) by both the test as well as the reference method.[4] Negative Predictive Value (NPV) was defined as the probability of a donor to have Hb value at or above the cut-off (passed/eligible) by both the test as well as the reference.[4]

Ethical Consideration

Ethical clearance was obtained from the Senate of Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS) with approval number MUHAS-REC-08-2018-50. Managers of selected blood donation centers granted permission to conduct the study. Study participants provided written consent. Confidentiality of the study participants was ensured by using codes instead of their personal names.

RESULTS

Socio-Demographic Characteristics of Study Participants

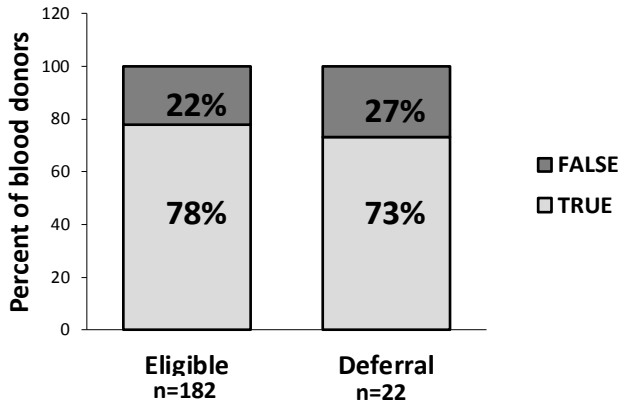
A total of 204 blood donors participated in this study. The median age was 30 years (IQR: 20-39). Males contributed 73.0% of the study participants. The majority of the participants, 39.2% (80/204), were from MNH, followed by 31.4% (64/204) from Temeke blood transfusion satellite site and 29.4% (60/204) from EZBTC.

The Proportion of False Eligible and Deferred Blood Donors by $CUSO_4$ Gravimetric Method

We compared the performance of the $CuSO_4$ gravimetric method with an automated haematology analyzer as a reference. We found that 182 (89.2%) out of 204 participants were eligible donors, whereas 22 out of 204 participants (10.8%) were deferral donors by the $CuSO_4$ gravimetric method. Of the 182 participants eligible by the $CuSO_4$ gravimetric method, 142 (78%) qualified by the automated haematology analyzer, whereas the remaining 40 (22%) were disqualified (Figure 1). Thus, 19.6% (40/204) of the study participants screened by the $CuSO_4$ gravimetric method were falsely eligible.

Furthermore, we observed that 16 out of 22 (73%) of the deferral blood donors were disqualified by both the $CuSO_4$ method and automated haematology analyser, whereas the remaining 6 out of 22 (27%) who were disqualified by $CuSO_4$ gravimetric method were qualified by the automated haematology analyser (Figure 1). These results indicate that 2.9% (6/204) of the blood donors screened by the $CuSO_4$ gravimetric method were false deferrals. Further analysis regarding the participants' demographic characteristics revealed that the proportions of male donors who were falsely eligible and falsely deferred were 55% (22/40) and 66.7% (4/6), respectively (Table 1). In addition, we observed that among the false eligible blood donors, 37.5% (15/40) were aged 21- 30 years, while among false deferred blood donors, the majority, 66.7 % (4/6) were aged 18 to 20 years. (Table 1)

FIGURE 1: Proportions of True and False Eligible and Deferral Donors by CuSO4 Method



Donors identified as eligible (n=182) and deferral (n=22) by the CuSO4 method were analysed for haemoglobin using the automated haematology analyser as the gold standard. The proportions of true and false eligible and deferral donors are indicated.

TABLE 1: Demographic Characteristics of False Eligible and Deferred Blood Donors

Variable	Deferred by $CuSO_4$ n = 22	Falsely Deferred Blood Donors n = 6 (%)	Eligible Blood Donors by $CuSO_4$ n = 182	False Eligible Blood Donors n= 40 (%)
Sex				
Male (55.0)	5	4 (66.7)	144	22
Female (45.0)	17	2 (33.3)	38	18
Age group				
18-20	18	4 (66.7)	40	7 (17.5)
21-30 (37.5)	1	0 (0.0)	48	15
31-40	1	1 (16.6)	53	8 (20.0)
41-50	1	1 (16.6)	27	7 (17.5)
> 50	1	0 (0.0)	14	3 (7.5)

Comparison of $CuSO_4$ and HemoCue Methods

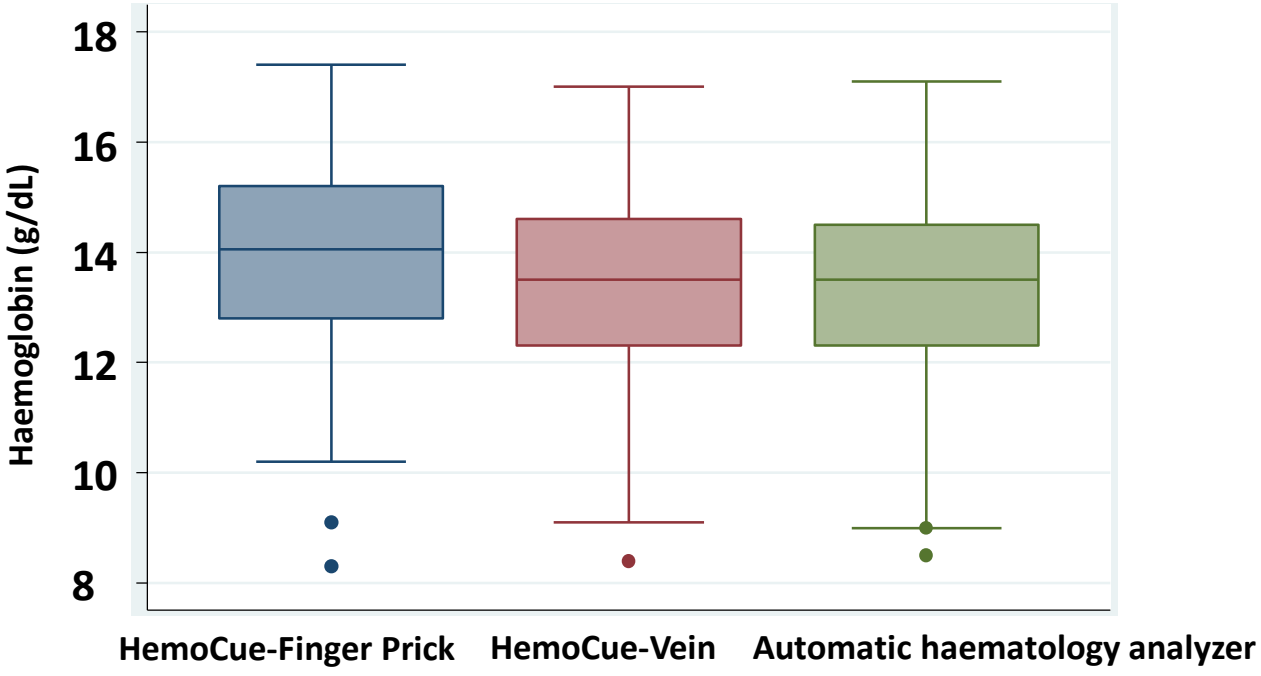
We then compared the performance of $CuSO_4$ and HemoCue methods with the automated haematology analyzer as reference. We observed that the sensitivity and specificity of $CuSO_4$ gravimetric method were 95.9 % and 28.6%, respectively, and the Kappa agreement was 0.1, suggesting a slight agreement (Table 2). In contrast, the HemoCue method had higher sensitivity and specificity of 98.6% and 62.5 %, respectively, and Kappa agreement of 0.63, suggesting a substantial agreement (Table 2). Furthermore, the positive predictive values (PPV) for $CuSO_4$ and HemoCue methods were 78.0% and 87.4%, and the negative predictive values were 72.7% and 94.6%, respectively (Table 2).

