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Photos top to bottom: (1) Liquid filing and sealing machine at Siphar Pharmaceutical, Bujumbura, Burundi ; (2) Equipment used in the production of drugs at Medipharm Industries at Bweyogerere, Kampala, Uganda and (3) EAHRC, Young East African Research Scientists Forum at Jakaya Kikwete Cardiac Institute, Dar es Salaam, Tanzania

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COMMENTARY

Corona Virus Disease 2019 Pandemic Contributed to Pregnancy Devastating Outcomes in Low Income Countries

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ABSTRACT

Corona Virus Disease 2019 (COVID-19) pandemic has been a public health threat of the 21st century. This pandemic has unexpectedly occurred, and countries have faced challenges to implement the preventive strategies against this unexpected killer. Pregnancy is a critical state among women, and special care should be provided to prevent pregnancy related complications as early as possible. COVID-19 pandemic has restricted services provided to pregnant women due to some prevention measures and treatment programs. Previous studies reported the high increase of obstetric complications among women infected or ever infected by COVID-19. Depression, suicidal intention, low quality of life during pregnancy, gestational hypertension and gestational diabetes mellitus, the premature rupture of membranes, miscarriage, preterm delivery, edema, maternal death, and hypoxia and other respiratory conditions were observed among women infected by COVID-19. Strategies for protecting pregnant women during pandemics should be enhanced to prevent pregnancy related complications and maternal death. There should be home health care nurses and midwives working with community health workers to assist pregnant women at home. Governments shoulddevelop policies and plans about maintaining maternal and child health during pandemics requiring travel ban and other prevention measures.

INTRODUCTION

At the end of December 2019, an outbreak of Corona Virus Disease was declared in Wuhan, China. As its spread was quick, the outbreak of the disease became a worldwide pandemic, declared by the World Health Organization (WHO) on March 11, 2020.[1]Corona Virus Disease, clinically occurs as severe pneumonia like symptoms with high fever and uncontrolled body weakness, was given a name of 'coronavirus disease 2019' (COVID-19) by WHO. COVID-19 is caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) invading the human vascular and respiratory systems.[2] The virus can be transmitted via droplets and air.[3]

Up to date, COVID-19 has contributed tolow living standards and 6,078,162 people havelost their lives from 470,783,178 cases that occurred worldwide.[4]In Africa, the first case was reported in Egypt on February 14, 2020. It was speculated that Africa would be the most affected continent due to the lack of standardized health infrastructure, human resource, and poverty.[5] However, the prevention measure that were put in place like lockdown, contact tracing, and COVID-19 testing contributed to the control and prevention of the epidemic.[6]

As the spread of COVID-19became critical, both high income and low and middle income countriesstruggled to put in place preventive measures including social distancing, the shift from face to face conferences to virtual conference, testing, screening, mask wearing, use of hand sanitizers and lockdown.[7]However, low income countries were directly forced to go to lock down to prevent the high spread of the pandemic which could lead to the failure of the control of the pandemic.[8]COVID 19 Pandemic has impacted important health services including maternal and child health services that contributed to adverse maternal and child health outcomes when delayed.[9]

Low and middle-income countries faced the double burden of the preparedness of COVID 19 policy prevention and increasing maternal and child health service during the pandemic. Maternal and child health care is still critical in low and middle income countries and not accessible for millions of women.[10]COVID 19 pandemic is likely to have contributed to the three main delays associated with adverse maternal and neonatal outcomes. These delays include, delay in the decision to seek care because of COVID-19 prevention measures that required permission in some cases,delay in arrival at a health facility because of the transport problem,since-

motorcycles, bicycles, and cars were all banned, and the delay in obtaining adequate treatment because hospitals and health centres were busy dealing with COVID-19 patients. Travel ban was a barrier to the work of community health workers on maternal and child health, and could leadto the negative maternal health outcomes.[11]

Pregnancy is a critical period and may expose womento the development of severe clinical conditions after coronavirus invasion due to pregnancy relatedimmunosuppression.[12]COVID-19 has contributed to the high fatality rate and other pregnancyrelated complications.[13]In 2004, during SARS pandemic, the rate of complications and deaths werehigh among pregnant women compared to non-pregnant women.[14] Similarly, adverse health complications were reported among women in gestation during the pandemic of H1N1influenza in 2009.[15]

The Centre for Disease Control and Prevention (CDC) has recommended that there should be rigorous precautionary measures to protect pregnant women against the COVID-19 pandemic due to their relatively low immunity that cannot deal with pregnancy and infections.[16,17] Previousstudies reported that there is an association between pregnancy immune-suppression and increaseof adverse health outcome when infected with viruses.[18]

Pregnancy outcomes during COVID-19 Pandemic

Previous studies about perinatal health during COVID-19 focused on pregnancy outcomes associated with SARS-CoV-2. The pregnancy outcomes such as caesarean section[19], foetal distress, preterm birth[20], and maternal death were observed among women infected by SARS-CoV-2 during pregnancy.[21]Researchers have investigated the negative consequences of COVID-19 pandemic on the mental health of pregnant women and foetal outcomes.[22]The fear of COVID-19 was reported to be associated with depression, suicidal intention, and the low quality of life during pregnancy.[23]

Other studies reported the high obstetric complications among pregnant women including few maternity places due to many COVID-19 patients in hospitals, high risk of pregnancy complications, and other maternal and neonatal outcomes. The COVID-19 pandemic contributed to gestational hypertension and diabetes mellitus (GDM)[24], and premature rupture of membranes among women.[25] During the pandemic, a higher admission rate of women was reported in intensive care unit compared to the period before the pandemic occurred. During lockdown, increased institutional stillbirth rate was reported across the world.[26]

Some studies reported the pregnancy outcomes associated with the COVID-19 pandemic. The study conducted by Li et al. reported four pregnant women out of seven with SARS-CoV-2 who faced spontaneous miscarriages.[27]In the Second/third-trimester, some studies reported that pregnant women with SARS were exposed to higher rate of maternal mortality.[14] Serious complications like miscarriage, preterm delivery, and small for gestational age neonates have been observed.[28]Systemic infections and inflammatory states contributed to preterm delivery among COVID-19 virus infected women. Pregnant women infected by COVID 19 virus should be highly considered for the mode and timing of delivery to avoid the comorbidity related obstetric complications.[29]

Challenges facing pregnant women in low-income countries during COVID-19 pandemic

Some countries in Africa have not established the new modalities that pregnant women can attendpregnancy related health care particularly intrapartum care that requires a period of hospitalization. Some other countries with good health maternal care system were not prepared because the pandemic occurred suddenly.[30] There was a dilemma of how safe deliverywas at the hospital during the pandemic and how possible the COVID-19 prevention measures such as wearing a face mask during the active phase of labour, hand washing, and social distancing could apply in the labourrooms.[31] This has contributed topregnancy related complications caused by the delay in seeking care resulting from the fear of the unknown source of SARS-CoV-2 transmission,lack of accessing care because of the national lockdowns and the delay in care provision resulting from the lean staff at the health facility maternity units.[32]

The women are required to attend antenatal care regularly at health centres, and hospitals under qualified and skilled health care professionals. This routine antenatal care became sharply curtailed since COVID 19 pandemic eruptedwhen most countries were not prepared to fight against it, which affected the foresight on essential care including pregnancy, intrapartum and postpartum care for mothers from pregnancy to childbirth. The fear of SARS-CoV-2 infection have been a barrier to pregnant women to attend antenatal care, and no special counselling and guidelines have been provided to prepare them and reassure them of their safety and that of their unborn baby.[33] If women skip the routine antenatal care, the aims of care during pregnancy are grossly affected. The aim of antenatal care includes the early detection and treatment of pregnancy related complications. Examples of such complications include urogenital tract infections, toxaemias of pregnancy, communicable and non-communicable disorders of pregnancy, and obstetric conditions complicating pregnancy and birth such as haemorrhage, obstructed and prolonged labour that could predispose to intrauterine infections.[34]

Community health workers provide home based care for pregnant women, but following the lockdown, they have not been authorised to carry out home visiting, to prevent the spread of SARS-CoV-2 infection.[35] Pregnant women have developedpsychological outcomessuch as depression, anxiety, and distress resulting from the lack of pregnancy related counselling during COVID-19 pandemic.[36] The nutrition of a pregnant woman is hampered by food supplies at the family unit, and if the head of the family is in employment that has been suspended temporarily due to COVID-19Pandemic, it may have contributed to nutritionalanaemia among pregnant women which predisposes them to preterm labour, low birth weight, maternal malnutrition, and weight loss which is a cause of early pregnancy loss.[37] Pregnant women need social support, motivation, and s-

ecurity. During lockdown, it has not been possible for pregnant women to receive social support which contributed to stigma among this group with critical health conditions.[38]

Staying at home contributes to poor circulation and persistent lower limb oedema among pregnant women, which is a cause ofadverse pregnancy outcomes including a poor quality of life related to discomfort due to lack of exercises and simple movements.[39] Pregnant women need good aeration and physiologically take deeper and more breaths to maintain normal oxygen circulation for themselves and the growing foetus. The fact that they sometimes suffer from breathing conditions, there are no special masks provided to help them maintainoxygenation without feelings of suffocation especially during the labour intensive second stage of labour.[40]

Masks could reduce the amount of oxygen that enters the lungs, and this could be the cause of negative respiratory health outcomes among pregnant women due to hypoxia and subsequent respiratory acidosis. Persistent low-level oxygen exposes women to congenital birth defects similar to the risk of smoking cigarettes during pregnancy. Persistent foetal hypoxia predisposes to complications such as microcephaly and mental retardation in the new born.[41] There was no special transport support provided by health facilities for emergency cases of pregnant women which could lead to maternal death due to interventional delays as discussed earlier.[42]

Evidence of the rise of pregnancy devastating outcomes during COVID-19 pandemic

During COVID-19 pandemic, the increase of pregnancy devastating outcomes was observed. In Nepal, the study conducted on the effect of the COVID-19 pandemic response on intrapartum care, stillbirth, and neonatal mortality outcomes reported the increase of institutional stillbirth rate that changed from 14 per 1000 total births before lockdown to 21 per 1000 total births during lockdown, intrapartum foetal heart rate care decreased by 13·4%.[26] In South Africa, Thrombocytopenia and lymphocytopenia was reported among 9% and 15% of the women, respectively.[43] Some other studies reported a comparative pregnancy outcomes where 14.7% maternal deaths occurred among COVID-19 admitted women, which is 8 times women (1.8%)admitted for other health outcomes.[44]

During this pandemic, adverse mental health outcomes among pregnant women were observed. In a study carried out on mental health outcomes in Nigeria among pregnant women, thesevere and extremely severe depression wereobserved in 7.2% (n=33) and 6.4% (n=29) of women, respectively. The study also reported that 3.3% (n=15) and 7.7% (n=35) of participants experienced severe and extremely severe anxiety, respectively. About 23% (n=105) of the participants have developed severe stress while 16.7% (n=76) experienced extremely severe stress.[45] A qualitative study carried out in Kenya reported the high maternal outcomes among womenrefugees. There was an increase of home delivery, and the delay of antenatal and post-natal services among refugees.[46] A study carried out in Nigeria reported that more than 10% pregnant women admitted for COVID-19 had difficulty in breathing, while 54.6% had caesarean section.[47]

In Romania, a study reported that 78.8% parti-cipants (n=439) mental affected by the pandemic. About 45.8% had the fear that their pregnancy could be affected by the virus.[48]

THE WAY FORWARD

Governments of low-income countries should establish policies to support pregnant women in sudden situations like COVID 19 pandemic. Phone based counselling could be provided during lockdown, health education could be emphasised especially on how pregnant women should behave during lock down. This can be provided through the ministry of health trough radio and television programs. Community health volunteers should be allowed to work closely with pregnant women in their community units to make sure that pregnant women are safe during the lockdown. Special care should be provided for pregnant women especially for mask wearing with the healthcare worker having to wear a double mask and allow the woman to take deep breaths that maintains oxygen saturation levels within normal particularly during labour.

Community midwifery or health visiting by nurses and midwives is a practice that has a chance for revival as health care systems leverage on the COVID 19 restriction whereby the provider meets women in their households to prevent their unnecessary travel to the hospital which can expose them to COVID 19 infection, hypoxia and other nosocomial infections.

Health visiting also improves the ability for personalised care and early diagnosis of conditions that are risky for the mother and foetus. If home visiting becomes a viable option, then home based nurse or midwife should be tested for COVID 19 before they make the home visit. Nutritional support should be emphasized for pregnant women during lockdown. Some families have suffered from poor nutrition even before COVID 19 pandemic in least developed countries. This is because COVID 19 pandemic has complicated the way of food availability and the supply chain for essential food commodities at the dining table. This has exposed pregnant women to poor nutrition and poor birth outcomes. Families need health education for decision making personal preferences and choices on contraception, pre-conception care, pregnancy, and labour care during lockdown for better reproductive outcomes.

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CASE STUDY

Tuberculous Meningitis Presenting with Stroke in an Immunocompetent Adolescent: A Case Report

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ABSTRACT

Background: Tuberculous meningitis (TBM) is a severe infection of the central nervous system that has high mortality.The disease predominantly affects young children and those who are immuncompromised. Strokes have been reported in about one-third of children with tuberculous meningitis and are associated with poor clinical outcomes.

Case report: A previously healthy 14-year-old girl living in Dar es Salam, Tanzania presented with one month history of weight loss associated with weakness, loss of appetite, apathy; without respiratory symptoms. Anti-TB therapy was started, based on radiological findings of the chest which showed multiple patchy centrilobular nodules with linear branching pattern bilaterally, mediastinum lymph node enlargement with punctate calcification. She then became aphasic and developed right-sided hemiparesis. Brain imaging showed infarction, hydrocephalus and meningeal enhancement. Diagnosis of tuberculous meningitis (TBM) with left sided ischaemic stroke was made and dexamethasone was added to her regimen. Treatment and rehabilitation was continued for 12 months with minimal improvement.

Conclusion: Tuberculous meningitis can present with non-specific features and has grave outcomes. Stroke is an uncommon complication in older immunocompetent children and results in severe morbidity. A high index of suspicion is required in adolescents with neurological symptoms that can be confused with behavioural symptoms.

INTRODUCTION

Tuberculous meningitis(TBM) in children is a deadly disease with a mortality rate of up to 30% in severe cases and about 50% of surviving patients have some form of neurological sequelae despite adequate -anti-tuberculous treatment.[1] Because of their relative inability to contain primary tuberculosis infection in the lungs, children younger than 5 years are more frequently affected by tuberculous meningitis. Risk factors including poverty, malnutrition, overcrowding, a compromised immune system and living in an endemic area are similar to those of pulmonary TB. It is very rare in immunocompetent patients.

Lymphohematogenous spread of the mycobacteria to the brain from the primary focus in the lungs results in the development of a Rich focus. This caseous granuloma ruptures into subarachnoid space leading to basal inflammatory exudates that cause cranial nerve palsies and obstruct cerebrospinal fluid pathways resulting in hydrocephalus and obliterative vasculitis which triggers infarctions.[1]

Signs and symptoms of TBM vary from non-specific symptoms such as headache, fever, vomiting and neck stiffness to more progressive meningitic symptoms such as focal neurologic deficits, with cranial nerve palsies (50%) and limb weakness (10%).[2]

Stroke is a common complication of TBM and has been reported in 13-57% of patients. [3] It is a poor prognostic predictor and about 1 in 5 patients with stroke die within 6 months of diagnosis. [4]

The diagnosis and management of TBM is challenging, particularly in low resource settings because the pathogenesis of the disease is still poorly understood. In addition, there are no rapid, sensitive and affordable tests to diagnose TBM.[5] Neuroimaging findings are non-specific and include:basal meningeal enhancement, hydrocephalus, tuberculomas and infarcts. [5]
Unlike pulmonary TB, the optimal therapy of TBM has not been established in clinical trials yet.[6]A study analysis done from nine trials showed that use of corticosteroids in patients with TBM reduced the risk of death by a quarter at two months to two years after the treatment was initiated.[7]

We report a case of TBM with stroke in an immunocompetent adolescent female. A summary of the timeline of events is provided in Table 1.

CASE REPORT

A 14- year-old girl of South Asian origin living in Tanzania for the past 8 years; who had been vaccinated with bacilleCalmette-Guérin (BCG) vaccine in infancy presented with a one month history of subjective gradual weight loss associated with loss of appetite, weakness, decreased activity and subjective fever. She also reported severe pain in her limbs. However, she had no history of cough, difficulty breathing, headache or night sweats. She had been to several outpatient clinics and was being treated for helicobacter pylori-associated gastritis with no relief. As the illness progressed, she developed extreme weakness and was not able to ambulate. She also had changes in her speech and personality where she would respond only with words and would respond selectively to the mother only.

Her past medical history was unremarkable with no previous hospital admissions or surgical procedures. She had normal growth and developmental milestones.
On examination, she was found to be weak, severely cachexic and pale with no palpable lymph nodes. She had no signs of respiratory distress. Her central nervous system findings were unremarkable except for the poor verbal response. The rest of her examination was normal.

A diagnosis of probable tuberculosis was made with a differential diagnosis of autoimmune disease and hematologic malignancy.

A complete blood count showed anaemia with haemoglobin of 6.5g/dl (range: 10.5-14.5g/dl) with microcytic hypochromic red cell indices. White blood cell count was normal at 5.63 $x10^9$/l (range: 4.5-13.0 $x10^9$/l), absolute neutrophils count - 4.93$x10^9$/l (range: 1.78-5.38 $x10^9$/l) and lymphocytes - 0.37$x10^9$/l (range: 0.4 - 4.0 $x10^9$/l). Peripheral smear revealed anisopoikilocytosis with target cells, there were no abnormal or immature cells seen.

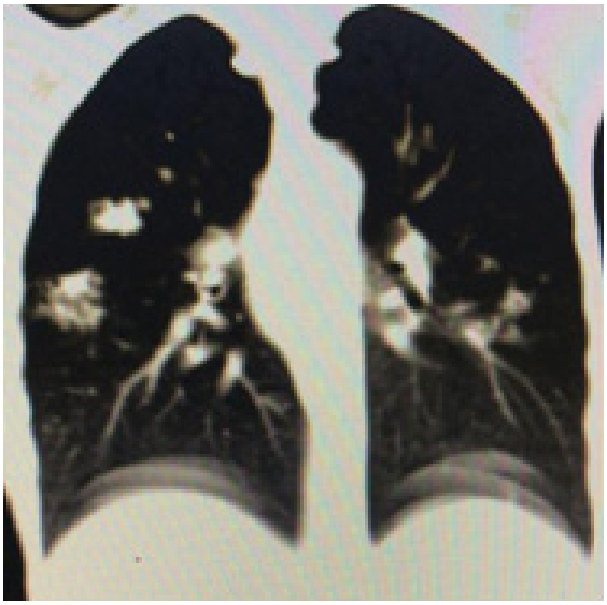
C reactive protein (CRP) was elevated at 91.5mg/l (normal: 0.5 – 5.0mg/l). Electrolytes and renal function were normal except for hyponatremia with sodium of 123mmol/l (range: 135-145mmol/l).

Liver functions showed severe hypoalbuminaemia - 18g/l (range: 35 – 48g/l) with normal liver enzymes.
Autoimmune disease workup was done and both anti double-stranded DNA (anti dsDNA) and antinuclear factor (ANA) were negative.

A chest x-ray showed multiple patchy micro lobular consolidations bilaterally and a subsequent Chest Computerised Tomography (CT) scan showed multiple patchy centrilobular nodules with linear branching pattern bilaterally, mediastinum lymph node enlargement with punctate calcification, highly suggestive of pulmonary tuberculosis. (Figure 1). Further history revealed open TB contact with an aunt, 2 years before moving to Tanzania and was not put on post-exposure prophylaxis.

A diagnosis of Pulmonary TB was made with severe acute malnutrition and anti TB drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) were initiated as per the national guideline together with nutritional rehabilitation provided by parenteral nutrition. No active management with iron supplement or blood transfusion was done for anaemia, as it was attributed to chronic inflammation.

FIGURE 1. A chest CT showed multiple patchy micro lobular consolidations bilaterally



Over the course of 3 days, she showed clinical improvement; she started feeding orally, started ambulating and gained 1.5 kg. Her repeat CRP was 11.1mg/l and Hb: 7.2g/l. However, despite her clinical improvement, her regular speech did not return and she remained disinterested and was thought to have depression.

On the 4th day, she suddenly became more lethargic, completely aphasic, had reduced oral intake and began drooling saliva. She was then referred to a higher level of care for further workup and possible intensive care unit (ICU) admission.

Upon review after referral, she was lethargic with a Glasgow coma score of 8/15, appeared cachexic, and moderately pale with a normal pulse (103 beats per minute), respiration (18 breaths per minute) and blood pressure (110/83). Her weight was 21kg which was less than the 3rd centile for age. On neurologicexamination, she had a stiff neck with bilaterally equal and reactive pupils and hemiparesis of the right upper and lower limb. She had an impaired gag reflex. Respiratory, cardiovascular and abdominal exam findings were unremarkable.

Magnetic resonant imaging (MRI) and magnetic resonant angiography (MRA) of the brain with contrast was performed which revealed subacute ischemic infarct affecting the left basal ganglia as well as the insular cortex with left frontoparietal leptomeningeal enhancement (figure 2). Hence a diagnosis of tuberculous meningitis with left-sided subacute ischemic infarct was reached. The MRA showed complete occlusion of the M2 segment of the left middle cerebral artery and its branches (figure 3). The gastric aspirate was taken for gene Xpertwhich detected Mycobacterium tuberculosis sensitive to rifampicin; however, gram stain for acid-fast bacilli (AFB) was negative. Her HIV test was also negative.

TABLE 1: Timeline of Key Events, Diagnostic Tests and Interventions

	Summary from initial and follow up visit	Diagnostic testing	Intervention
Day 1	1 month history of gradual weight loss, increasing weakness, loss of appetite, limb pain and selective mutism. On examination: pale, cachexic, poor verbal response and unremarkable systemic exam. **Diagnosis:** severe acute malnutrition and pulmonary TB	Haemoglobin 6.5g/dl, White blood cell count 5.63 x109/l, C reactive protein 91.5mg/l sodium 123mmol/l Peripheral smear: anisopoikilocytosis with target cells, albumin 18g/l chest x-ray and CT suggestive of pulmonary TB	Initiation of anti TB drugs (rifampicin, isoniazid, pyrazinamide & ethambutol)
Day 4	Became lethargic, aphasic and drooling saliva. On exam: Glasgow score 8/12, lethargic, cachexic, stiff neck, impaired gag reflex, hemiparesis of the limbs on the right. Diagnosis: tuberculous meningitis with subacute ischaemic infarct.	MRI and MRA of the brain: subacute ischaemic infarct of the left basal ganglia. Gastric aspirate: mycobacterium tuberculosis sensitive to rifampicin. HIV test: negative	Referral to higher centre & admission in ICU. Continue with anti TB and pyridoxine. Initiation of dexamethasone and lamotrigine. Speech & physiotherapy with nutritional rehabilitation.
Day 9	Significant improvement with some weight gain	Albumin: normal Haemoglobin: 6.4g/dl	Discharged home on 1 year dose of anti TB, tapering dose of oral prednisolone & rehabilitation.
Follow up after 1 year	Still aphasic with right-sided weakness and stiffness on physiotherapy and baclofen. Dependent on caregivers for daily activities.		

She was admitted to ICU, started on lamotrigine 12.5mg (0.6mg/kg/day) for seizure prophylaxis and dexamethasone 4mg 8 hourly (0.5mg/kg/day) was initiated to reduce cerebral oedema. A neurosurgical consult was sought and non-surgical conservative treatment was advised. Her anti TB drugs were continued and 20mg daily of pyridoxine was added. Occupational, speech and physiotherapy was initiated on the following day of admission. Nutritional rehabilitation was continued with high-calorie nasogastric tube feeds.

She showed gradual improvement and on the 9th day was discharged with the weight of 22.1kg, her electrolytes had normalized, albumin was normal though she still had anaemia (Hb: 6.4g/dl). She was on oral prednisolone 2mg/kg per day to be taperedfor over a month, anti- TB drugs for 1 year and to continue with rehabilitation.

On follow up after completing her anti-TB treatment, she remains aphasic; she still has stiffness and weakness on the right side of her body and is on physiotherapy and using baclofen. She has not been able to return to school and is still dependent on caregivers for daily activities.

DISCUSSION

Tuberculous meningitis has been commonly reported in children in countries with a high burden of TB. However, the peak age of occurrence is common in children under the age of 5 years.[1] It is rare in older children with no underlying immunosuppression. Our patient was a 14 y-

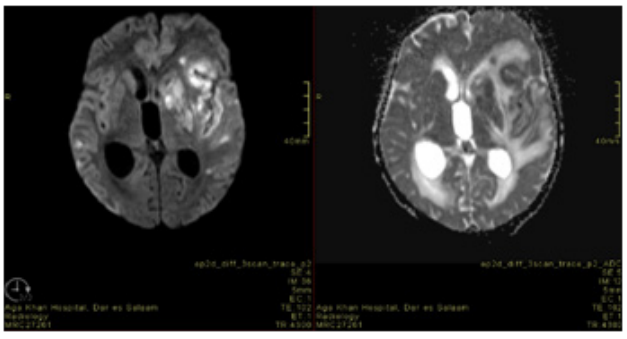
ear-old female with no underlying medical history and hence TBM with a stroke was an unexpected occurrence.

The initial presentation of TBM is usually with non-specific symptoms like fever and headaches. As the disease progresses, they may present with signs of raised intracranial pressure, cranial nerve palsies and neurologic deficits.[1] Our patient presented initially with non-specific symptoms of weight loss, poor appetite and limb pain without any pulmonary symptoms for more than a month. This led to a delay in diagnosis of TB and subsequently, consideration of other diagnoses such as autoimmune disease and hematologic malignancy in addition to tuberculosis.

She subsequently showed speech disturbance with poor verbal response and changes in her personality. This was initially presumed to be a behavioural or emotional problem. Behavioural problems are common in adolescents with chronic illness and girls with chronic illness have been found to be more at risk of emotional disturbances.[8] Her symptoms were only attributed to being neurological when she developed further neurological signs like reduced level of consciousness and drooling. The delay in recognition of the neurological signs and symptoms also led to further diagnostic delay of TBM.

The common triad of neuroradiological findings in TBM are: Basal meningeal enhancement, hydrocephalus and infarctions in the supratentorial brain parenchyma and brain stem.[5] Tuberculosis related infarcts commonly occur in the "TB zone" supplied by the medial lenticulostriate and thalamoperforating arteries. However, other studies have also described infarcts in multiple areas and involving perforators as well as cortical branches.[3] Our patient had an infarct involving the basal ganglia which is the commonest site of infarction in TB.

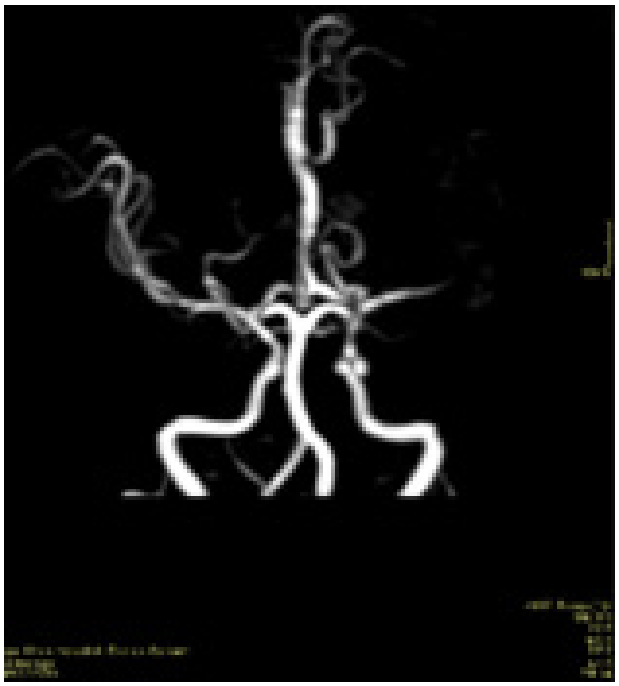
FIGURE 2. Magnetic Resonant Imaging (MRI) of the Brain



Arterial occlusion in TB usually involves the terminal branches and the complete occlusion of the M2 segment of the middle cerebral artery that was seen in our patient is a rare finding. Our patients' neuroimaging also did not demonstrate the vasospasm and vasculitis that occurs frequently in TB associated strokes.

Despite adequate anti TB treatment and supportive care, our patient did not make a complete recovery and had significant residual neurological symptoms. This is similar to what is described in the literature with only about 35 % of patients making a complete recovery. Poorer outcomes are described in those diagnosed with late stages of disease.[1]

FIGURE 3. magnetic Resonant Angiography (MRA) of the Brain



CONCLUSION

TBM in children remains a devastating disease, associated with substantial morbidityand mortality. The diagnosis of TBM remains challenging for clinicians especially in a resource-limited setting, mainly due to difficulties in the directdetection of *M. tuberculosis* bacilli in CSF and other specimens. The non specific nature of initial symptoms also contributes to diagnostic delay.A high index of suspicion is required for early diagnosis and treatment in countries with high burden of TB irrespective of age and immune status; more so in adolescents where neurologic symptoms can be confused with behavioural problems.