Correlation of Haemoglobin from Venous and Finger Prick Blood Samples

In the present study in order to investigate whether the difference in the performance of haemoglobin measurement was due to the blood sampling site or the analytical instrument used, we assessed capillary and venous Hb by HemoCue Hb301. We observed that the median Hb estimated from capillary samples was relatively higher (14.1 g/dL) compared to that from venous route (13.5g/dL) (Figure 2a). We performed a linear regression analysis which showed a positive strong correlation (with r = 0.913) between Hb estimate of venous blood and that of finger prick. Through this we determined the prediction formula of the venous blood from the Hb estimate of finger prick through equation of $y = 0.913x + 0.685$ (Figure 2b).

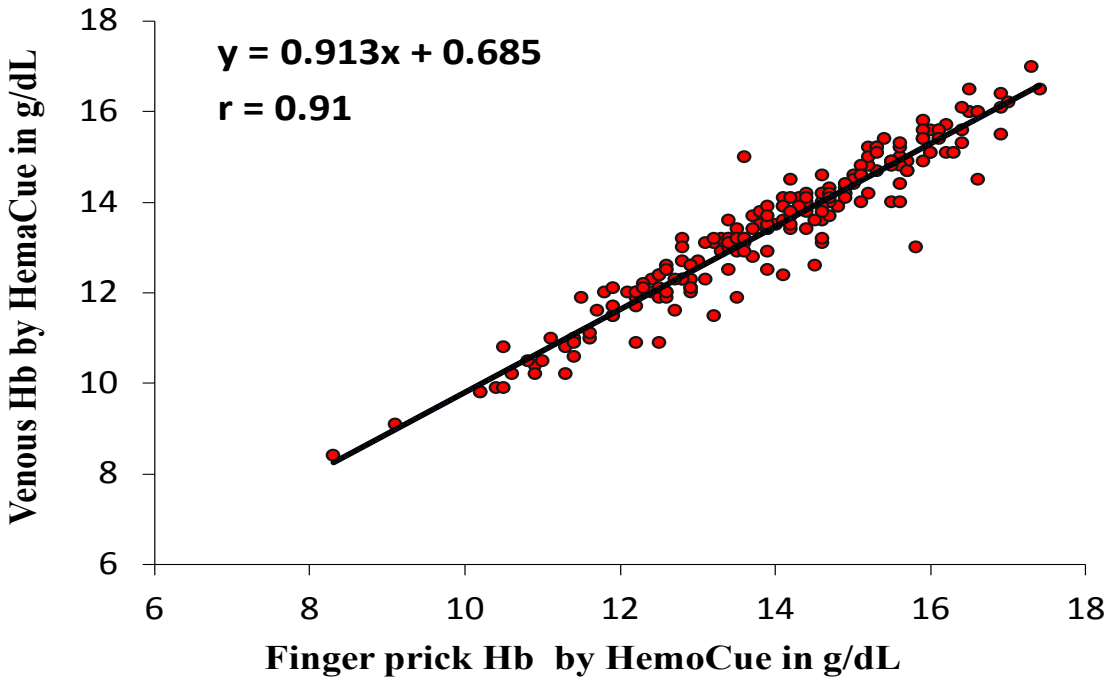
In addition, paired T- test between Hb from finger prick capillary and vein blood samples was performed, whereby the mean difference of Hb between those blood samples from venous route versus finger prick method was 0.53 g/dl and this was statistically significant with *P* value <.001 (Table 3).

FIGURE 2a: Median Quartile and Interquartile Range Using Finger Prick Sample and Venous Sample



The figure depicts the comparison of the distribution of the Hb values estimated by HemoCue method from the finger prick and venous samples.

FIGURE 2b: Correlation of Hb Estimations via Finger Prick and Venous Route as Site of Blood Collection by HemoCue



The figure depicts the correlation of Hb estimation between capillary sample and venipuncture sample by HemoCue

TABLE 2: Performance of $CuSO_4$ and HemoCue Methods Using Automated Haematology Analyser as the Gold standard

	Sensitivity (%)	Specificity (%)	PPV (%)[a]	NPV (%)[b]	Cohen's Kappa (K)
Method					
CUSO4	95.9	28.6	78.0	72.7	0.1
HemoCue	98.6	62.5	87.4	94.6	0.63

[a]Positive predictive value; [b]Negative predictive value.

TABLE 3: Paired T- test Between Haemoglobin From Finger Capillary and Vein Blood Samples

Variable	Standard Error	Standard Deviation	95% CI	T-value	P Value
HemoCue – Finger Prick	0.12	1.70	13.69 - 14.15	15.75	<.001
HemoCue – Vein	0.11	1.62	13.17 - 13.62		
Difference	0.03	0.48	0.46 - 0.59		

DISCUSSION

Accurate estimation of haemoglobin among blood donors is paramount to ensuring the safety of both the donors and recipients. Here, we evaluated the commonly used $CuSO_4$ gravimetric method for haemoglobin estimation in three leading blood donation centers in Dar-es-salaam, Tanzania. Our findings revealed that the method had suboptimal specificity (28.6%), PPV (78.0%), NPV (72.7%), and a slight (0.1) Kappa agreement with automated haematology analyzer; consequently, leading to a high proportion of false eligible donors of 19.6%. On the other hand, the HemoCue method had superior specificity (62.5%), PPV (87.4%), NPV (94.6%), and substantial (0.63) Kappa agreement with automated haematology analyzer.

The present study revealed that 19.6% of blood donors screened by the $CuSO_4$ gravimetric method in the three blood donation centers with the highest number of donors in Dar es Salaam, Tanzania, were falsely eligible. This proportion is higher than that reported in other studies that used the $CuSO_4$ gravimetric method, such as the study by Gupta *et al.* in India that reported a smaller proportion of 3.8% false eligible donors;[20] and that by Guracha *et al.* in Ethiopia at 9.2% of false eligible blood donors.[26] and another study by Chaudhary *et al* found 6.9% of the blood donors were false eligible.[4] Such difference could be due to variations of practice in preparations, quality control measures in the use of $CuSO_4$ gravimetric methods as previously reported to affect the performance.[4, 25] The high proportion of false eligible donors in our study setting increases the risks for donation-induced anemia among donors and inadequate blood transfusions to recipients.

We observed that 2.9% of blood donors screened by $CuSO_4$ gravimetric method and failed were false deferrals. This proportion was higher when compared to the study done by Gupta *et al.* in India, where the proportion of the deferred blood donors was only 1.4%.[20] The high proportion of the false deferred donors in our study may also be due to inadequate quality control checks, as shown previously, to influence the performance of the $CuSO_4$ gravimetric method.[20] The finding suggests that in order to improve the performance of the $CuSO_4$ gravimetric method to estimate haemoglobin among potential blood donors, one needs to improve the frequency and efficiency of the quality control checks.

With regard to $CuSO_4$ gravimetric method sensitivity and specificity, our study showed that the $CuSO_4$ method had high sensitivity. The findings are in line with the sensitivity reported in other previous studies conducted by Sobhy *et al*, Pistorious *et al*, Wilkinson *et al*, Gupta *et al.*, Guracha *et al.*, and Agnihotri *et al*. that reported a sensitivity of 97%, 94%, 95.7%, 98.4%, 94.4%, and 96.55%, respectively.[20, 25-29] In contrast, the specificity of $CuSO_4$ gravimetric method in our study was low compared to that reported by Gupta *et al* (98.8%) and Agnihotri *et al* (74.42%).[20, 25] Furthermore, our study revealed low PPV and NPV for the $CuSO_4$ gravimetric method compared to PPV of 95.8%, 92.3%, and 80% and NPV of 81.0%, 90.7% and 99% reported in previous studies.[20, 28, 30] The difference in PPV between the present study and the previous studies might be due to the non-conformity of the Standard Operating procedure (SOP).