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EAHRC
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ORIGINAL ARTICLE

Knowledge, Attitude and Practices of Hand Hygiene among Students and Nurses Staff in Mwanza Tanzania: A Cross-Sectional Hospital-Based Study during Global COVID-19 Pandemic

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ABSTRACT

Background: Hand hygiene (HH) is a critical component of infection prevention and control (IPC) which aims at preventing microbial transmission during patient care hence reducing the burden of healthcare associated infections (HCAIs). Information on the level of HH knowledge, attitudes and practices among healthcare workers (HCWs) from low- and middle-income countries is scarce. This study determined knowledge, attitude and practices of HH among students and staff nurses in Mwanza, Tanzania.

Methods: This cross-sectional hospital-based study was conducted between August and October 2020 among student and staff nurses from 2health centres, 2district hospitals, 1regional referral hospital and 1zonal referral hospital. Self-administered pretested structured questionnaires were used for data collection. All data was transferred to Microsoft excel spreadsheet for cleaning and coding, then to STATA software version 13.0 for analysis.

Results: A total of 726 nurses aged 18 to 59 years with median (IQR) age of 29(24-38) years were enrolled. About 3 quarters 76.4% (555/726) of nurses had good level of knowledge on HH as most of them 88.3% (641/726) had received rigorous IPC trainings during COVID-19 pandemic. About 42.0% (305/726) of the participants reported that, the action of HH was effortless. Majority of the participants, 81.1% (589/726) practiced hand washing more than hand rubbing routinely. Being a student nurse [OR: 0.30, 95%CI: 0.21-1.44, p<.001], working in inpatient department [OR: 0.38, 95%CI: 0.27-0.55, p<.001], high level of education i.e., degree and above [OR: 1.74, 95%CI: 1.36-2.24, p<.001] and having working experience of 5 years and above [OR: 2.41, 95%CI: 1.52-3.82, p<.001] was associated with being knowledgeable of HH.

Conclusion: Majority of the participants had good level of knowledge on HH because they had received rigorous training on IPC, notably HH during the global COVID-19 pandemic.

INTRODUCTION

Hospital Acquired Infections (HAIs), also known as nosocomial infections or health care-associated infection (HAIs) are defined by the World Health Organization (WHO) as infections occurring in a patient during the process of care in a hospital or other health care facility, which were not present or incubating at the time of admission. This includes infections acquired in the health care facility, but appearing after discharge, and also occupational infections among Health Care Workers (HCWs) of the facility.[1, 2]The acquisition of an infectious agent causing HAI is aided by 3 factors, namely; 1) source of the organism e.g., contaminated hospital environment, 2) presence of a susceptible host e.g., patient with impaired anatomical barriers and 3) mode or vehicle of transmission of the infectious agent to susceptible host i.e., contaminated hands of healthcare workers (HCWs).[3]Most HAIs can be prevented by simple measures of Infection Prevention and Control (IPC) such as Hand Hygiene (HH).[3] Practicing HH by using alcohol-based hand rub for 20 to 30 seconds or hand washing with running clean water and soap for 40 to 60 seconds is very effective against pathogens causing HAIs including Multidrug Resistant (MDR) pathogens.[4] Alcohol-based hand rub is recommended when hands are not visibly soiled while washing hands with running clean water and soap is recommended when hands are visibly soiled with blood or other body fluids.[4] In 2009, the World Health Organization (WHO) introduced the 5 moments of HH in healthcare facilities.[4] These include; 1. before touching a patient; 2. before clean/

aseptic procedure, 3. after body fluid exposure/risk; 4. after touching a patient; and 5. after touching patient surroundings.[4] These moments of HH aims at preventing the transmission of pathogens between patients, from patients to HCWs and vice versa, from patients to hospital environment and from contaminated hospital environment to patients.[4]

In most low- and middle-income countries, the level of knowledge as well as attitude and practice of HH among HCWs is reportedly poor. Inadequate HH facilities and lack of adequate and appropriate training are the major factors contributing to poor knowledge, attitude and practice of HH among HCWs.[5] In Nigeria, about 55.8% and 68.8% of HCWs washed their hands before patients' palpations and giving injections, respectively.[6] In Ethiopia, about 65.9% of HCWs are reported to be knowledgeable about HH and 56% have poor practices of HH.[7] In Tanzania, Wieden mayer *et al*., reported a compliance of 56.1% and 30.5% to HH practices among healthcare workers in healthcare facilities with and without HH interventions, respectively.[8] Wieden mayer *et al*., proved that whenever IPC trainings i.e., HH is offered among HCWs, there is always a room for improvement, definitely reducing the burden of HAIs.[8] We therefore hypothesised that, the level of HH knowledge, attitudes and practices among HCWs improved dramatically during the global COVID-19 pandemic. This is because, during the COVID-19 pandemic, HH was among employed strategies for preventing the spreading of the virus known to cause COVID-19. Therefore, this study determined the knowledge, attitude, and practices of hand hygiene among students and nurses in 6 healthcare facilities in Mwanza, Tanzania.

METHODS

Study Design, Duration, and Settings

This was a cross-sectional, hospital-based study conducted from August to October 2020 in primary, secondary, and tertiary healthcare settings in Mwanza, Tanzania. Primary healthcare facilities included 2 health centres; secondary healthcare facilities included 2 District Hospitals; and tertiary healthcare facilities included 1 Regional Referral Hospital and 1 Zonal Referral Hospital. The estimated number of nurses(employees) in each Health Centre was 20 and 14 in the 2 health centres; 125 and 87 in the 2 District Hospitals; 163 in Regional Referral Hospital, and 513 in Zonal Referral Hospital respectively. Student nurses pursuing bachelor's degree in Nursing practice their clinical subjects in tertiary healthcare facilities (Regional Referral and Zonal Referral Hospitals).

Study Population, Sample Size Estimation and Selection Criteria

The population of this study comprised mainly nurses (staffs and students in clinical rotations) because it's the nurses that mostly provide medical care services (e.g., samples collection, giving injections and cleaning of wards/clinics) to patients both in wards and clinics. Therefore, they possess the major risk of transmitting pathogens between patients, and patients and contaminated hospital environment resulting to patients' acquisition of HAIs. The minimum sample size for this study was 384, obtained by using Kish Leslie formula (1965) and a prevalence of 50%. We used a prevalence of of 50% to calculate the sample size because there was no similar study conducted in Tanzania before. All nurses (staffs and students in clinical rotation) in the selected healthcare facilities who were on practice and consented to participate in this study were enrolled. A total of 750 nurses were enrolled in the study but 24 were excluded due to incompleteness of their data collection tool. Therefore, only 726 participants were considered for final data analysis.

Sampling Procedure and Data Collection

Simple random sampling method was used, whereas participants in the respective healthcare facilities under this study were enrolled consecutively until overall sample size was met. Due to unequal staffing levels of the selected study sites, the distribution of enrolled participants is not equal.

Data Collection, Management, and Analysis

Self-administered pretested structured questionnaire was used for data collection. The questionnaire was sectioned into 4 parts namely; PART A: socio-demographic questions, PART B: comprised with 15 knowledge-based questions, PART C: comprised with 7 attitude based questions, and PART D: comprised with 10 practice-based questions. For knowledge based questions, scores were expressed in percentages and for each correct answer one point was awarded. To get percentages, points earned from correct answers by participant were dived by total points the participant was supposed to earn and then multiplied by 100%. Thus, the level of knowledge on HH among participants was categorised into good knowledge (>75%), moderate knowledge (50%-74%) and poor knowledge (<50%) as reported in previous studies.[9]

All data were transferred to Microsoft excel spreadsheet for cleaning and coding and then to STATA software version 13.0 for analysis. Percentages and fractions were used to present categorical data while median (interquartile range: IQR) were used to present continuous data. Chi square and logistic regression analysis were simultaneously performed to determine the association between categorical outcome i.e., knowledge level on HH and categorical predictors i.e., socio-demographic data. To facilitate analysis of association between categorical outcome and categorical predictors, 2 levels of knowledge (poor and moderate) were categorised to "not knowledgeable" while good knowledge was categorised to "knowledgeable". A p-value of less than .05 at 95% Confidence Interval (CI) was considered statistically significant.

Ethical Considerations

This study was approved by the joint CUHAS/BMC Research Ethics and Review Committee (CREC) with certificate number: 1590/2020. Permission to conduct this study was sought for from the administrations of the respective healthcare facilities. All participants were requested to sign informed written consent forms before being enrolled into the study. To ensure that confidentiality is observed throughout the study, unique identification codes were used to identify participants as opposed to use of participants' names. During data collection, physical distancing, wearing of face masks and use of alcohol-based sanitisers were observed to prevent

the possible spreading of COVID-19.

RESULTS

Socio-Demographic Characteristics of Study Participants

A total of 726 nurses aged between 18 to 59 years with median [IQR] age of 29[24-38] years were enrolled to the study. The majority of participants were females (60.6%; n=440), working in the inpatient departments (83.5%; n=606), enrolled from tertiary healthcare settings (81.7%; n=593), and staff nurses (56.2%; n=408) with working experience of more than 5 years (75.2%; 307/408).(Table 1).

Levels of knowledge on HH among Nurses

More than 3 quarters (76.4%; 555/726) of the study participants had good knowledge on HH and had received training on HH(88.3%; 641/726) during the COVI-19 pandemic from March to May 2020 prior to the study. Two thirds (68.5%;497/726) of the participants reported patient exposure to colonised surface and another two thirds (66.7%; 484/726) of respondents reported germs already present on patient body as the main route of cross-transmission and infections. The majority of participants acknowledged that, HH actions prevent transmission of germs to patients if well practiced: before touching the patient (96.1%; n=698); immediately after a risk of body fluid exposure (91.2%; n=662); after exposure to immediate surroundings of a patient (87.2%; n=638); and immediately before a clean/aseptic procedure (90.4%; n=656). The majority of respondents reported that HH protects HCW from pathogens when it is practiced: after touching a patient (93.8%; n=681); immediately after a risk of bodily fluid exposure (92.1%; n=668); immediately after a clean/aseptic procedure (100%; n=726); and after exposure to the immediate surroundings of a patient (89.7%; n=651). Most of the participants (79.9%; n=580) admitted that, if you touch the patient's environment you have essentially touched the patient.

On the other hand, only a quarter (25.3%; 184/726) and nearly two quarters (60.1%; 436/726) of nurses correctly answered the minimal time needed for alcohol-based hand rub (20-30 seconds) and for hand washing with water and soap (40-60 seconds), respectively. However majority (87.7%; 637/726) of the participants reported that, hand washing should be done using water and soap. Most of the nurses agreed that, wearing jewellery (91.1%; n=661), damaged skin (100%, n=726), artificial finger nails (92.2%; n=669) and regular use of hand creams (82.8%; n=601) should be avoided as they increase the likelihood of becoming colonised with harmful germs. Majority of the nurses also admitted that watches/bracelets (93.5%; n=679) and rings (91.5%; n=664) should be removed; wrist (95.3%; n=692) should be washed; and all cuts/lacerations (83.2%; n=604) should be covered with waterproof dressing during hand washing, and that hands (96.6%; n=701) need to be dried after hand washing(Table 2).

The Attitudes of Nurses (Staffs and Students in Clinical Practices) towards Hand Hygiene

More than one-third (42.0%; n=305) of the participants said it requires no effort to practice good HH while more than a quarter (29.2%; n=212) said it requires big efforts to practice good HH. More than half (56.6% 411/726) of the participants said that they require no reminders so as to practice HH. The rest (43.4%; 315/726) who requires a reminder, the majority of them (63.5%; 200/315) said the availability of posters is enough to remind them to practice good HH. On the other hand, more than half of the nurses believed that; hand rubbing is more rapid for hand cleansing than hand washing (53.4%; n=388), hand rubbing causes skin dryness more than hand washing (50.3%; n=365), hand washing is more effective against germs than hand rubbing (54.4%; n=395), and that hand washing and hand rubbing should not be performed in sequence (53.2%; n=386). Furthermore, more than half (53.2%; 386/726) and nearly three quarters (74.2%; 539/726) of the respondents reported that, the use of gloves damages the skin and that the purpose of HH is to prevent transmission of infections from patients to HCWs, respectively (Table 3).

TABLE 1: Socio-Demographic Characteristics of Study Participants

Variables	Frequency (n)/ median (IQR)	Percentages (%)
Median age [IQR] in years	29 [24-38]	N/A
Gender		
Females	440	60.6
Males	286	39.4
Status		
Student nurses	318	43.8
Staff nurses	408	56.2
Facility		
Primary healthcare	32	4.4
Secondary healthcare	101	13.9
Tertiary healthcare	593	81.7
Education level		
Certificate	128	17.6
Diploma	353	48.6
Degree and above	245	33.8
Profession		
Nurse	103	14.1
Midwife	10	1.4
Nurse and midwife	274	37.7
Intern nurse	20	2.8
Student nurse	318	43.8
Department		
Outpatient departments	120	16.5
Inpatient departments	606	83.5
Working experience of staff nurses		
<5 years	101	24.8
>5 years	307	75.2

The Practices of Nurses (Staffs and Students in Clinical Practices) on Hand Hygiene.

More than three quarters (81.1%; 589/726) of the respondents routinely practiced hand washing than hand rubbing. Further, majority of the respondents (97.5%;-

TABLE 2: The Level of Knowledge on HH Among Study Staff Nurses and Student Nurses in Clinical Practices

Question	Response	Frequency (n)	Percentage (%)
Did you receive formal training in HH in the last three years?	Yes	641	88.3
	No	85	11.7
Which of the following is the main route of cross-transmission of potentially harmful germs between patients in a health-care facility?	Health-care workers hands when not clean	159	21.9
	Air circulating in the hospital.	30	4.1
	Patients exposure to colonised surfaces (i.e. beds, chairs, tables, floors)	497	68.5
	Sharing non-invasive objects (i.e. stethoscope, pressure cuffs etc.) between patients.	40	5.5
What is the most frequent source of germs responsible for health care-associated infections?	The hospitals water system	48	6.6
	The hospital air	33	4.6
	Germs already present on or within the patient	484	66.7
	The hospital environment(surfaces)	161	22.2
Which of the following HH actions prevents transmission of germs to the patients?			
Before touching the patient	Yes	698	96.1
	No	28	3.9
Immediately after a risk of body - fluid exposure	Yes	662	91.2
	No	64	8.8
After exposure to the immediate-surroundings of a patient	Yes	638	87.9
	No	88	12.1
Immediately before a clean/aseptic-procedure	Yes	656	90.4
	No	70	9.6
Which of the following HH actions prevents transmission of germs to the health-care worker?			
After touching a patient	Yes	681	93.8
	No	45	6.2
Immediately after a risk of bodily-fluid exposure.	Yes	668	92.1
	No	58	7.9
Immediately before a clean/aseptic-procedure	Yes	726	100.0
	No	-	-
After exposure to the immediate-surroundings of a patient	Yes	651	89.7
	No	75	10.3
If you touch the patient's environment you have essentially touched the patient	True	580	79.9
	False	146	20.1
What is the minimal time needed for alcohol-based hand rub to kill germs on your hands?	20 seconds	184	25.3
	3 seconds	156	21.5
	60 seconds	206	28.4
	10 seconds	180	24.8
What is the correct duration for hand washing with water and soap?	20-30 seconds	245	33.8
	40-60 seconds	436	60.1
	90 seconds	16	2.2
	120 seconds	29	3.9

Continued

TABLE 2: Continued

Question	Response	Frequency (n)	Percentage (%)
With what you wash your hands?			
	Only water	14	1.9
	Water with soap	637	87.7
	Water with ash	71	9.8
	Others	4	0.1
Which of the following should be avoided, as associated with increased likelihood of colonization of hands with harmful germs?			
Wearing jewellery	Yes	661	91.1
	No	65	8.9
Damaged skin	Yes	726	100
	No	-	-
Artificial finger nails	Yes	669	92.2
	No	57	7.85
Regular use of a hand cream	Yes	601	82.8
	No	125	17.2
Watch and bracelet should be removed during hand washing?	Yes	679	93.5
	No	47	6.5
Rings should be removed during hand washing?	Yes	664	91.5
	No	62	8.5
Wrist should be washed during hand washing?	Yes	692	95.3
	No	34	4.7
Hands need to be dried after washing?	Yes	701	96.6
	No	25	3.4
All cuts and lacerations shall be covered with a waterproof dressing.	True	604	83.2
	False	122	16.8
Level of knowledge on HH	Poor (scored <50%)	0	0
	Moderate (scored 50-74%)	171	23.6
	Good (scored >75%)	555	76.4

708/726) changed gloves when serially contacting different patients, of whom (91.5%; 664/726) practiced HH before putting on gloves to attend to the next patient. Moreover, almost all respondents (98.7%; 716/726) practiced HH after removal of gloves, however they believe gloves protects them from contamination. In the following situations: -before palpation of the abdomen, more than 3 quarters of nurses preferred hands rubbing (78.1%; 567/726); before giving an injection, more than half of the respondents preferred hands washing (52.6%; 382/736); after emptying a bedpan, more than 3quarters of respondents preferred hands washing (88.8% 645/726); after making patients' bed, more than half of the respondents preferred hands washing (58.9%; 428/726); and after visible exposure to blood, more than 3quarters of respondents preferred hands washing (81.3%; 590/726). About 94.5% (686/726) of the respondents reported that HH facilities are always available at their duty stations, while majority 90.7% (658/726) reported that hands washing facilities are always available compared to alcohol-based hand rub facilities. However, only 61.2% (444/726) of the respondents practiced hand washing correctly in terms of the minimum time (40-60 seconds) one should take while washing their hands while only a quarter 26.6% (193/726) practiced hand rubbing for correct minimal time (20-30 seconds). Lastly, about 93.8% (681/726) declared that, adherence to hands hygiene standards are discussed during staff orientations and handovers (Table 4).

Factors Associated with Knowledge level on Hand Hygiene among Staff and Student Nurses in Clinical Practices.

Being a nurse student [OR: 0.30, 95%CI: 0.21-1.44, *p<.001*], working in an inpatient department [OR: 0.38, 95%CI: 0.27-0.55, *p<.001*], with a degree in formal education or above [OR: 1.74, 95%CI: 1.36-2.24, *p<.001*] and being a staff nurse with working experience of >5 years [OR: 2.41, 95%CI: 1.52-3.82, *p<.001*] was associated with being knowledgeable on HH among nurses (Table 5).

TABLE 3: The Attitudes of Nurses (Staffs and Students in Clinical Practices) Towards Hand Hygiene

Question	Response	Frequency (n)	Percentage (%)
What effort is required for you to perform good HH?	0 (No effort)	305	42.0
	1	151	20.8
	2	27	3.7
	3	31	4.3
	4 (A big effort)	212	29.2
Do you need reminder to perform HH practice at various point of care?	No	411	56.6
	Yes	315	43.4
If yes (from the above question), who/what do you want to remind you to perform HH?	Posters	200	63.5
	In-charge/Matron	32	10.2
	Demonstration	83	26.3
Which of the following statements on alcohol-based hand rub and hand washing with soap and water are true?			
Hand rubbing is more rapid for hand cleansing than hand washing	False	338	46.7
	True	388	53.4
Hand rubbing causes skin dryness more than hand washing	True	365	50.3
	False	361	49.7
Hand rubbing is more effective against germs than hand washing	True	331	45.6
	False	395	54.4
Hand washing and hand rubbing are recommended to be performed in sequence	True	340	46.8
	False	386	53.2
Does the use of gloves damage your skin?	Yes	386	53.2
	No	340	46.8
What is the reason for you to practice HH?			
To prevent contact of COVID-19		45	6.2
To prevent transmissions of infections from patients to you		539	74.2
To prevent transmissions of infections from you to patient		142	19.6

DISCUSSION

This is the first study on HH knowledge level, attitude and practices among nurse (staffs and students in clinical practices) during the global COVID-19 pandemic in this region. This study found out that majority of the participants were female nurses with working experience of more than 5 years, working in inpatient departments and enrolled from tertiary healthcare settings. These findings are similar to studies conducted in Tanzania[8] and Nigeria,[6] before the global COVID-19 pandemic. Majority of the participants were females because of the nature of the profession (nursing) being preferred mostly by females. Majority of the participants were staff nurses because few students are enrolled to pursue Bachelor of Nursing in the few Medical Universities available in the country and only senior students were eligible for clinical rotations. Furthermore, majority of the nurses were working in inpatient departments, this may be because the department requires significantly a higher number of work force (HCWs) to take care of hospitalised patients. As student nurses were excluded from working experience, majority of staff nurses had experience of more than 5 years. Lastly, majority of the nurses were enrolled from tertiary healthcare facilities because of the large bed capacities of these hospitals coupled with large number of patients attended to per day compared to lower tier (primary and secondary) healthcare facilities. For example, a Regional referral hospital has about 350 bed capacity while Zonal Referral hospital has over 950 beds capacity (https://www.bugandomedicalcentre.go.tz/index.php?bmc=1). Also, student nurses practice their respective clinical subjects in these tertiary healthcare settings and this also increased the number of participants enrolled from the tertiary healthcare facilities.

Three quarters of nurses enrolled in this study had good level of knowledge on HH contrarily to a study by Wiedenmayer *et al*, which was conducted before the global COVID-19 pandemic in the same region in Tanzania[8]. The higher level of knowledge on HH among nurses in this study can be attributed to the fact that, nearly 9 out of 10 nurses enrolled in this study received formal training on IPC during the global COVID-19 pandemic from March to May 2020. The IPC training package received by HCWs included but not limited to hand hygiene, contact precaution and use of Personal Protective Equipments (PPEs). It was evidenced in a study by Wiedenmayer

TABLE 4: The practices of nurses (staffs and students in clinical practices) on Hand Hygiene

Question	Response	Frequency (n)	Percentage (%)
Do you routinely use an alcohol-based hand rub or hand washing with soap and water?	Hand rub	137	18.9
	Hand washing	589	81.1
Do you change gloves when contacting different patients?			
	Yes	708	97.5
	No	18	2.5
If yes (from the above question), do you practice HH before putting on gloves for the next patient?	Yes	664	91.5
	No	62	8.5
Since gloves can prevent the contamination of the hands, do you always perform hands hygiene after taking off the gloves?	Yes	716	98.7
	No	10	1.3
Which type of HH method you may practice in the following situations?			
Before palpation of the abdomen	Rubbing	567	78.1
	Washing	140	19.3
	None	19	2.6
Before giving an injection	Rubbing	334	46.0
	Washing	382	52.6
	None	10	1.4
After emptying a bedpan	Rubbing	71	9.8
	Washing	645	88.8
	None	10	1.4
After making a patients bed	Rubbing	286	39.4
	Washing	428	58.9
	None	12	1.7
After visible exposure to blood	Rubbing	127	17.5
	Washing	590	81.3
	None	9	1.2
Are the HH facilities always available?	Yes	686	94.5
	No	40	5.5
If yes (from the above question), what facilities are always available?			
	Alcoholic hand rub	51	7.0
	Water and soap	658	90.7
	Water only	17	2.3
How much minimal time do you use to rub/sanitise your hands with alcohol-based hand rub?			
	20 seconds	193	26.6
	3 seconds	177	24.4
	60 seconds	161	22.2
	10 seconds	195	26.9
How much minimal time do you use for hand washing with water and soap?			
	20-30 seconds	253	34.9
	40-60 seconds	444	61.2
	90 seconds	11	1.5
	120 seconds	18	2.5
Is the adherence to HH standards discussed during staff handovers?			
	Yes	681	93.8
	No	45	6.2

et al.,[8] that provision of training on IPC i.e., HH among HCWs is proportion to their improved knowledge, practices and attitude. Therefore, IPC trainings i.e., may bring positive impact in minimising the emergence and spreading of HAIs as reported previously in a study conducted in Taiwan.[10] The good level of knowledge on HH among nurses in this study was evidenced through the results from a basic knowledge-based questionnaire administered to the participants. The questionnaire included questions such as; types of HH actions which

TABLE 5: Factors Associated with Knowledge level on HH among Staff and Student Nurses in Clinical Practices

Variable	Knowledge level		Chi-square analysis		Logistic regression analysis	
	Not knowledgeable	Knowledgeable	X2	p-value	OR[95%CI]	p-value
Gender Male	66 (23.1)	220 (76.9)				
Female	105 (23.9)	335 (76.1)	0.0569	0.807	0.96[0.67-1.36]	.807
Student nurses vs staff nurses						
Student nurse	40 (12.6)	278 (87.4)				
Staff nurse	131 (32.1)	277 (67.9)	37.8534	0.000	0.30[0.21-1.44]	.000
Level of education						
Certificate	44 (34.4)	84 (65.3)				
Diploma	91 (25.8)	262 (74.2)				
Degree and above	36 (14.7)	209 (85.3)	19.9760	0.000	1.74[1.36-2.24]	.000
Working experience of staff nurses						
<Five years	49 (48.5)	52 (51.5)				
>Five years	83 (27.0)	224 (73.0)	14.4214	0.000	2.41[1.52-3.82]	.000
Facility						
Primary (Level 2)	5 (15.6)	27 (84.4)				
Secondary (Level 3)	27 (26.7)	74 (73.3)				
Tertiary (Level 4 & 5)	139 (23.4)	454 (76.5)	1.6883	0.430	0.95[0.67-1.32]	.751
Department						
Inpatient	112 (18.5)	494 (81.5)				
Outpatient	45 (37.5)	75 (62.5)	28.2941	0.000	0.38[0.27-0.55]	0.000

prevents the transmission of germs causing HAIs between patients, patients and HCWs; removal of watches, bracelets, and rings during hand cleaning; and covering of all cuts and lacerations on HCWs' hands. Majority (>80%) of nurses got these knowledge-based questions correct. However, the minority who received no formal training on IPC measures; basically on HH (about 11%) and those who got wrong the knowledge-based questions in the administered questionnaire (~20%) should not be ignored. Thus, strategic trainings and retraining following assessments to determine improved level of knowledge on IPC measures, mainly HH is recommended at all levels of healthcare tiers in this region. Since they are not correctly knowledgeable, basically they maybe incorrectly practicing HH, hence, their hands may potentially act as vehicles in cross-transmission of harmful germs between patients, and patients and their immediate environments resulting to the emergence and spread of HAIs including MDR pathogens.

Nearly, half of the nurses enrolled in this study considers HH as an effortless action however the rest considered this action as laborious. The attitude of nurses to HH as a laborious action, may negatively affect effectiveness of HH compliance. A study by Engdaw *et al*., found that, positive attitude towards HH increases the likelihood of HH compliances.[10] Furthermore, at least one in two nurses believed that the presence of reminders viz., posters at their work stations will make them recall good HH practices. Multimodal interventional studies by Lam *et al*.,[11] and Alp *et al*.,[12] found that the use of reminders including posters increase compliance to HH among HCWs. In this study, more than half of the nurses believed hand rubbing using alcohol based agents is more rapid but it is not as effective as hand washing. Although, an experimental study by Ehrenkranz and colleague found that, alcohol-based hand rubbing is superior to hand washing in prevention of transfer of Gram-negative bacteria to catheters by the hands of HCWs.[13] However, WHO recommends hand washing whenever hands are visibly soiled with blood or other body fluids.[4] In this study, nurses believed that, hand rubbing causes skin dryness. This may happen when plain alcohol-based hand

rub is used. Therefore, the use of glycerol (humectant for skin care) supplemented alcohol-based hand rubs is recommended as reported by the WHO.[14] Moreover, more than half of nurses reported that, the use of gloves damage their skin. This happens when latex-examination powdered gloves are worn for a long duration, resulting into skin dryness and roughness. Also, hypersensitivity to natural rubber latex (NRL) have been reported.[15, 16, 17] The use of powder-free gloves (if possible) and the presence of petroleum jelly at all hand washing stations is recommended in cases of hypersensitivity to NRL or skin dryness and roughness, respectively.[16, 17] Lastly, about 3quarters of nurses think that HH aims at preventing transmission of infections from patients to HCWs. Therefore, they need to be updated on the risk of their hands acting as vehicles in cross-transmission of infectious agents (e.g., bacteria, fungi and viruses) between patients, and patients and their immediate environments as previous reported in "my five moments of HH" by the WHO.[4]

Majority of nurses in this study practiced hand washing than hand rubbing. A similar observation was reported elsewhere.[18] This may be because hand washing facilities are always readily available as reported by nurses in this study. However, participants' attitude that hand washing is more effective against harmful germs than hand rubbing may also explain why the majority of nurses prefer hand washing. Moreover, most of the nurses change gloves in between when attending to different patients and before put on gloves for the next patient, nurses practice hand cleaning. Nurses in this study may be knowledgeable that long duration of gloves wearing facilitates re-colonisation of hands as reported by Grasso s *et al*.,[19] and Wistrand *et al*.,[20]. Therefore, hand cleaning is recommended whenever gloves are removed. Further, during situations like palpation of the abdomen, giving an injection, emptying a bedpan, making of patient's bed, and visible exposure to blood, most nurses prefer to practice hand washing over hand rubbing as recommended by WHO.[21] This may be because facilities for hand washing are always available or due to the participants' attitude that hand washing is more effective than hand rubbing. Despite the fact that, the majority of nurses reported that HH standards are discussed during shift handovers, nearly a half and about one third of the participants practiced hand rubbing and hand washing in less than the recommended minimum time, respectively. Thus, frequent trainings and retraining on IPC measures i.e., HH are recommended to make sure the majority if not all nurses practice hand cleaning (rubbing and washing) correctly.