The implication of low PPV and NPV may result in donation-induced iron deficiency (DIID) anemia,[9] and lead to an increased risk of adverse events reporting from the blood donors.[31]

In this study, we observed that the HemoCue method performance was superior to that of $CuSO_4$ gravimetric method. This observation is similar to what has been reported elsewhere.[5, 15, 20, 24, 30, 32] Therefore, these findings suggest HemoCue method may be a reasonable substitute for the $CuSO_4$ gravimetric method in our setting.

CONCLUSION

The present study is the first to reveal the high magnitude of false eligible and deferral blood donors through the $CuSO_4$ gravimetric method in our study settings. This study shows that the $CuSO_4$ method performed poorly compared to the reference method in estimating haemoglobin among blood donors, leading to high levels of false eligibility for donation. On the other hand, the HemoCue method performed better in the same settings. As $CuSO_4$ pentahydrate is still the most affordable method, our findings call for healthcare providers and stakeholders to formulate strategies to improve the screening of blood donors to enhance the quality of donated blood in our setting and other resource-limited settings. More studies are needed for the purpose of quality improvement of this method as it is still the most widely available and most appropriate technique considering the different environments that blood donation is done. Assessment on the frequency of quality control checks and how that affects the accuracy of the haemoglobin estimation could help to find the optimal frequency and points at which quality checks should be done to increase the effectiveness of this method. Investigating the accuracy of the technique with different concentrations of copper sulphate solution is an alternative method that may improve the quality of the technique. In addition, we suggest that in order to save inappropriate deferrals, when $CuSO_4$ method be used for massive screening, and subsequent testing should be done with HemoCue in situations where there is high demand for blood.

Limitation

The present study had a limitation. This study used the gold standard that is only applied to the venous sample due to the fact that Cell dyne analyser need a larger amount of blood and therefore the cell dyne analyser could not be used to test for the finger prick samples.

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ORIGINAL ARTICLE

Assessment of Biochemical Parameters of Graft Survivors Post Renal Transplantation at King Faisal Hospital in Rwanda

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ABSTRACT

Background: Chronic kidney disease (CKD) remains a public health concern of 21st century. Each year, over million people die from CKD resulting from the lack of proper diagnosis and treatment of this terrible disease of the urinary system. Non-communicable diseases (NCDs) cause roughly 60% of all deaths worldwide. There is insufficient data in Rwanda for the management of kidney diseases and other NCDs for all health facilities. Renal substitution therapy appears to be the best solution for long–suffering patients with end-stage renal illness who want to survive. The study's purpose was to find out the serum creatinine and blood urea nitrogen (BUN) concentrations among kidney transplanted patients at King Faisal Hospital of Rwanda, and to show the consequences linked with the transplantation of kidney.
Methods: This was a retrospective study carried from November 2018 to December 2019. The data were collected from medical records at King Faisal Referral Hospital, and analysed with SPSS version 22.
Results: BUN and serum creatinine concentrations ranged from 77.4 to 93.5% and 67.8 to 87.1%, respectively. BUN levels that were abnormally high ranged from 3.2 to 19.4%, while creatinine levels that were abnormally high ranged from 6.5 to 29.0%. BUN and creatinine levels that were abnormally low, ranged from 0.0 to 6.5 and 3.2 to 9.7%, respectively. Diabetes mellitus affected 19.35% of the study population, hypertension affected 35.48%, and antibody-mediated rejection affected 6.45%.
Conclusion: The slight change in biochemical parameters may be a problem after kidney transplantation. There should be a monitoring of biochemical parameters tests to prevent the post kidney transplantation complications.

BACKGROUND

Worldwide, one of the vital public health issues is chronic kidney disease (CKD), whose final outcome is end-stage renal disease (ESRD). Ten percent of people worldwide are suffering from CKD. The progress of CKD toward ESRD can be prevented via early diagnosis and suitable management. The only treatments of choice available for ESRD patients are dialysis and kidney transplantation. CKD is defined as a declined working capacity of kidneys with a velocity of glomerular filtration of eGFR/1.73 m^2 < 60 ml/min. ESRD is a reduction in kidney function that is incapable of being repaired and it is mandatory to save the life of patients by renal replacement therapy (RRT). The increase of ESRD cases shows that it is a threatening health issue.[1]

Every year, more than a million people die as a result of inability to get a proper and vital CKD treatment. CKD data in countries under development are limited and mortality rates are significantly higher compared to high-income countries. In low-income and middle-income countries (LMICs), many persons are undiagnosed and a high proportion of those with CKD develop ESRD. In addition, most of them don't have accessibility to life-saving RRT.[2] In Rwanda, the prevalence of CKD varies from 4 to 24% of population-based importantly on the protein in urine as a marker.[3] Hypertension and diabetes are two important factors leading to CKD. HIV and phytodrugs were also reported to have a similar role.[3] To control and manage CKD patients and kidney transplants, follow-up is needed.[4]

Usually, serum creatinine and BUN are biological markers that are measured several times for patients who had experienced renal transplantation. Such biomarkers help to evaluate how healthy the kidneys are, after renal transplantation.[5] Urea, normally considered as BUN when it is measured in the blood is a metabolism product of protein. BUN is defined as a natural byproduct of non-protein nitrogenous waste. The protein breakdown delivers amino acids and are deaminated to produce ammonia. Through liver enzymes, ammonia is subsequently converted to urea. Consequently, the urea concentration is

dependent on protein intake, body capability for protein catabolism, and sufficient urea excretion by the renal system.[6]

Creatinine is basically a creatine phosphate metabolite. It is a composite that acts as an energy source in the muscle. It is formed at a moderately steady velocity in the body, though this does differ depending on the body mass. Due to the bigger skeletal mass, men are likely to have higher creatinine levels than women.[7-9] Creatinine is freely filtered and secreted through the glomerulus and proximal tubules. It is used in several formulas to obtain the eGFR. The decreasing kidney function can cause the increasing tubular secretion of creatinine and cause also extra renal elimination of creatinine.[10] Thus the evaluation of serum creatinine and the level of BUN is necessary for the graft survival following renal transplantation.

In LMICs, lifestyle is changing, plus rapid urbanization, then NCDs impacts becoming more and more recognized. After all, only some epidemiological studies have been done on the prevalence, incidence, and the cause of these diseases. Between 1990 and 2010, CKD was almost twice a cause of death internationally and it was ranked the 18th highest death cause globally in 2010.[11] Estimations show that in 2030, patients with ESRD greater than 70% globally will be in LMICs unless key problems and concerns are solved.[12]

More than half of all patients necessitating RRT worldwide die resulting from the lack of access to dialysis or kidney transplantation. In Africa, predominantly in Sub-Saharan Africa, there is the biggest disparity in access to renal replacement, and among people requiring RRT only less than 3% can receive it. Consequently, the rising saddle of CKD falls on the least equipped countries and to provide the expensive but life-saving therapies of dialysis and/or transplantation is not easy. Therefore, patients with ESRD continue to die although treatment options are established. The enormous price together with giving RRT provides a forceful economic motivation for enhancing the prevention, detection, and management of CKD in LMICs.[13]

Reports show that people with HIV/AIDs have the highest risk for CKD in the world. Sub-Saharan Africa is the region with the highest number of HIV-positive people. Kidney can change its function during antiretroviral (ARV) treatment. Previous studies did not give confirmation of the high risk of CKD on Africans with HIV and close renal function control and surveillance in patients with high blood pressure and other risk factors.[14] Besides, most studies that were conducted in East Africa about BUN and serum creatinine concentrations were based on patients with CKD only.[3,10,15,16]

This study was conducted on kidney transplant patients. It was aimed to quantify the BUN and serum creatinine levels during the period of follow-up, and to investigate the associated consequences (diabetes mellitus, hypertension, and antibody-mediated rejection) with kidney transplantation. Therefore, two research questions of the present study are "What are BUN and serum creatinine levels among kidney transplanted patients during follow-up period? and "What are the risk factors associated with renal transplantation?

METHODS

Study Area

This study was conducted in renal unit department at King Faisal Hospital, Kigali City (Rwanda). The renal unit provides close attention to patients with kidney failure and other renal-related complications. The department has a haemodialysis unit that deals with the elimination of waste products like urea and creatinine as well as water from the blood of patients when the kidneys fail to do their functions. They thus do follow-up of kidney transplant patients.

Study Design and Period

This was a retrospective study. BUN and serum creatinine levels at the beginning after transplantation, and at every appointment (15 days, 1st month, 3rd, 6th, 9th and 12th months) post-transplantation were considered. The study was conducted from November 2018 to December 2019.

Study Population

The study population was 31 patients with kidney transplants at King Faisal Hospital that had completed at least 12 months of follow-up, post-renal transplantation. Only 31 patients were available during the study period.

Inclusion and Exclusion Criteria

The present investigation included kidney transplanted patients at King Faisal Hospital for follow-up. It excluded CKD patients that had no renal transplant, and patients who did not complete at least 12 months of follow-up. Patients with pre-existing diabetes and hypertension before receiving renal transplants were also excluded.

Ethical Consideration

This study was checked and approved by the ethical review committees of INES Ruhengeri and King Faisal hospital. The approval letter from INES-Ruhengeri was presented to King Faisal hospital administration, and the hospital granted the authorization to collect data. The information of the patients and data were collected anonymously and kept confidential.

Data Collection

Data were collected using paper forms. Urea and creatinine data were collected from the data record system of the hospital in the department of biochemistry and in medical records unit. Information was recorded starting from the first day until 12 months of follow-up. The information regarding NCDs especially diabetes mellitus and high blood pressure were recorded indicating if the patients had been affected before or after kidney transplantation.

Statistical Analysis

The data were presented in tables. The SPSS version 22 was used for data analysis. Descriptive analysis was calculated in terms of mean ± SD and some using frequencies and percentages. Additionally, the significance was considered based on *p-value* of < 0.05.

RESULTS

Levels of BUN among Kidney Transplanted Patients

In the present study, serum creatinine and BUN at King Faisal hospital were assessed, post-transplantation of the kidney. The normal range of BUN varies between 2.50 and 7.50 mmol/l. Table 1 shows that patients with normal ranges were more than those with abnormal levels. Within abnormal values, the ones with upper limits were more than those with lower limits. The mean BUN in the first 3 months was lower than in the subsequent months with a significant increase starting from the 6th to 12th month.

Levels of Serum Creatinine among Kidney Transplanted Patients

Serum creatinine levels were determined among kidney transplanted patients. Data were stratified into 6 groups according to days and months. The normal range of serum creatinine in King Faisal hospital in Kigali was between 60 and 130 µmol/l. Patients with normal creatinine levels were more than those with abnormal levels. Patients with high creatinine levels were more than those with low creatinine. The levels of creatinine were raised progressively for some patients. This was noticed from the 3rd to the 9th month (Table 2).

Consequences Associated with Kidney Transplantation

Kidney transplantation may be associated with some adverse effects. The results in Table 3 show that the study population had hypertension more than diabetes mellitus. Few were with antibody mediated rejection. All these risk factors were statistically significant.

TABLE 1: BUN Within First Year post Kidney Transplantation Follow-up

Period (post transplantation)	Normal (7-30 mg/dl)	High	Low	Mean	Std. Deviation
15 days	24 (77.4%) 29 (93.5%)	5 (16.1%) 2 (6.5%)	2 (6.5%) 0	4.87 4.63	2.76 2.18
1 month	28 (90.3%) 26 (83.9%)	2 (6.5%) 5 (16.1%)	1 (3.2%) 0	4.70 4.92	1.75 2.54
3 months	28 (90.3%) 29 (93.5%)	2 (6.5%) 1 (3.2%)	1 (3.2%) 1 (3.2%)	4.69 4.62	1.66 1.52
6 months	27 (87.1%) 27 (87.1%)	4 (12.9%) 3 (9.7%)	0 1 (3.2%)	5.22 5.10	2.05 2.59
9 months	26 (83.9%) 26 (83.9%)	5 (16.1%) 5 (16.1%)	0 0	5.42 5.42	3.30 3.34
12 months	24 (77.4%) 25 (80.6%)	6 (19.4%) 6 (19.4%)	1 (3.2%) 0	5.59 5.27	3.76 2.95

TABLE 2: Levels of Serum Creatinine Within First Year post Renal Transplantation

Period (post transplantation)	Normal (0.7-1.2 mg/dl)	High	Low	Mean	Std. Deviation
15 days	26 (83.9%) 27 (87.1%)	2 (6.5%) 3 (9.75)	3 (9.7%) 1 (3.2%)	98.77 97.72	32.68 29.79
1 month	26 (83.9%) 23 (74.2%)	4 (12.9%) 5 (16.1%)	1 (3.2%) 3 (9.7%)	97.79 96.52	33.11 33.37
3 months	27 (87.1%) 23 (74.2%)	2 (6.5%) 6 (19.4%)	2 (6.5%) 2 (6.5%)	97.87 98.97	25.91 30.85
6 months	26 (83.9%) 27 (87.1%)	4 (12.9%) 3 (9.7%)	1 (3.2%) 1 (3.2%)	101.06 100.37	30.77 31.52
9 months	25 (80.6%) 23 (74.2%)	5 (16.1%) 7 (22.6%)	1 (3.2%) 1 (3.2%)	105.18 108.94	42.26 47.45
12 months	21 (67.7%) 21 (67.7%)	9 (29.0%) 9 (29.0%)	1 (3.2%) 1 (3.2%)	127.93 117.21	104.79 51.89

TABLE 3: Consequences Within First Year Post Kidney Transplantation

Consequences	Number of subjects	Positive cases(%)	P-value	Degree of freedom	X2
Diabetes mellitus	31	6 (19. 35)	.0006	1	11.64
Hypertension	31	11 (35.48)	.001	1	2.61
Antibody mediated	31	2 (6.45)	.000	1	23.51

DISCUSSION

Serum creatinine and BUN of graft survivors post the transplantation of kidney were analysed at King Faisal hospital. It is more helpful to evaluate the function of kidneys by using a biomarker which is BUN which it must be measured several times for patients who were renal transplanted.[17] The change rate of BUN concentrations was recorded recurrently over time following renal transplantation. Patients with normal ranges were higher than those with abnormal levels. The patients with high levels of BUN increased gradually as days post renal transplantation increased. The longitudinal study done by Jaffa *et al.*[5] on renal outcome analysis, following transplantation and demographic factors, demonstrated that the BUN levels change was only influenced by the donor's vital status. The advantage was to put together recipient patients from living donors (as opposed to deceased donors). Urea levels can be changed by many factors including diet but the main cause can be the degree of damage to kidneys which have the role of urea excretion.