Finally, in this study we observed that, being a nurse student, working in the inpatient department, increased level of formal education i.e., degree and above, and being a staff nurse with working experience of >5 years was associated with being knowledgeable on HH among student and staff nurses. Similar observations were reported in previous studies.[22, 23] Student nurses had received training on IPC measures recently during their lectures as a part of strategy to prevent transmission of COVID-19 among University community and during clinical practices in wards or clinics. They were also more likely to adhere to clinical guidelines including HH as they are at all times under supervisions during their respective clinical rotations. Nurses working in the inpatient departments may have evidenced critical cases and outcomes of infectious diseases such as COVID-19 than those from outpatient departments. From this phenomenon, their alertness to seeking for more information on IPC measures including HH may have increased and definitely increasing their level of knowledge. Increasing level of formal education among nurses is proportional to having sufficient knowledge on infectious diseases, therefore increasing the likelihood of seeking further knowledge of infection prevention and control i.e., HH.[22] Lastly, as reported by Asadollahi *et al*.,[23] our study also observed that working experience of 5 years and above is associated with increased level of HH knowledge among the nurses which may be explained as they have received more trainings on IPC measures notably HH.

CONCLUSION

Majority of the nurses exhibited good level of knowledge about hand hygiene. This could have been enhanced through the extensive and frequent trainings on IPC notably HH during the global COVID-19 pandemic between March and May 2020.

We recommend that, HCWs are reminded on the correct duration required for effective HH because only two thirds and a third practiced hand washing and hand rubbing within recommended duration respectively. We also recommend further studies focusing on the compliance of HCWs on HH.

Study Limitations

Recall bias among study participants maybe a limitation of this study.

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ORIGINAL ARTICLE

Practice and Prevalence of Antibiotic Self-Medication among Undergraduate Students at Kilimanjaro Christian Medical University College, Tanzania

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ABSTRACT

Background: Antibiotic self-medication has been on the rise in different parts of the world. Antibiotic self-medication causes excessive antibiotic exposure to humans which is associated with many health risks including antibiotic resistance. The objective of this study was to assess practice and determine the prevalence of antibiotic self-medication among undergraduate students.

Methodology: This was a descriptive cross-sectional study conducted at Kilimanjaro Christian Medical University College. A self-administered questionnaire was used to assess the practice and knowledge of antibiotic self-medication among undergraduate students. A total of 300 undergraduate students were purposively sampled. The association between categorical predictors and antibiotic self-medication waspresented as Odds Ratios (OR) with 95% Confidence Intervals (95% CIs) using logistic regression.

Result: The prevalence of antibiotic self-medication among undergraduate students is 191(63.7%) with amoxicillin 103(53.9%) being the most used antibiotic for treatment of respiratory disorders 109(57.1%) and gastrointestinal disorders 50(26.2%). Pharmacy is the major source of antibiotics used for self-medication 165(86.4%). Delayed/queue in seeking hospitals services was the main reason for practicing antibiotic self-medication 74(38.7%).

Conclusion: The study observed a high prevalence of antibiotic self-medication among undergraduate students. This calls for immediate implementation of public health programs aimed at increasing awareness of consequences that may results from antibiotic self-medication. At the policy-making level, there is an urgent need to legislate and enforce laws restricting access to antibiotics in Tanzania.

INTRODUCTION

Self-medication with antibiotics is frequently practiced in many parts of the world and has been one of the major factors contributing to the development of antibiotic resistance. World Health Organization (WHO) defines self-medication as self-care based on the selection and use of antibiotics by individuals to treat self-recognised symptoms or illnesses.[1] Antibiotic self-medication has negative consequences as incorrect diagnosis with inappropriate treatment can lead to disease progression, life-threatening conditions[2], and increased emergence of resistant bacteria that would be challenging to eliminate.[3]

Undergraduate students in universities or colleges, who are the future health care workers, play a pivotal role in educating the community or patients on the advantages and disadvantages of self-medication.[4] Different studies have reported prevalence of antibiotic self-medication among undergraduate students in different parts of the world.[2,4–10] Antibiotic self-medication tendency is a common problem among healthcare college students during their junior years of study. This is due to their expanding awareness of diseases and therapeutics.[11] It has been reported that healthcare students usually practice self-medication based on their limited knowledge and as a result the prevalence of antibiotic self-medication has been reported to range from 45.8% to 77.1% in Ethiopia[12–16] and 38.8% to 92.3% in Nigeria.[17–19]

Studies in Kuwait[20] and Pakistan[4] have reported prevalence of up to 98% and 99% respectively. Moreover, factors such as easy access to medical guides, health writings, opinion from their colleagues and self-prescription are among the main drivers for self-medication among healthcare college students.[21]

In Tanzania, like in many other African countries, antibiotics are available in pharmacies (usually locate-

d in health facilities) and accredited drug dispensing outlets (ADDO). Antibiotics are more available to communities in the drug-specific retailers through the prescription and over-the-counter dispensing mechanisms.[22,23]

Several studies have been conducted elsewhere to highlight the problem of antibiotic self-medication in the general community[22,24,25], students[26,27] and children through their parents/caretakers.[28,29] There is limited data on antibiotic self-medication practices among undergraduate students in Tanzania. Therefore, there was a need to conduct this study to determine the prevalence of the problem and emphasise more knowledge and practice on antibiotic self-medication as it threatens public health. Hence, findings from this study will provide baseline findings that would help in formulating strategies for control of antibiotic consumption in the medical and non-medical communities.

MATERIAL AND METHODS

Study Area, Design and Population

A descriptive,cross-sectional study was conducted at Kilimanjaro Christian Medical University College (KCMUCo) from April to May 2017. KCMUCo is a private medical institution which is a constituent college of Tumaini University-Makumira located in Kilimanjaro, Tanzania. The main campus is located in the urban area of Moshi district. The study included both male and female medical students from Year 1 to Year 3. The study excluded year 4 and year 5 students because they were in their clinical rotations/ practice and thus were not readily available.

Sampling Method and Data Collection Tool Questionnaire

The questionnaire was adopted from Araia *et al.*,[30]. Non-probability (purposive) sampling technique was used to get the sample of 300 students. The students were approached during class hours and the questionnaires were self-administered. The questionnaire was set in English language and it composed of closed-ended questions. The questions were structured into subsections that guided data analysis and interpretation. The questionnaire was composed of 3 sections. Section 1 composed of socio-demographic information such as; sex, gender and year of study.

Section 2 composed of 12 questions. Participants were asked questions regarding their knowledge on self-medication. The following questions were asked; "Can self-medication be practiced in all illnesses? Do you think that self-medication is better than medical consultation? Can the same prescription be shared between two people having different complaints? Do you think that self-medication can result in harmful consequences,Do you think that self-medication can delay seeking medical advice? Do you think that antibiotic resistance is an outcome of self-medication without prescription and can self-medication lead to emerging of a new problem like new complaint?".

Section 3 composed of 14 questions regarding self-medication practice with antibiotics. Participants were asked which medical condition (s) assessment prompted them to self-medicate with antibiotics, what were the reasons for self-medication and the sources of the antibiotic they use(d).

Validity of the Questionnaire

To maximise validity, the questionnaire was pretested on relevant respondents before distribution. 10 students filled the questionnaire as a pilot study, and in-depth cognitive interviews were carried out to examine how the students understood and responded to the questions. In addition, 2 experts in the field of survey design approved the quality of the questionnaire. After the pretest, adjustments in phrasings were made so as to make the questionnaire simple to answer and yet give accurate and credible data.

Data Analysis Plan

Data was analysed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to summarise the data. The association between categorical predictors (sex, mode of entry, year of study and course) and antibiotic self-medication was presented as odds ratios (OR) with 95% Confidence Intervals (95% CIs) using logistic regression. Only one predictor was significantly associated with self-medication in the bivariate analysis (set to $p < .1$). Therefore, multivariate analyses were not performed. A $p < .05$ was significant.

Ethical Considerations

Ethical approval to conduct this study was obtained from KCMUCo ethical committee, Certificate Number 2473. Before administration of the questionnaire, written consent was sought from the participants. All measures to protect privacy and confidentiality were considered. Neither names nor students' registration number were mentioned during data collection and final publication.

RESULT

Socio-Demographic Characteristics of the Study Participant

A total of 376 questionnaires were administered, 300 undergraduate students filled and returned the questionnaire, giving a response rate of 80%. Among these 187(62.3%) were males and 113(37.7%) were females. The mean age was (Mean ± SD) 23.3 ± 2.6 years. 180(36.0%), 107(35.7%) and 85(28.3%) were in their 1st, 2nd, and 3rd year respectively, while 98(32.7%), 85(28.3%), 67(22.3%) and 50(16.7%) were in BSc laboratory Medicine, BSc Physiotherapy and BSc nursing classes respectively, Table 1.

Knowledge of Antibiotic Use Based On Antibiotic Self-Medication

Over 90% of the respondents were aware that self-medication should not be practiced in any illnesses and that seeking for medical consultation is the best treatment practice. Majority of the respondents knew that the same prescription cannot be shared between two people with different complaints. As expected, majority reported that antibiotic use for self-medication can lead to antibiotic resistance. Few respondents 16(5.3%) reported that self-medication can be practiced in all illnesses, 20(6.7%) reported that self-medication is better than seeking for medical consultation, and 19(6.3%) reported "no" to a statement that self-medication can result in harmful consequence. Only 6(2.0%) reported "no" to a statement

that self-medication can cause a delay in seeking medical advice, Table 2. All respondents 300(100.0%) had heard about antibiotics.

Antibiotic Self-Medication Practice

A total of 191(63.7%) respondents practiced antibiotic self-medication, 109 (57.1%) used antibiotics to treat respiratory disorder, 50(26.2%) gastrointestinal disorders, 24(12.6%) pain in case of injury, 6(3.1%) skin disease and 2(1.0%) fever. When asked about the reason for antibiotic self-medication, 74(38.7%) mentioned delayed/ queue in seekinghospitals services, 54(28.3%) emergency illness, 28(14.7%) said it is convenient, 16(8.4%) used their experience, 12(6.3) reported health facility being too far, lastly, 7(3.7%) reported that there is no medicine in the health facility.

Regarding the source of information/antibiotics for self-medication, 165(86.4%) got an opinion from a pharmacist, 11(5.8%) got an opinion from a friend, 6(3.1) called a doctor by phone satisfaction with the previous prescription and 5(2.6) used leftover treatment from a previous illness, Table 3. Among those who practiced antibiotic self-medication (191), when they were asked about the outcome of self-medication, 143(74.9%) reported that their condition improved while 2(1.0%) reported that their condition got worse. 13(6.8%) reported adverse reactions such as vomiting, dizziness and headache.

TABLE 1: Demographic Characteristics of the Studied Population (N=300)

Variable	n	%
Sex	187	62.3
Male		
Female	113	37.7
Mode of entry		
Direct from school	279	93.0
In-service	11	7.0
Year of study		
Year 1	108	36.0
Year 2	107	35.7
Year 3	85	28.3
Course		
Medicine	85	28.3
BSc Laboratory	98	32.7
BSc Nursing	50	16.7
BSc physiotherapy	67	22.3

Common Antibiotic Used for Sell-Medication

Antibiotics commonly used by undergraduate healthcarestudents are shown in Figure I. The most commonly used were amoxicillin 103(53.9%), followed by metronidazole 41 (21.5%), erythromycin 24(12.6%), doxycycline 12(6.3%), chloramphenicol 6(3.1%), and lastly tetracycline 5 (2.6%).

Association of Self-Medication with Socio-Demographic Characteristics

In the univariate analysis of predictors for self-medication, only one variable qualified for further analysis. Respondents in their 2nd year of study were more likely to self–medicate themselves with antibiotics as compared to 3rd-year students (OR = 2.68; 95% CI: 1.42 – 5.04). Since only one variable qualified for further analysis, the multivariable analysis was not performed, Table 4.

DISCUSSION

Currently, there are increasing reports of antibiotic resistance in different parts of the world[31], its prevalence is increasing and among the major drivers of resistance is antibiotic self-medication.[32] Equally, Self-medication is reported to be an increasing problem among medical and non-medical students.[33]

Though the inappropriate use of antibiotics as a result of self-medication is common worldwide, developing countries are most affected due to the higher prevalence of diseases and limited resources.[34] In this study, more than half of the respondents who practicedself-medication preferred amoxicillin 53.9%. Amoxicillin is from the penicillin group of antibiotics. The choice of antibiotics used by majority of the respondents demonstrates that these groups of antibiotics are more prone to misuse since they are readily available and at low cost. A big percentage of the respondents got their medicines from pharmacies 86.4%, more importantly, these antibiotics are the most used and prescribed by clinicians in the region.[35]A study conducted in Nigeria reported that antibiotic is the most frequently mis-useddrug among undergraduate students[17], specifically amoxicillin, which has been reported to be used for self-medication elsewhere.[18,35] Due to inappropriate treatment, there is probable risk of antimicrobial resistance as well as adverse events for individuals.Resistance to amoxicillin has been reported in the setting.[36,37] The inappropriate use of antibiotics in the area resulted in the observed resistance to antibiotics.

The prevalence of antibiotic self-medication in thisstudy (63.7%) is lower than that observed in a study conducted in the Democratic Republic of Congo which reported a prevalence of 73.4%.[38] Moreover, studies conducted in Sudan (79.5%)[2], Pakistan 76%[4], Palestine 98%[39] and Eritrea 79.2%[30] found that the prevalence of antibiotic self-medication among undergraduate students was considerably higher as compared to results from the present study. This could be attributed to their high knowledge aboutmedications and, consequently, think that their knowledge is adequate to practice self-medication[40], and that they do not need medical consultation.[41] In areas where self-medication is common among the general population, also undergraduate students have the same behaviours. In Tanzania, a prevalence of 58% was reported among the general population.[24] Therefore, the prevalence is generally similar. In Ethiopia, a study conducted among households showed the prevalence of self-medication to be at 50.2%.[42] In other settings such as Saudi Arabia, Ras Al-Khaimah, India and Pakistan, the prevalence was reported to be 35.4%, 52.1%, 69.6% and 84.4% respectively.[43–46]

The practice of self-medication is common among both the general population and students. Undergraduate students attempt to practice their acquired knowledge of pharmacology and related subjects and this has resulted

FIGURE 1: Common Antibiotic Used for Self-Medication

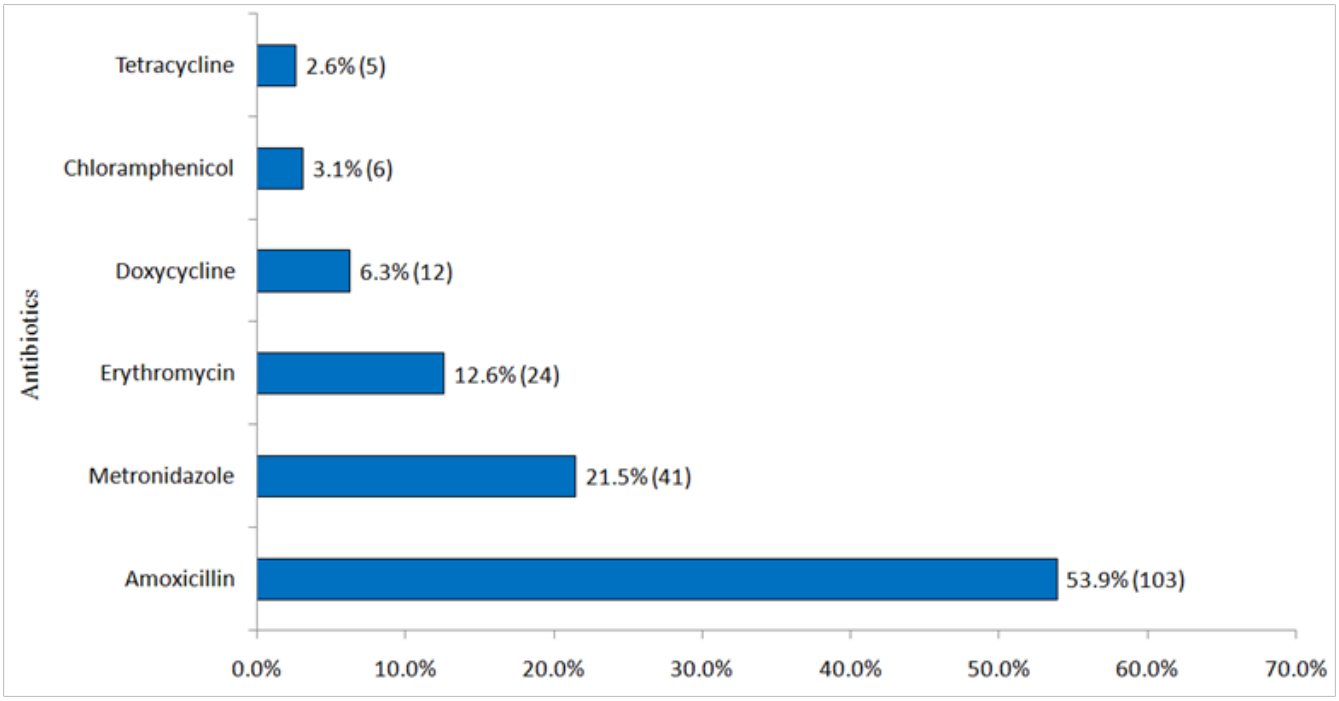

TABLE 2: Knowledge of Self-Medication (N=300)

Knowledge statement	Response	n (%)
Can self-medication be practiced in all illness	Yes No	16(5.3) **284(94.7)**
Do you think that self-medication is better than medical consultation	Yes No	20(6.7) **280(93.3)**
Can the same prescription be shared between two people having a different complaint	Yes No	16(5.3) **284(94.7)**
Do you think that self-medication can result in harmful consequences such as antimicrobial resistance	Yes No	**281(93.7)** 19(6.3)
Do you think that self-medication can delay seeking medical advice	Yes No	**294(98.0)** 6(2.0)
Do you think that a resistance is an outcome of self-medication without prescription?	Yes No I don't know	**295(98.3)** 3(1.0) 2(0.7)
Can self-medication lead to emerging of a new problem like the new complaint	Yes No I don't know	**296(98.7)** 2(0.7) 2(0.7)

Bolded responses are correct unless indicated otherwise

TABLE 3: Practice Regarding Self-Medication of Antibiotics among Undergraduate Students (n=191)

Variable	Response	n (%)
Common ailments leading to self-medication	Respiratory disorder	109(57.1)
	Gastrointestinal disorder	50(26.2)
	Injury	24(12.6)
	Skin disease	6(3.1)
	Fever	2(1.0)
Reasons for self-medication	Delayed/queue in seeking hospitals services	74(38.7)
	Emergency illness	54(28.3)
	Convenience	28(14.7)
	Experience	16(8.4)
	Health facility being too far	12(6.3)
	No medicine in the health facility	7(3.7)
Source of information/antibiotic	Opinion from a pharmacist	165(86.4)
	Opinion from a friend	11(5.8)
	Calling a Doctor/Satisfaction with the previous prescription	6(3.1)
	Leftover from a previous illness	5(2.6)
	*Media	4(2.1)

* Such as TV, Internet, newspapers, radio, promotions

TABLE 4: Association of Self-Medication with Antibiotics and Socio-Demographic Characteristics

Variable		Yes n(%)	No n(%)	COR (95% CI)	p-value
Sex	Male	117 (62.6)	70 (37.4)	1.00 (0.28-3.50)	>.05
	Female	74 (65.5)	39 (34.5)	Reference	
Mode of entry					
	Direct	176 (63.1)	103 (36.9)	0.68 (0.25-1.81)	>.05
	In-service	15 (71.4)	6 (28.6)1		
Year of study					
	Year 1	58 (53.7)	50 (46.3)	0.85 (0.48-1.51)	>.05
	Year 2	84 (78.5)	23 (21.5)	2.68 (1.42-5.04)	.002
	Year 3	49 (57.6)	36 (42.4)	1	
Course	Medicine	52 (61.2)	33 (38.8)	0.67(0.33-1.32)	>.05
	BSc Laboratory	61 (62.2)	37 (37.8)	0.70 (0.36-1.36)	>.05
	BSc Nursing	31 (62.0)	19 (38.0)	0.69 (0.32-1.50)	>.05
	BSc Physiotherapy	47 (70.1)	20 (29.9)	1	

COR: Crude odds ratio

in a higher prevalence of self-medication among undergraduate students of up to 98% in Kuwait[20] and 99% in Pakistan.[4]

Clinical Features and Self-Medication

The main reasons for self-medication reported by respondents in this study were delayed/queue in seeking

hospitals services, emergency illness and experience. Reasons for delayed/queue in seeking hospitals services could be due to the small number of health workers employed at the hospitals, late coming among the hospital employees, and corruption among health workers where these workers are bribed by well-off patients andplace their appointment cards before others.[47] Similar findings were observed in studies conducted in Ethiopia[5,48] and Pakistan.[4]

In this study, the most common health problems which lead to self-medication among undergraduate students were respiratory and gastrointestinal disorders at 57.1% and 37.1% respectively. This observation is in agreement with previous studies.[40,49–51] This study observed that respiratory disorders such as flu and cold were the most common ailment that provoked self-medication among the respondents. Consequences of illnesses such as; cold, flu and fever could be due to viral infections, and thus these conditions are usually wrongly treated using antibiotics.[52] This indicates inappropriate antibiotic use as treatment of viral infections. Such inappropriate antibiotic use leads to development of resistant microbes, increased treatment cost and adverse reactions. The rational use of antibiotics is thus of utmost importance to limit the increase in bacterial resistance.

Association of Self-Medication with Socio-Demographic Characteristics

Self-medication with antibiotics was observed with no significant association with socio-demographic characteristics variables such as sex as well as the mode of entry. Results show that students in year 2 were more likely to practice self-medication as compared to students in year 3 of study. There is no direct explanation as to why the second-year undergraduate students were more likely to self-medicate, this may have happened by chance. Otherwise, we expected that third-year students could have practiced self-medication more as compared to others because they are more exposed to the field as compared to the second- and first-year students. In other studies, conducted in Tanzania, an association of Self-medication with antibiotics with socio-demographic characteristics was reported.[24,25] However, these findings were in the general population.

Limitation of the study

The main limitation of this study is that the data collected was self-reported. This may introduce some bias in the behaviour of the respondents studied. Prevalence of self-medication was studied for one month only, results could have been different in other periods as well as in different seasons. The study population was only undergraduate students of year 1 to year 3, other students were not included because the time of data collection coincided with the time of their clinical rotation in peripheral hospitals. The inclusion of other undergraduate students could have presented differences in analysed data. However, this has no effect on the validity of the results observed concerning the parameters assessed in this study. Some variables were not stretched enough to provide multiple responses, this could also have provided a different perspective on antibiotic self-medication among undergraduate students.

CONCLUSION

The study observed a high prevalence of antibiotic self-medication among undergraduate students. This calls for immediate implementation of public health programs aimed at increasing awareness of consequences that may results fromantibiotic self-medication. There is need for review of educational programs especially in the teaching of clinical pharmacology to include modules on self-medication and rational use of medicines. At the policy-making level, there is an urgent need to legislate and enforce laws restricting access to antibiotics in Tanzania. More importantly, a national commitment forsolving the problem of antibiotic misuse in Tanzania is urgently required.

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ORIGINAL ARTICLE

Prevalence and Predictors of HIV Infection among Under Five-Year Children Born to HIV Positive Mothers in Muheza District, North-Eastern Tanzania

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ABSTRACT

Background: Human Immunodeficiency Virus (HIV) pandemic has become a serious public health concern worldwide. The prevalence of paediatric HIV infection is largely unknown in many countries in Sub-Saharan Africa (SSA). We aimed to determine the prevalence and predictors of HIV infection among under-5 years children in Muheza District, Tanzania.
Methods: A facility-based study among mothers/guardians with their under-5 years children exposed to HIV infection was conducted from June 2015 to June 2016. Information on HIV status, socio-demographic and other family characteristics was collected using a structured questionnaire. Data analysis was performed using STATA version 13.0.
Results: A total of 576 HIV-exposed under-5 years children were recruited together with their respective mothers/guardians. The HIV prevalence among under-5 years children was 10.6% (95% CI: 8.1-13.1%). The burden of HIV infection was observed among older children aged 25 to 59 months (AOR= 8.0, 95% CI 2.5-26.0) than in the younger children. There was a four-fold (AOR=3.9, 95% CI 1.7-9.1) risk of HIV infection among children born to mothers of unknown HIV status at conception than among children born to mothers with known HIV status. The odds of HIV infection were higher among children who were delivered from home (AOR=2.6, 95% CI 1.0-6.5), received mixed feeding (AOR=2.4, 95% CI 1.2-4.9), and those living far from a health facility (AOR=3.0, 95% CI 1.4-6.5).
Conclusion: The prevalence of HIV among under-5 years children in Muheza is higher among older children. The high prevalence is associated with being born to mothers with unknown HIV status at conception, received mixed feeding, home delivery, and living far from the health facility. Campaigns that provide health educational massages addressing risk factors of HIV need to be emphasised in order to promote the control and prevention of HIV among children.

INTRODUCTION

The HIV pandemic has become a major global public health concern.[1] An estimated 1.7 million children worldwide were infected with HIV by the end of 2020. In the same year, approximately 150,000 new HIV infections occurred in children worldwide with more than 90% of the infections residing in Sub-Saharan Africa (SSA).[2] Tanzania is among the top ten countries in the world worst affected by HIV. In 2016, out of the total number of people living with HIV in Tanzania, 18% of these infections were due to Mother-To-Child Transmission (MTCT).[3] Due to increased accessibility to cost-effective Prevention of Mother To Child Transmission (PMTCT) interventions in Tanzania, the number of children born with HIV had decreased from 26,900 in 2009 to 10,000 in 2016.[2,3] The prevalence of paediatric HIV in SSA varies from country to country. In Nigeria, a prevalence of 5.3% was reported among healthy children presenting at immunisation clinics.[4] A similarly lower prevalence (3.1%) has also been reported among children with unknown HIV status in Cameroon.[5] In Mali, a prevalence of 10.1% among HIV exposed children aged 18 months and below presented at paediatric clinics was reported.[6]

A hospital-based study in Kenya among HIV exposed infants aged below 12 months found a higher prevalence of 11- 41%.[7] A similar higher prevalence (25%) has been reported among hospitalised children aged above 18 months in Zambia.[8] The strategy of testing all children presenting at health facilities to know their HIV status is very important for providing immediate and appropriate care to those found being HIV infected. However, the World Health Organization (WHO), recommends provider-initiated HIV testing and counselling for all infants and young

children presented at health facilities irrespective of HIV epidemic settings.[9] In addition, Early Infant Diagnosis (EID) of HIV provides an opportunity for HIV exposed infants to receive virological testing between the age of 4 to 6 weeks or at the earliest opportunity thereafter, and those found infected to start on Antiretroviral Therapy (ART) as soon as possible.[9]

Mother-to-child transmission, either during pregnancy, childbirth, or breastfeeding is the most (15 to 45%) predominant source of HIV infection in young children.[10] Maternal immunological status and biological factors have been pointed to increase the risk of MTCT as are the ones through which the underlying socioeconomic and proximate factors operate to exert an impact on child health.[11,12] The risk of MTCT is high in children born with low birth weight or born before 34 gestation weeks. Although the mechanism of MTCT in these low birth weight neonates is unclear, this could reflect prematurity due to inadequate passive or active immunity at that age, combined with significant transmission during labour or delivery.[13,14]

The risk is higher in vaginal deliveries than in caesarean section due to direct contact between infant and HIV-infected maternal body fluids (blood, vagina, and cervical secretions).[15,16] The presence of Sexually Transmitted Diseases (STDs) like gonorrhoea, chlamydiosis, trichomoniasis, or genital infections during pregnancy have been shown to increase HIV transmission.[13] There are pieces of evidence that mothers presenting with malaria during pregnancy have an increased risk of MTCT.[17,18] Several factors that increase the risk of MTCT do exist. However, studies on the determinants of HIV infection among children have focused primarily on maternal viral load.[19]

It has been pointed out that the risk is strongly associated with high maternal viral load and advanced stage of maternal HIV infection during labour and at delivery.[14] High maternal viral load and low Cluster of Differentiation 4 (CD4) count are highly associated with an increased risk of HIV infection in children.[20,21,22] Studies have shown that maternal CD4 cell count of less than 200 cells per mm^3 near delivery and those who have been diagnosed with the severe clinical disease are more likely to transmit the virus than those who are less severely affected by HIV infection.[14,23]

Studies have shown that breastfeeding duration, as well as maternal immune status, are the major determinants of increased risk of MTCT of HIV.[24] The risk of HIV transmission increases drastically among mixed-fed children especially if breastfeeding is mixed with solids during the first 2 months of life and the risk is even more if breastfeeding duration is continued for more than 4 months.[25] The use of Antiretroviral (ARV) drugs by HIV-infected mothers and infant ARV prophylaxis has been shown to reduce the risk of postnatal HIV transmission through breastfeeding.[26,27,28]

Since its inception in 2000, the PMTCT program has made several advancements in most settings including here in Tanzania. In 2010, the WHO recommended Option A regimen. This involved the use of Zidovudine (AZT) from 14 weeks of pregnancy until 7 days post-delivery and infant Nevirapine (NVP) from birth until one week after cessation of breastfeeding.[29] Later, these guidelines were updated, and Option B regimen was introduced which involved triple ART during pregnancy from 14 weeks of pregnancy until the end of breastfeeding and infant NVP daily from birth up to age 4 to 6 weeks. This regimen was further modified to involve initiating triple ART for life during pregnancy and breastfeeding women irrespective of clinical stage of disease or CD4 count and infant NVP up to the age of 4 to 6 weeks. The regimen was named PMTCT Option B+ and is the best recommended regimen that is currently in use in most settings.[29]

In Tanzania, Option B+ was adopted in 2013, available in a fixed dose combination regimen of one pill taken once per day.[30] However, the current introduction of the Dolutegravir (DTG)-based ART regimen in most settings including Tanzania has dramatically shown to reduce the risks of transmission of HIV, though its safety and efficacy are still under development.[30,31.32] In addition, the use of Cotrimoxazole Preventive Therapy (CPT) among pregnant women with CD4 cell count ≤350 cells/mm^3 has been shown to reduce the risk of HIV transmission to the child.[30]

Data on the prevalence and predictors of HIV infection in children below 5 years born to HIV positive mothers presenting at health facilities in Muheza, Tanzania is largely unknown. Without knowing the predictors and the burden of HIV among HIV exposed children below 5 years, the policy will not be informed, and as a consequence interventions will not be focused effectively. Therefore, continuous understanding of the contributing factors associated with the epidemiology of paediatric HIV may reveal opportunities to reduce the risk of MTCT of HIV.