Renal allograft dysfunction occurs most commonly after one year following renal transplantation. It is often asymptomatic, and is typically detected by an increase in serum creatinine level.[18] Patients with normal creatinine were more than those with those with abnormal values. Creatinine levels increased progressively, with an important increase between 3rd and 6th month. Similar to investigations conducted by Donald *et al.*[19] and Hariharan *et al.*[20], serum creatinine concentrations increased at one year post-transplantation. Those studies evaluated the effect of a number of variables on graft survival and it was confirmed that there is an important independent relationship between serum creatinine concentrations and kidney graft loss. The impaired kidneys and use of high doses of anti-rejection drugs can cause a rise in serum creatinine. They can cause nephrotoxicity and kidney damage. Generally, there is an agreement among the investigations in nephrology domains concerning the importance of BUN and serum creatinine levels in influencing graft non-success following kidney transplantation.

Many nephrons can be damaged by high blood sugar levels leading to the incapacity of kidneys to maintain fluids and electrolytes homeostasis.[21] The study population had hypertension more than diabetes mellitus. In the report of Alalawi *et al.*[22], diabetic kidney disease (57%) and high blood pressure (12.4%) were rising as the most usual causes of ESRD. In that study, 10.9% of patients had undetermined causes and f4.6% had transplant rejection reactions. Similar to the present study, patients with diabetes were 19.35% and those with hypertension were 35.48% one year post renal transplantation, while 6.45% had antibody-mediated rejection. Furthermore, Donald *et al.*[19] indicated that for at least 6 months, the majority of patients with kidney transplants had hypertension regardless of their degree of renal function. In one year after transplantation, 75% had systolic blood pressure (SBP) greater than 130 mmHg. The increased SBP was independently and importantly related to chronic graft non-success within seven years of monitoring. There was significant link of SBP in the absence or presence of acute rejection with the loss of long-term graft. One of the main causes of renal transplant to be vulnerable to developing hypertension and diabetes is the use of anti-rejection drugs that can damage organs in the body.

Limitations of the Study

This was a retrospective investigation conducted at one referral hospital in Rwanda (Kind Faisal hospital) from November 2018 to December 2019. As it was retrospective, laboratory data records were gathered from the department of biochemistry and in the medical records unit and missing data from the records could not be recovered. As the results are from one hospital, they cannot be generalised to the entire country.

CONCLUSION

The first years post renal transplant follow-up are of great importance for improving graft survival. It is essential to use a combination of serum creatinine and BUN as makers, and it is very important to take care of the consequences associated with kidney transplantation. This provides an opportunity to carry out secondary interventional assessment early to delay graft failure in this population. Creatinine and urea were found to be vital indicators of normal kidney function. It is recommended to King Faisal hospital to advise all renal transplantation patients to attend follow up monthly, inorder to be evaluated, and access services for the control renal failure risk factors to ensure that their renal grafts are working properly.

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ORIGINAL ARTICLE

Importance and Limitations of Healthcare Verification for Accelerating Implementation of Universal Health Coverage in Burundi

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ABSTRACT

Introduction: As one of the avenues for implementing universal health coverage, healthcare verification for financing health facilities is receiving increased attention. Verification is the process of ensuring that healthcare services provided to users meet the predetermined criteria for billing and payment. The objective of this article was to examine the Burundi health system practices in healthcare verification for financing health facilities in order to identify strengths, limitations, and potential solutions for more advancement in universal health coverage implementation.
Methods: A critical case study was used as the overall methodological approach and a narrative review design to draw conclusions about the case.
Results: The results show that verification helps visualise the country's level of progression in implementation of universal health coverage. While it promotes efficiency in healthcare service reimbursement by allowing payment for quality care services, verification has proven to be a resilient function to fraud, abuse, and waste in the demand for, and supply of, healthcare services. However, verification has some limitations in terms of services and population targeting, and technical effectiveness of the verification team. The most important way identified for alleviating these limitations is to separate the demand for, and supply of, healthcare services.
Conclusions: More investments in research are required to recognise verification as an essential sub-function of health financing for universal health coverage implementation.

INTRODUCTION

Since the 2005 World Health Assembly on financing health for Universal Health Coverage (UHC), developing a country-specific health financing policy has emerged as a crucial step toward UHC implementation.[1] The most important component of UHC implementation proposed by this Assembly was increasing financial access to quality care for everyone in need. Making progress in this component has become a global political journey.[2,3] Verification prior to reimbursement of health facilities for healthcare services delivered is receiving extended interest as one of the health financing avenues for this journey. That said, the interest in investing in healthcare verification to ensure that health facilities provide quality care without financial barriers to access is more likely to increase than decrease in the coming years.[4]

In developed countries, verification is introduced in pay-for-performance or Performance-Based Financing (PBF) schemes to enforce quality care improvement and enhance efficiency in healthcare payment.[5,6,7] As a piece of evidence, healthcare verification is used in the United Kingdom for a standardised cost recovery system,[8] while it helps mitigate the risk of fraud, abuse, and waste in the United States of America health insurance shemes.[9,5]

In developing countries, healthcare verification is promoted as a crucial function in PBF schemes to enforce quality improvements and increase data accuracy for healthcare payment.[10,11] In health facilities, verification refers to the process of controlling the conformity of healthcare services provided with the pre-set criteria for billing and payment.[6] While it is a prerequisite for payment by a health financing scheme, verification is changing into an essential function to authenticate healthcare services payment.[12,13]

In Africa, there is limited and fragmented evidence about the importance of healthcare verification for universal health coverage implementation.[14]

In Burundi, the Low-Income African and 10th most aid-dependent country worldwide,[15] healthcare verification has been nationally implemented since 2010 to legitimate healthcare payment by linking this payment with the quality and volume of healthcare services provided in health facilities.[16]

To analyse the practice of healthcare verification for UHC implementation, first the verification process is described, as well as the problem statement in Burundi. Next, the description of data collection and analysis method is provided. Finally, findings are discussed and ways for improvement are suggested.

BACKGROUND – Verification as a Process for Proving Data Accuracy in Burundi

Healthcare verification for financing health facilities in Burundi context is associated with the introduction of PBF in 2006. Since 2010, Burundi has employed mixed healthcare verification teams comprised of civil servants and contractual experts hired by development partners such as Cordaid, the European Union, and the World Bank. This mixed healthcare verification teams have been established in each province and integrated into each health province office in order to enable PBF appropriation by the Ministry of Public Health.

Within the Burundi PBF, healthcare verification is based on standard service-oriented contracts in health facilities. The contracts specify the healthcare services package and the guidelines for delivering services. In this context, verification is known as the process of reviewing and triangulating multiple data sources that are available in health facilities to prove that the healthcare services claimed by providers meet the payment criteria stated in the contracts.

PBF is one of the popular strategies used for health systems strengthening.[10] In Burundi, PBF has evolved from a simple health system strengthening tool to a health financing policy for three main reasons. First, PBF and its crucial function of healthcare verification have received national political priority since 2010. Second, PBF complies with the four essential health financing functions: policymakers use PBF to solicit and collect financial support from various donors; funds collected from various sources are virtually pooled before being redistributed to health facilities; findings of healthcare verification inform the amount to allocate to each health facility; and all stakeholders involved in healthcare payment agree on the package of healthcare services to be paid for on the national scale. Third, health facilities are paid in the form of a financial incentive for making progress on quality care, as well as reimbursement for free care provided to pregnant women and children under the age of five. Figure 1 depicts healthcare verification as an essential function of implementing PBF policy and making progress on UHC.

The **eligibility** test consists of determining whether or not healthcare services claimed by health facilities are eligible for the predetermined healthcare services package. Through this test, healthcare verifiers ensure that providers deliver quality care to the target population by checking the accuracy of the healthcare service user identity and clinical data.[15] Checking the identity of women giving birth in maternity services in 2021, for example, revealed that 78.8% of institutional deliveries were eligible for the national maternity service package.

The **compliance** test consists of detecting the conformity of claimed healthcare services with the pre-set healthcare services package and the provision standards.[11]

For example, in 2021, the test guided in rejecting healthcare services for 10.3% of pregnant women who visited the consultation service because the healthcare services they received did not comply with the pre-set package. Forging diagnostics, using unskilled personnel, ignoring treatment protocols, etc. contributed to the rejection of these services for reimbursement.

The satisfaction test aids to collect critical feedback from healthcare service users on services they received.[17] In 2021, for example, 22.7% of users were dissatisfied with the services they received. According to previous experience, a lack of sufficient medicines in health facilities is the most critical feedback and key source of dissatisfaction frequently reported by users. This is true because the national health policy document 2016-2025 indicates that essential medicines coverage in Burundi, remains below 50%, as it is in the rest of Africa's poorest countries.[18]

These classical tests of health coverage, which constitute the verification process, determine the healthcare services that comply with the pre-set national healthcare services package for billing and payment. Healthcare verification plays an important role in the mitigation of fraud, abuse, and waste in healthcare delivery system.[19,20] To clarify these terms further, fraud is an intentionally false statement of facts or identity to obtain payment[21] such as reporting wrong benefits, giving incorrect information to get free healthcare services, etc. Abuse refers to inappropriate practice that results in an unnecessary reimbursement.[22] This is exemplified by clinical data forgery when claiming benefits payment. Waste includes spending on services that cannot be formally justified[23] such as non-quality care payment, overtreatment, etc.

PROBLEM STATEMENT

Two important issues. One is the absence of inter-sectoral collaboration when defining the package of healthcare services as proposed by the 2005 World Health Assembly for UHC.[1] Additionally, there are restricted relationships between policymakers and local population when elaborating contracts. As a result, contracts are more "people-using" than "people-building" for financial access to quality care. Contracts focus more on the stimulation of the target population to use healthcare services than on building the population's capacity to organise their own financial protection systems (community-based health insurances, for example). The other contract-related challenge is the break in continuity of coverage throughout an individual's life time. As they are not included in the process of contract elaboration to defend their rights, women (especially those affected by inequities such as indigenous women) are only covered during the period of pregnancy and children before the age of five. This implies that contracts do not offer the possibilities to keep financial coverage in case of changes in the social status of the target population.

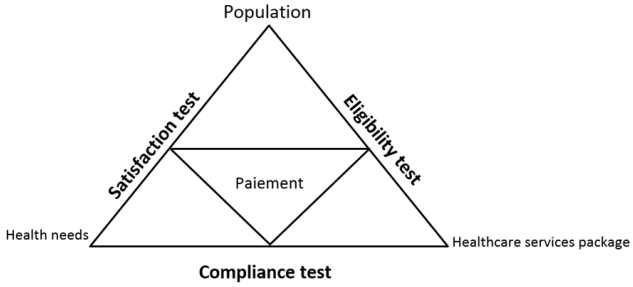
The other important issue is that quality scores from healthcare verification do not reflect reality on the field. For example, the 2021 PBF report showed that the quality assessment score used to calculate the amount of payment for quality care improvement in Burundi hospitals ranged between 80% and 90% in 2020. However, experience has shown that when performing extemporized quality

assessment, the score rarely exceeds 50%.

In this situation, the relevance of healthcare verification for financing health facilities remains debatable in terms of implementing UHC in Burundi.

The objective of this paper was to examine the Burundi health system practices in healthcare verification for financing health facilities in order to identify strengths, limitations, and potential solutions for more advancement in UHC implementation.

FIGURE: Description of the Function of Healthcare Verification in Burundi



METHODS

The World Health Organization (WHO) published a framework in the 2010 World Health Report that can be used to assess countries' progress toward UHC implementation. The framework, known as the UHC cube, consists of two main axes of progress on UHC implementation: the axis for quality healthcare services and the axis for financial risk coverage or health insurance.[23,24] The two axes were used to collect, synthesise and analyse data. Burundi was selected as a study setting because of it's uniqueness: the country has implemented PBF as a health financing policy. A critical case study approach was selected because is more effective compared to other methodological approaches,[25] such as statistical and structured surveys, for achieving the research objective.

Under the case study approach, we used a narrative review design to draw conclusions about the case. This design was helpful in identifying strengths, limitations, and potential solutions[26] to healthcare verification improvement. We used a qualitative method to collect and summarise data for this narrative review design. To do so, we collected a sample of information available on the online database, which can be found at: www.fbpsanteburundi.bi. This database is the most appropriate and significant for finding information about the present case because it contains documents, reports, and opinions from different actors (with different perspectives such as the Ministry of Public Health authorities, local researchers, development partners, etc.) concerned with the healthcare verification for financing health facilities in Burundi. So, there is no concern about the representativeness and validity of the information contained in this database. We used a purposive sample of information to build the case. This means that, for each of the two axes of progress in the UHC, we progressively checked additional information in the database until the ability to obtain new ideas is reached (saturation).[27] Considering the nature of the current study design, we used an unsystematic strategy to search for information in the database. We used the qualitative content analysis technique to extract and present information published between 01/01/2010 and 31/12/2022 in the database. To reduce the risk of information bias from a single source of data, we triangulated the information extracted from the database with what we know from our own professional experience in healthcare verification. We excluded information that we were aware had already been published somewhere. We narrated the case using both comparative and theory building structures. The comparative structure consisted of presenting results from the database in comparison to the two major axes of progress on UHC implementation. The theory building structure included an explanation of how the case contributed or not contributed to UHC implementation.

RESULTS AND DISCUSSION

Findings from healthcare verification determine which services, population, and costs are covered in each health facility. Those findings help in visualising the country's level of progress on the two axes of UHC implementation.

The Importance of Healthcare Verification

Healthcare verification is contract-oriented. In other words, contracts specify quality and quantity targets for each contracted health facility. According to the 2021 PBF report and 2021 yearbook of health statistics, 963 or 69.7% of the 1381 operational health facilities (public and private combined) in the country were contracted. The more the health facilities progress towards the targets, the more they get paid. As an outcome, health facilities progressively create new services where none previously existed in order to meet contract agreements, reduce disparities in the healthcare delivery system, and make the nationally defined healthcare services package effective.

Grids used for healthcare verification are permanently available in the 963 contracted health facilities for eventual self-assessment. The results of healthcare verification are synthesised in a standardised bill format that includes the package of healthcare services nationally covered. When analysing the billed services for each health facility (unavailable services are billed for zero dollars or claimed void or not applicable), it becomes simple to identify the available services in each locality of the country compared to the nationally pre-set package of healthcare services. In 2021, for example, the service of screening and treatment of malnutrition for children under the age of five was unavailable to the 66,313 population of Kanyosha Health Centre's catchment area (Bujumbura province).

Policymakers use findings from healthcare verification to periodically define and redefine evidence-based benefits package. This means that, based on the level of progress of health facilities toward national targets, quantity and quality indicators used for healthcare verification are regularly revised to stimulate further progress in various aspects of the healthcare delivery system. The 2014 amendment to the healthcare services package clearly

illustrates this point. While preventive services in hospitals were reimbursed before 2014, they were removed from the reimbursable healthcare services package in 2014.