The main context of the study is mainly to understand the progress made with the PMTCT intervention in Muheza District, Tanzania, in relation to the risk of MTCT of HIV among exposed under-5 years children. Continuous epidemiological surveys are crucial in this paediatric population, as they will help to know how interventions are working and keep track of the progress and utilize lessons learned for modification of intervention measures and/or development of new interventions. Hence this study aimed to determine the prevalence and predictors of HIV infection among exposed children below 5 years in Muheza, Tanzania.

MATERIALS AND METHODS

Study Area, Design and Population

A facility-based study was conducted in Muheza district in north-eastern Tanzania (4^0, 45'S; 39^000'E) from June 2015 to June 2016. The health care system of Muheza district is comprised of 46 health facilities which includes; one hospital, 4 health centres, and 41 dispensaries of which 36 offer PMTCT services and 28 offer EID services.[33] Study population involved HIV exposed under five-year children and their respective mothers/guardians. A guardian in this study was defined as the child's main primary caregiver living with the child in the same household.

This included either the child's biological parent, grandparent, sister, brother, aunt or uncle. The inclusion criteria were based on the following characteristics:

Mother/guardian who agreed to participate and with an under five-year child born to HIV positive mother (maternal HIV status was confirmed from the district HIV database); under five-year child with a confirmed HIV positive test result. A mother/guardian with HIV exposed under five-year child who was not permanent residents of Muheza district were excluded from this study.

Sampling and Sample Size Determination

The participants were selected by employing a multistage sampling approach. The initial step involved the selection of the district purposively. Muheza district was chosen because it is among the leading district with high HIV prevalence among pregnant women in Tanga Region.[34] Next, a list of health facilities (N=46) was obtained from the district according to their geographical position. Health facilities (clusters) serve as Primary Sampling Units. These health facilities were listed by name and numbered from 1 to 46. Finally, the sampling interval was obtained. From the list, we randomly selected 18 health facilities that provide PMTCT and EID services. Before initiation of data collection, a list of HIV exposed under five-year children was obtained from the registers/database at each health facility. The list was numbered and all eligible under five-year children each with their respective mother/guardian were selected randomly using the lottery method and enrolled based on the inclusion criteria. The sample size (n) was calculated based on the formula that accounted for simple random sampling and the design effect, where by $n = z^2\ p\ (1\text{-}p)*DEFF/d^2$).[35]

The Design Effect (DEFF) was adjusted by a factor of 2, at a 95% Confidence Interval (CI) with a Z value of 1.96, and the desired level of absolute precision (d) was taken at 5%. The transmission rates of HIV from mother to child range between 20 and 45%.[36] The highest exposure of infection risk (p) was taken at 45% and a response rate of 90%. Hence the estimated sample size $[(1.96)^2*45(100\text{-}45)*2/5^2]$ was 760 + 76 = 836 under-five year children. However, the minimum and maximum numbers of mother/guardian-child pairs in each cluster was set at 20 and 50 respectively. But, the proportion to size was employed based on the estimated total number of HIV-exposed under five-year children at a particular health facility. In this study a total of 576 mother/guardian-child pairs were enrolled. More details of this study have been described elsewhere.[37]

Data Collection

Socio-demographic factors of the mother/guardian-child pair (age, sex, residence, marital status, occupation, knowledge of HIV and distance from a health facility (which was defined by time taken in minutes to reach the health facility on foot) were collected using a structured questionnaire. Mother/guardian's knowledge on HIV was assessed based on 4 key questions that addressed general MTCT knowledge; prevention of MTCT; timing of post-exposure prophylaxis to HIV exposed infant and factors affecting HIV transmission. Maternal information which included CD4 cell count levels during pregnancy, HIV status before conception and history of taking CPT during pregnancy was also collected. The child's HIV status was extracted from the HIV database available at the district hospital. In addition, during recruitment, more data on children and their biological mothers were extracted from registers, HIV database, hospital case files for children under five-year or antenatal cards, and PMTCT records to supplement the collected primary data.

Data Management and Analysis

All data were double entered in Epi Data database version 3.1 by two separate data clerks. Data were compared directly at entry with a previous entry of the same data. After finishing data entry, data were validated in Epidata, and any discrepancy observed was clarified by editing the data and comparing it with original data forms, and redoing the validation. The amount of discrepancy allowed was 2%. The validation process continued until all data were compared. After validation of data was completed, data were exported to STATA version 13 (Stata Corp, College Station, Texas, USA) for analysis. The analyzed data was validated by the Minitab version 19 statistical software and the results were comparable. Data were summarised using descriptive statistics and graphical summary, whereby, continuous variables were described using median and Inter-Quartile Range (IQR).

Categorical variables were described using frequencies and percentages. The child's HIV status was categorised into a binary variable; 'HIV positive or HIV negative'. A composite variable on knowledge on HIV was developed based on 4 key questions by the use of recoding and computing commands to form a single unit composite variable with a binary outcome 'good' or 'poor'. The 4 variables composition for good knowledge comprised of; 1) MTCT can occur and can be prevented, 2) MTCT can be prevented by taking ARV drugs during pregnancy, 3) HIV can be transmitted in utero, during delivery, and through breastfeeding, 4) post-exposure prophylaxis to HIV exposed infant should be given soon after birth within 6 to 12 hours post delivery. The 4 variables composition for poor knowledge comprised of 1) MTCT can occur and can be prevented, 2) MTCT can be prevented by taking fansidar (*Sulphadoxine Pyrimethamine*), ferrous and traditional medicines during pregnancy, 3) HIV can be transmitted by mosquito bites, evil spirits and by sharing food with a person who has AIDS, 4) did not know the correct timing of post-exposure prophylaxis to HIV exposed infant. The development of a composite variable for good or poor knowledge of HIV comprised of all the 4 data sets as described above.

All variables from univariate analysis with P values of ≤0.2 were fitted to the multivariable logistic model. Multiple logistic regression analyses were used to examine the associations between various socio-demographic factors of the mother/guardian-child pairs and the child's HIV infection status. A stepwise Logistic Regression was employed by removing variables that were statistically non-significant at *P- value* of ≤ *.05*. The final modal comprised of variables with Adjusted Odd Ratios at 95% Confidence Interval (CI) which was taken as significant predictors for HIV infection in under five-year children. Crude Odds Ratios (COR) with corresponding *P-values* were also presented.

Ethical Considerations

Ethical approval was obtained from the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania with reference number: -

NIMR/HQ/R.8a/Vol. IX/1978. Permission to conduct this study was given by Muheza District Council Authority. Written informed consent was obtained from each mother/guardian before the recruitment of study participants. All participants were identified by numbers throughout the study. No names were used to identify participants during data collection, report, or publication of study findings

RESULTS

Socio-Demographic characteristics of Study Populations

A total of 576 mothers or guardians each with HIV exposed under five-year child were involved. Out of 576 under five-year children, 281 (48.8%) were males and 295 (51.2%) were females. The majority 528 (91.7%) of under-five children were delivered at health facilities and 48 (8.3%) at home. Out of 576 children, 251 (43.6%) were born to mothers with unknown HIV status at conception. A total of 433 (75.1%) of the under-five year children received exclusive breastfeeding, 130 (22.6%) received mixed feeding and 13 (2.3%) received replacement feeding. A total of 83 (14.4%) children did not receive NVP prophylaxis at birth and the period thereafter. Out of 576 mothers/guardians, 549 (95.3%) were the biological mothers of the respective children. Slightly more than half of the mother/guardian child pair had to walk for more than 30 minutes to the nearest health facility.

HIV Prevalence among Children

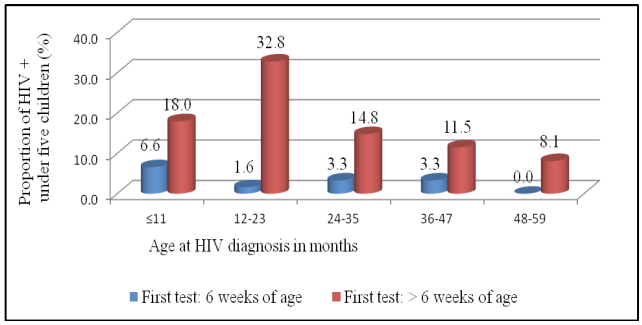
Out of 576 under-five year children, 61 were confirmed to be HIV positive. The HIV prevalence was reported to be 10.6% (95% CI: 8.1-13.1%) A total of 46 (75.4%) HIV-positive under five-year children were diagnosed at ≥ 12 months old. Among 61 HIV-positive under-five year children, 52 (85.2%) reported receiving their first HIV testing at more than 6 weeks of age (Figure 1). The Median age at diagnosis among those confirmed to be HIV positive was 20 months (IQR: 12.5-35 months). Accordingly, 4 (6.6%), 12 (19.7%), and 45 (73.7%) HIV-positive under five-year children were in the ≤ 11 months, 12 to 23 months, and 24 to 59 months age group respectively (Figure 1).

Predictors of HIV Infection in Children

In multiple logistic regression model, children who were older, delivered at home, born with low birth weight, received mixed feeding practices, born to mothers with unknown HIV status at conception, born to mothers with known HIV status at conception with the absence of CPT during pregnancy and lived far from the health facility were significantly and independently associated with HIV infection (Table 1).

Higher odds of HIV infection were observed among older children aged 25 to 39 months (AOR= 8.0, 95% CI 2.5-26.0) than in younger children. The risk of HIV infection was 2.6 (AOR=2.6, 95% CI 1.0-6.5) times higher among children delivered at home than among those delivered at a health facility. Children born with low birth weight of < 2500g had a 3.4 (AOR=3.4, 95% CI 1.2-9.3) times higher risk of HIV infection compared to those born with ≥ 2500g. The risk of HIV infection in children was 2.4 (AOR=2.4, 95% CI 1.2-4.9) times higher among those who received mixed feeding compared to those who were exclusively breastfed. There was a 4-fold (AOR=3.9, 95% CI 1.7-9.1) risk of HIV infection among children born to mothers with unknown HIV status at conception compared to those born to mothers with known status. There was a 3-fold (AOR=2.7, 95% CI 1.1-7.2) risk of HIV infection among children born to mothers with known HIV status at conception with the absence of CPT compared to those with the presence of CPT during pregnancy. The risk of HIV infection was 3.0 (AOR=3.0, 95% CI 1.4-6.5) times higher among those who lived far from a health facility as compared to those living close to a health facility (Table 1).

FIGURE 1. Proportion of HV positive under five-year children according to age at HIV diagnosis and access to first HIV testing in Muheza district, Tanzania



The odds of infection were reduced among children who had received NVP after delivery (AOR=0.3, 95% CI 0.1-0.5) compared to non-recipient of *Nevirapine*. Similarly, the odds of infection were reduced among children born to mothers with a CD4 cell count of ≥ 350 cell/µl (AOR=0.3, 95% CI 0.1-0.8) compared to those with low CD4 cell count (Table 1). The Guardian's marital status, occupation, knowledge of HIV, and residence did not show any association with the child's HIV infection.

DISCUSSION

Results from this study indicate that nearly 3 quarters of under- five-year children diagnosed with HIV infection in Muheza district, the diagnosis was done at more than one year of age. The majority of HIV-positive children were in the age group, between 13 to 59 months and accessed their first HIV testing at more than 6 weeks of age. Studies from Ethiopia and Tanzania reported that HIV transmission was higher in infants enrolled late than in those who were presented earlier.[39,40]

This suggests that late HIV testing in children at older ages may be triggered by symptoms that make their parents/guardians bring them to the health facility for diagnostic testing. Children born to mothers with unknown HIV status at conception were more at risk than those born to mothers with known HIV status. A study in Mozambique reported that EID programs target infants born to mothers with known HIV-positive status.[41] This implies that most of the HIV-infected children will not be identified if EID services are offered only to children born to mothers with

TABLE 1: Predictors of HIV infection among under five-year children in Muheza district, Tanzania

Child and maternal variables	n (%)	OR (95% CI) Unadjusted	Adjusted
Child age (months)			
≤ 12	224 (38.8)	1	1
13-24	155 (26.9)	5.9 (1.9-18.1)**	3.7 (1.1-12.7)**
25-59	197 (34.2)	14.9 (5.2-42.4)*	8.0 (2.5-26.0) #
Place of delivery			
Health facility	528 (91.7)	1	1
Home	48 (8.3)	3.7 (1.8-7.5)*	2.6 (1.1-6.5)**
Birth weight			
≥ 2500	525 (91.2)	1	1
< 2500	51 (8.9)	1.6 (0.7-3.7)	3.4 (1.2-9.3)**
Mode of feeding			
Exclusive breastfeeding	433 (75.1)	1	1
Replacement feeding	13 (2.3)1.4	(0.2-10.9)	0.5 (0.1-5.0)
Mixed feeding	130 (22.6)	6.0 (3.4-10.5)*	2.4 (1.2-4.9)**
NVP prophylaxis			
0 day	83 (14.4)	1	1
1-90 days	493 (85.6)	0.08 (0.04-0.14)*	0.3 (0.1-0.5)*
Maternal CD4 cell count			
≤ 349 cell/µl	189 (32.8)	1	1
≥ 350 cell/µl	214 (37.2)	0.3 (0.1-0.6)#	0.3 (0.1-0.8) #
Unknown	173 (30.0)	1.3 (0.7-2.3)	0.9 (0.4-2.0)
Maternal HIV status at conception			
Known	325 (56.4)	1	1
Unknown	251 (43.6)	8.0 (4.0-16.0)*	3.9 (1.7-9.1)#
Maternal known HIV status at conception with			
Presence of CPT during pregnancy	480 (83.3)	1	1
Absence of CPT during pregnancy	(96) 16.7	14.2 (7.9-25.7)*	2.7 (1.1-7.2)**
Distance to the health facility			
Near (≤30 minutes)	267 (46.4)	1	1
Far (>30 minutes)	309 (53.6)	3.2 (1.7-6.0)*	3.0 (1.4- 6.5)#

Notes: (a) Total sample size N=576 (b) CPT=Cotrimoxazole Preventive Therapy; NVP=Nevirapine (c) OR: Odds ratio, CI=Confidence interval (d) P values: #p<.01, * p<.001, ** p<.05

known HIV status.