Quantity indicators enable monitoring the evolution of service utilisation, determining the volume of services consumed by each category of the target population, and highlighting services that are under or over utilised in comparison to the targets per locality. For example, according to the 2021 PBF report, the target is 2 new visits per inhabitant per year for curative services in children under the age of five. Cankuzo province (7.0 new visits) outperformed the target, while Bujumbura city underperformed (1.2 new visits) in 2021.

Quality indicators provide practical guidelines for meeting quality standards in the healthcare delivery system. The guidelines are used to attribute quality scores to contracted healthcare providers. These providers must receive at least a 70% quality score in order to receive financial incentives for meeting quality standards in healthcare service delivery. Following quality standards contributes to meeting the health needs of the population, resulting in increased credibility and trust on the side of users. For example, the 2021 PBF report indicates that trust in healthcare facilities increased the use of immunization services, resulting in 82% of target children being fully vaccinated nationwide by 2021.

There are also indicators used for assessing perceived quality or the level of satisfaction by healthcare service users. Those indicators serve as a reference to measure the level of acceptability of the national package of healthcare services in each health facility. According to our professional experience, users frequently suggest lowering the costs of laboratory tests. It means that those tests are financially unaffordable. This informs the need for price reductions in health facilities, expanding financial coverage to a larger group of the population, or revising the amount of user fees for the health insurance schemes.

Based on the arguments presented above, we endorse the idea that healthcare verification serves as a fraud and abuse mitigation intervention in the quality healthcare delivery system.[28] Each healthcare system is expected to have more than 10%[29] fraud and abuse, as well as 20-40% wasted resources.[23] While it promotes efficiency in healthcare service reimbursement by allowing payment for only quality care services, healthcare verification in Burundi has proven to be a resilient practice to reduce fraud, abuse, and waste[30] in both the demand for and supply of healthcare services. During monthly verification visits, healthcare verification experts provide technical advice to healthcare providers about the health data reporting system in order to enforce data accuracy improvements that inform payment of the supply of healthcare services (or health facilities). In the demand for healthcare services, the healthcare verification protects health insurance schemes from bankruptcy that may arise from the overconsumption of healthcare services. According to Sun *et al*, verification can detect and avoid approximately 70% of wrong healthcare services usually claimed for reimbursement in healthcare delivery systems.[28]

To increase healthcare providers' accountability towards health insurance schemes, healthcare verification in Burundi includes financial sanctions in case of claiming wrong data and financial incentives when obtaining at least a 70% of quality score.[31] The payment amount for each health facility is determined by the volume of quality healthcare services provided and the number of users of those services. Since prices of healthcare services vary from one province to another, findings from healthcare verification inform price adjustments among provinces for equity in resource allocation. That said, the level of annual budget consumption per health facility per province informs the next budget per capita to mobilise, and the total amount to pool annually for each province. Although healthcare verification appears promising for moving toward UHC implementation, it has limitations that must be alleviated in order to make further progress.

Limitations of Healthcare Verification

Healthcare verification focuses on targets and acts on a limited set of indicators. As a result, healthcare providers focus their efforts on paid indicators and change their behaviours based on the services that will be assessed and the amount of awards attached to targets. In terms of quantity, for example, providers prioritise high-priced indicators over unpriced or low-priced ones. In terms of quality, providers prioritise indicators with high grades and neglect those with low grades. Progress in UHC implementation is limited to contractual indicators, and our professional experience has shown that it is difficult to estimate real targets for each indicator in health facilities. The use of three different population reference estimates (520,252 or 535,491 inhabitants according to the 2021 PBF report, and 488,867 inhabitants according to the 2021 yearbook of health statistics) for calculating targets in Bururi province is a good illustration of this wrong estimation of targets in the healthcare delivery system in Burundi.

A serious concern is that policymakers frequently define healthcare service packages without considering how the target population will receive and accept them in each region of the country. To illustrate this further, community actors who could assist in informing the acceptability of healthcare services in various regions of the country face some challenges: (i) Health Committees, which are supposed to represent the population in decision-making about the primary healthcare delivery system, continue to be manipulated by healthcare providers. As a result, they are concentrating their efforts on assisting providers rather than representing the population; (ii) PBF uses community-based organisations to assess the cost-acceptability of healthcare services. However, they represent a small number of users (80 per health facility) and cannot inform change; (iii) Community Health Workers lack robust policy support to carry out their mission. Sekhon *et al* suggested that wrong targets combined with unacceptability of healthcare services widen disparities in healthcare service access.[32]

Another critical issue is the limited technical effectiveness of the healthcare verification team. The team is integrated into the supply side of the health system and is over dependent on provider's guidelines. Too often, the team faces adversarial pressure from the health system's authorities (health district or province officers for

example) to tolerate errors (incomplete recording of clinical data for example) when billing or scoring healthcare services. As verification became routine for the team, regular visits in health facilities have increased familiarity between the team and the health staff, which does not help to sanction some observed imperfections in healthcare service claiming or reporting. The 7% average discrepancy (documented by the 2022 survey on verification cost-effectiveness) observed between routine, extemporized, and counter-assessment findings exemplifies this situation.

One more criticism is the use of unrealistic indicators to verify healthcare services for some health facilities, which results in under or over scoring of quality indicators. Maternity services, for example, are not available in some health facilities and are therefore scored as inapplicable during the verification process. Furthermore, according to the PBF implementation tools (especially healthcare verification grids), quality assessment requires at least 26 reports of activities or meetings per month per health facility. Our professional experience has taught us that healthcare providers fabricate fictive reports to obtain quality score without making any improvements. Since the indicators are defined in such a way that only the availability of well-established reports is required (what we refer to as unrealistic indicators), providers are often given a high quality score that does not reflect reality.

These critics raise concerns about the payment efficiency of healthcare services. This is why some policymakers are thinking about direct health facility financing and the use of supportive supervision teams of health districts for healthcare verification to increase efficiency. Direct financing refers to funds received in health facility bank accounts from the government, donors, or other sources via output-based payment.[33] These funds are managed independently by health facilities to meet the needs of the population in their respective catchment areas. There are no national standardised indicators guiding verification in this form of financing. Independently, health facilities choose to focus on indicators that are relevant to their local context. This means that, health facilities develop healthcare service packages in their own context based on the needs of the local population.[33] It is important to keep in mind that in the context of Burundi, supportive supervision is a crucial component of the provision of healthcare services. Using the supportive supervision teams of the health districts for healthcare verification could lead to more conflicts of interest between the provision and the purchasing functions of the health system. One of the most important ways to implement UHC, in our viewpoint, is to separate the demand for and supply of healthcare services. In this way, linking capitation with PBF has shown positive effects on maintaining quality standards in practice,[34] and on reducing fraud, abuse, and waste, which may result in increased healthcare payment efficiency.

WAY FORWARD

1. Combining PBF with capitation payment (pre-payment based on the expected volume of healthcare services consumption per year, per inhabitant, and per health facility) for a risk-based verification. The following are five proposed steps to follow:

Step 1: Inviting the target population of the catchment area of each health facility to a mandatory electronic enrolment for health insurance (Territorial-based registration).

Step 2: Provide the enrolled people with an electronic health insurance card with a unique identification number. The card could be presented to the healthcare provider holding a recognised professional identity number in case of healthcare service demand. This could help the healthcare provider automate the verification of the patient's identity.

Step 3: Based on the analysis of the history of verification findings, it may be possible to estimate per capita per year, the budget to virtually allocate to each health facility for healthcare service provision while taking into account a certain proportion (capped) of patients who may come from outside the catchment area – this is to ensure that the population's right to choose the preferred healthcare provider is not violated.

Step 4: Training healthcare providers to understand that the capitated budget has been virtually allocated (but not transferred) for one-year coverage. Consuming the entire budget before the end of the year would result in a shortage of healthcare provision payments for the rest of the year (for greater provider accountability). The amount to be paid monthly for provided healthcare services could be calculated during the verification process.

Step 5: Establishing a risk-based or targeted verification system (not systematically based on all health facilities).[20] To put this into practice, there are two tenets. First, quantity verification based on financial risk for cost-control: verification visits could focus on health facilities whose historical data show a tendency to consume the capitated budget before the due date. Second, verification based on the risk of no or low quality care in order to maintain an acceptable level of quality care in health facilities. To stimulate improvements, verification visits could target health facilities with a persistently low quality score (less than 50% for example). A bonus (financial incentive) could be given to healthcare providers who maintain a quality score of more than 70% after three successive verification processes (pay-for-qualitative performance).

2. Improving healthcare verification techniques by making it an intersectional function between the existing fragmented health financing schemes

To succeed in this coalition for healthcare verification, all the existing health financing schemes need: (i) a harmonised basic healthcare services package, (ii) the same target population and eligibility criteria, and (iii) harmonised pricing and payment methods.

The advantages of pooling healthcare verification techniques include reduced operational costs and the risk of payment duplication, resulting in increased payment efficiency. (Figure 2)

3. Removing the healthcare verification function from the supply side of the health system in order to make it independent and to mitigate the adversarial relationship between the provision and purchasing functions in the health system (Figure 3)

FIGURE 2: Pooling Verification Techniques

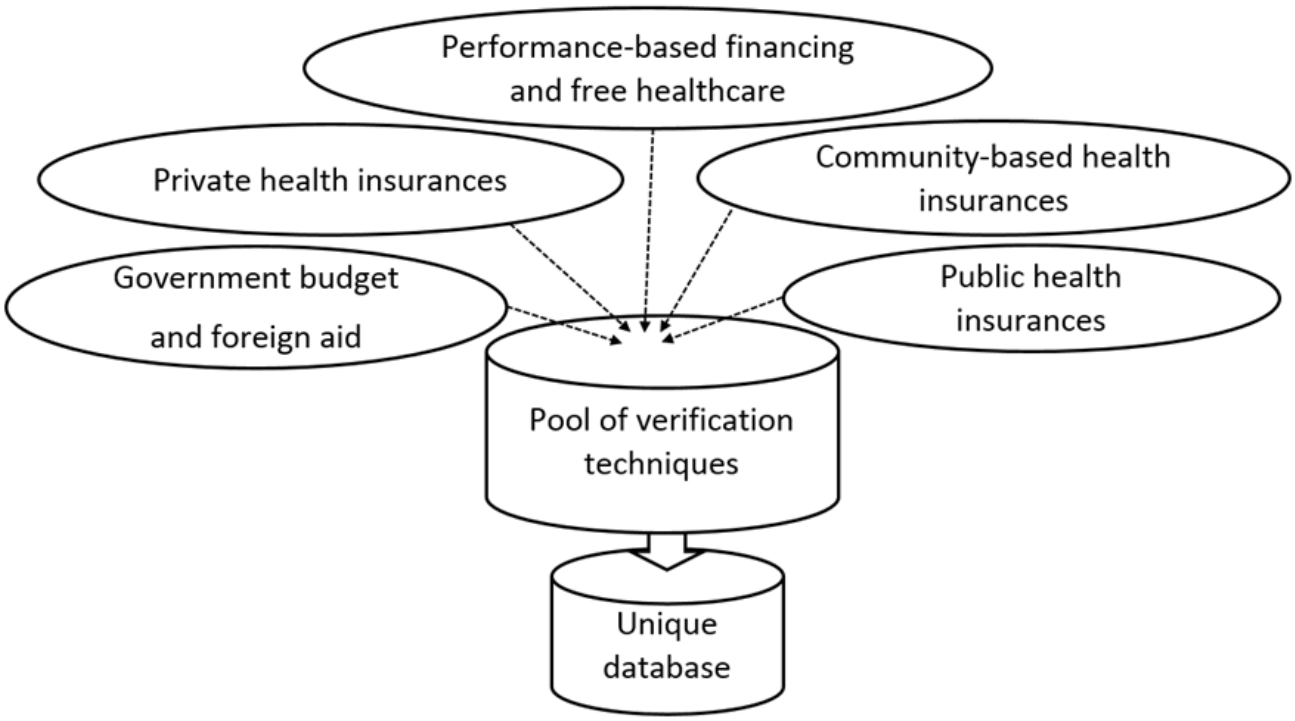
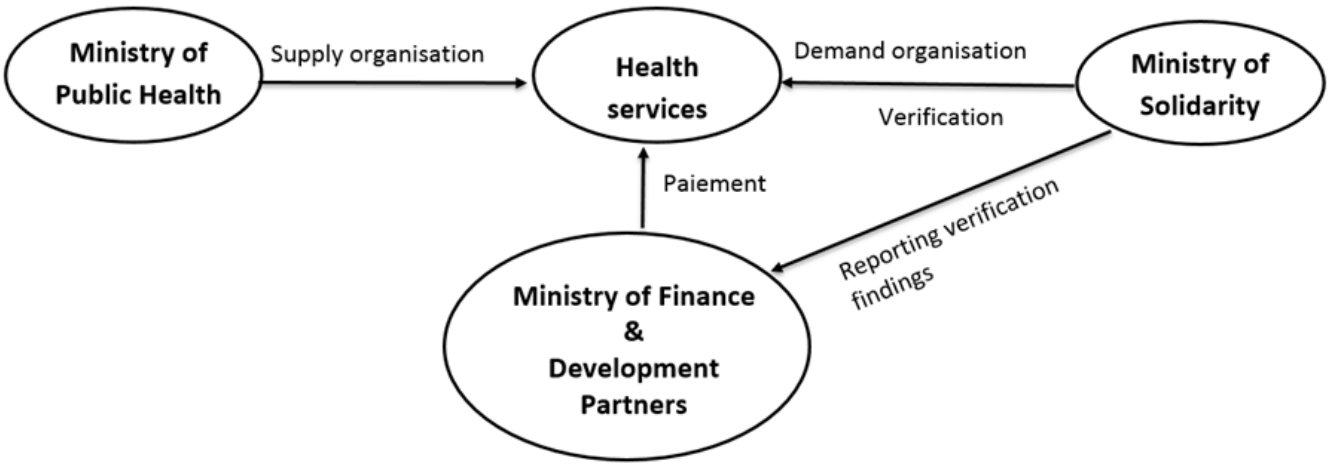


FIGURE 3: Proposed Institutional Design of Healthcare Verification in Burundi



As the authorised organiser of the demand for healthcare services in Burundi, the Ministry of Solidarity should secure the verification function.[35]

The Ghanaian model, the most advanced African country in strategic purchasing of healthcare services for UHC, is worth considering in Burundi. The package of healthcare services to pay is nationally defined by act, healthcare provision standards are defined by the Ministry of Public Health as the organiser of the supply of healthcare services, and the accredited national authority for health insurance enforces those standards through a contract-oriented verification system.[36]

The advantages of this power balance between supply and demand for healthcare services include the separation of institutional functions in purchasing healthcare services, which results in an impartial verification system and, thus, resilience to fraud, abuse, and waste.

CONCLUSION

This study has argued that when properly implemented, healthcare verification facilitates visualising a country's coverage situation and informing timely interventions that are required to advance UHC implementation. More investments in research are required to recognise healthcare verification as an essential sub-function of health financing for UHC implementation. Before getting there, setting a global agenda for healthcare verification would be an expert solution for mutual understanding and the development of this function, which is currently under-exploited.

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