Similar to our findings, vertical transmission of HIV has been considered to be higher among children delivered at home, living far from health facilities, and among those who received mixed feeding.[39,42] It has been reported that, in mixed-fed children, the risk of HIV transmission is high throughout breastfeeding and the risk is even more if breastfeeding duration is prolonged for more than 4 months.[25] However, children born from rural mothers or hard-to-reach areas are more prone to mixed feeding, the choice that is commonly practiced throughout Africa.[43,44,] In this study, place of residence was not a significant risk factor after adjustment with other variables. Recent studies in Tanzania, Rwanda, and Ethiopia reported less HIV transmission among urban children than in rural ones.[39,40,45] This could be due to inefficient PMTCT services or inaccessibility to health facilities in rural areas when compared to urban areas. Most women in low-income countries do not have access to antenatal services and present late with symptoms at health facilities which could affect the health outcome of both the mother and child.[46]

In our study, children who received ARV prophylaxis were found to have a reduced risk of acquiring HIV infection than those who were not taking ARV prophylaxis. The same is reported in studies conducted in Zimbabwe, Kenya, and Ethiopia.[47,48,49] Children born to HIV-positive mothers who did not take CPT during pregnancy had a higher risk of vertical transmission in this study. A study in Ethiopia and elsewhere in Tanzania also found that la-

ck of maternal PMTCT interventions was significantly associated with MTCT of HIV.[39,50] It was observed in this study that children born to mothers with a high CD4 cell count of more than 350 cells/µl were at reduced risk of being infected. The risk of MTCT of HIV has been reported among mothers with advanced disease who presented with low CD4 counts in several studies.[53,54,55,56]

The risk of HIV transmission from mother to child was observed in mothers with CD4 cell count of less than 200 cells/mm3 despite the use of maternal ARV treatment.[3,51,52] Low birth weight has been associated with advanced maternal HIV status in studies conducted elsewhere in Tanzania and Côte d'Ivoire. [56,57] This is consistent with this study's findings.

Limitations

The study could not establish temporal or causal relationships between exposure and outcome, the study only describes associations. Selection bias, since sample population selection only considered those who attended health facilities within the district. Mothers and children who were identified within communities through other means were left out, resulting in a sample that is not representative of the whole population in the study area.

The findings could also be affected by a shortfall of 30% of the estimated sample size of the study. The wider 95% confidence interval of the older age group (25 to 59 months) implies that the sample size for this variable was relatively small, and so reducing the precision of the finding for this variable. Information bias, since some mothers/ guardians may not report correctly some information regarding mother-child pair such as the history of taking CPT during pregnancy, the date which the HIV status of the mother was confirmed, or the history of taking infant NVP. However, this was minimised by verifying received information using hospital case files, children under 5 years or antenatal cards, and PMTCT records.

However, while factors included in the multiple variables logistic model were each potentially important factors associated with predictors of HIV infection among under 5 years children, it is difficult to eliminate the probability that the results were influenced by unmeasured confounders.

CONCLUSION

The burden of HIV among under-5 years children in Muheza district is higher among the older age category. The high prevalence is associated with being born to mothers with unknown HIV status at conception, absence of CPT during pregnancy of the index child, absence of infant NVP prophylaxis, mixed feeding of infants, home delivery, and living far from the health facility. This does not only show limitations in testing efforts, but it suggests the need for reshaping the current national HIV testing policies for women and men so that pre-pregnancy HIV status is prioritised. However, the findings underscore the need to focus on improved preventive health care before pregnancy, pre- and post-natal period for mothers and babies in order to reduce the risk of MTCT of HIV in areas with similar settings. Strengthening health and education programmes will have a direct benefit as it increases awareness of their children's needs to prevent them from acquiring infection.Although there has been some development in this area, the available evidence in this study population, the under five-year children is still limited. Hence, these pieces of evidence obtained from this study will save as a reference baseline to compare with other epidemiological surveys that will help to know the efficiency of interventions.

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ORIGINAL ARTICLE

Isoniazid and Rifampicin Tuberculosis Drug Resistance in HIV Endemic Region of Western Kenya

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ABSTRACT

Background: Tuberculosis drug resistance is often associated with inadequate anti-tuberculosis treatment regimen resulting to mutations that confers resistance to anti-tuberculosis agents. This is aggravated by synergetic relationship between Tuberculosis and HIV (Human Immunodeficiency Virus). Over 25% of Global Tuberculosis deaths occur in Africa and Kenya is one of the 30 high burden countries that together account for more than 80% of the world's TB cases. According to World Health Organization, in 2018, Multi drug resistant Tuberculosis prevalence in Kenya was 1.3% in new cases and 4.4% in retreatment cases. Kisumu County recorded the second highest HIV prevalence at 18.6% against the national prevalence of 4.5% in 2020. The extent of regional burden of DR-TB and HIV co-infection has not been exactly well-defined in Western Kenya.

Methods: This was a prospective cross sectional study that aimed to explore the association between Tuberculosis drug resistance and HIV status among new and previously treated pulmonary tuberculosis cases in Kisumu County, Kenya. Tuberculosis clinical suspects were recruited into the study and classified as HIV positive or negative based on their clinical data. Sputum samples from tuberculosis clinical suspects were subjected to fluorescent microscopy, phenotypic culture and line probe assay.

Results: Out of a sample of 256, response rate was 216 of which HIV positive cases were 119(55.1%) and negative were 97 (44.9%). The study found that out of 11 that were phenotypic Isoniazid resistance 8(6.7%) were from HIV positive cases while 3 (3.2%) were from HIV negative cases. Phenotypic rifampicin resistance among the HIV positive were 8 (6.7%) while HIV negative were 2 (2.1%). All the 2(1.7%) MDR cases were from HIV positive participants. The study found out that HIV status and Tuberculosis cases were significantly associated at p<.05. HIV positive cases were more likely associated with retreatment cases (OR=1.4,95CI:1.00-1.90) compared to new cases.
The study found out that the common mutant probe among the HIV positive was katG MUT1 4(2.6%), while mutant probes among the HIV negative were in hA MUT1 1(0.7%), katG MUT1 1(0.7%) and roB MUT2A 1(0.7%). Wild type gene deletion among the HIV positive cases were observed in probes katG WT 3(2.1%), roB WT7, katG WT 1(0.7%) while wild type gene deletion among the HIV negative cases were inhA WT1 1(0.7%), in hA WT1/inhAWT2 1(0.7%), katG WT 1(0.7%).

Conclusion: Interventions specific to HIV-endemic areas are urgently needed to block tuberculosis drug resistance transmission. Development and improvement of the efficacy of interventions will require a greater understanding of the transmission of multidrug-resistant tuberculosis in HIV-endemic settings like Kisumu County, Western Kenya.

BACKGROUND

The emergence and spread of Drug-resistant TB (DR TB) and Multidrug-resistant TB (MDR TB) have become a global health problem positioning tuberculosis as one of the top 3 infectious disease killers. Globally, the prevalence of drug-resistant tuberculosis (DR-TB) has increased substantially in the past 2 decades making Tuberculosis the leading cause of death among people living with HIV infection, accounting for approximately 40% of deaths among this population.[1,2] Tuberculosis has been associated with morbidity and mortality, especially in poor resource settings and is often the first indicator of HIV infection.[3] According to the World Health Organization (WHO), there were an estimated 9.0 million incidence cases of TB globally in 2018.[3] More than half of these cases (56%) were in South-East Asia and Western Pacific regions, while 29% were in the African region.[4] Additionally, WHO estimated that, 8.6% (7.4% to 10%) of 10 million (range, 9 to 11.1 million) incident cases with active TB were also coinfected with HIV in 2018,[5] while rifampicin-resistant (RR) or multidrug-resistant (MDR-TB) occurred among 3.6% new cases, 18% previously treated and 5.6% among all TB cases. Inappropriate use of antibiotics in treatment of drug-

susceptible patients, sub-optimal treatment regimens and failure to complete treatment in drug susceptible patients leads to drug resistance.[6] This has therefore resulted in high treatment failures and death rates due to the complexities in diagnosis and treatment.[7] Kenya is among the 14 countries globally that are in all the 3 lists of high burden countries (HBC) for TB, TB/HIV and MDR-TB and the fifth highest burden country in Africa.[8] The estimated incident for TB in Kenya is 348/100,000 population, translating to about 169,000 TB cases occurring annually, the mortality rate (excluding HIV+TB) is 60/100,000 of the population.[9] According to WHO, in 2018, the MDR-TB prevalence in Kenya was 1.3% in new cases and 4.4% in retreatment cases.[10] Tuberculosis drug resistance affects all age groups, but has greatest toll in the most productive age group of 15 to 44 years and the major factor responsible for the large TB disease burden in Kenya is the concurrent HIV epidemic.[11]

A study done on the prevalence and detection of drug resistant mutations in Mycobacterium tuberculosis among drug naïve patients in Nairobi between 2015 and 2017 showed that out of the 132 study participants screened for tuberculosis, only 2 showed resistance associated with first line and second-line anti TB drugs.[12] Of the 2 patients that had resistance to first and second line, one showed resistance to *isoniazid*, while the other depicted an MDR-TB case resistant to both *rifampicin* and *isoniazid*.[12] Out of a total of 132 patients that were tested for resistance to second-line antituberculosis agents, one showed cross-resistance to *kanamycin*, *amikacin*, and *capreomycin*.[12] The same study showed that, MDR-TB cases had an additional second-line anti tuberculosis drug resistance while mono-resistant cases had no additional second-line drug resistance.[12] In Western Kenya, anti-tuberculosis drug resistance is an emerging health problem especially in Kisumu County where cases of HIV and TB co-infection are predominant.[13] Human Immunodeficiency Virus and TB are synergistic with HIV increasing the incidence of TB and TB associated with increased mortality among people living with HIV, and as an indicator of Acquired Immunodeficiency Syndrome (AIDS) defining illness. Other studies show that the risk of TB infection is 16 to 27 times greater in People Living with HIV (PLHIV) than in the general population.[12] According to the 2019 report from Kenya National Tuberculosis, Leprosy and Lung Disease program, Kisumu County had the third highest TB co-infection rate in Kenya at 59% after, Homa bay 64% and Siaya 63% which is way above the national co-infection rate of 28%.[11] The report further states that the TB prevalence rate in Kisumu is 379 out of 100,000 people which is higher than the average National TB prevalence of 223. According to the Ministry of Health report on Kenya HIV Estimates, Kisumu County has the second highest HIV prevalence of 18.6% after Homa bay 20.2% and Siaya 17.8% against the national prevalence rate of 4.5%.[11] This high prevalence possess a greater challenge in Tuberculosis control and drug resistance surveillance in Kisumu County as HIV is more likely associated with TB.[10]

The emergence and transmission of drug-resistant tuberculosis epidemic is a threat to regional control of Tuberculosis. Globally, various studies show that, HIV has a profound effect on the progression of tuberculosis hence elevated transmission. The extent of regional DR-TB and HIV co-infection burden has not been well-defined and data on the relationship between HIV status and Tuberculosis drug resistance specific to HIV-endemic areas like Kisumu County in western Kenya are urgently needed to bridge tuberculosis transmission gaps and modulate continued spread of drug resistance.

The Genus Mycobacterium

The *genus Mycobacteria* belong to the family *Mycobacteriaceae* which are characterised by elevated lipid content, most notably a high level of waxes called *mycolic* acids.[14] These *mycolic* acids are responsible for the organism being resistant to decolonisation by acid alcohols hence are referred to as being acid-fast.[14] The importance of the *Mycobacteria* cannot be overemphasised and continued study is required to further delineate the role of these organisms in disease. Tuberculosis is a disease caused by the bacillus *Mycobacterium* tuberculosis species which typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB).[15] Tuberculosis is one of the top 10 causes of death worldwide and the leading cause of death from a single Infectious agent (ranking above HIV/AIDS).[16] Globally, an estimated 10.0 million people got infected with TB in 2018 of which there were an estimated 1.2 million TB deaths among HIV-negative people and an additional 251000 deaths among HIV positive people.[17] Studies shows that Tuberculosis affects all sexes across all age groups, however the highest burden is in men of 15 years and above who account for 57% of all TB cases in 2018. By comparison, women accounted for 32% and children (aged <15 years) for 11%. Among all TB cases, 8.6% were people living with HIV (PLHIV). Geographically, most TB cases in 2018 were in the WHO regions of South-East Asia (44%), Africa (24%) and the Western Pacific (18%), with smaller percentages in the Eastern Mediterranean (8%), America (3%) and Europe (3%).[18] Drug-resistant TB continues to be a public health threat. In 2018, there were about half a million new cases of *rifampicin*-resistant TB (of which 78% had multidrug resistant TB).[19] The 3 countries with the largest share of the global burden were India (27%), China (14%) and the Russian Federation (9%).[10] Globally, 3.4% of new TB cases and 18% of previously treated cases had multidrug resistant TB or *rifampicin*-resistant TB (MDR/RR-TB), with the highest proportions (>50% in previously treated cases) in countries of the former Soviet Union.[10] In a study that was conducted by Nduba and others, Kenya ranks 10th globally among the TB burden countries. The high prevalence of HIV is a major contributing factor to high TB incidence.[20]

Anti TB Drugs

Tuberculosis can be managed effectively by first line anti-tuberculosis agents. However, this first line therapy may fail to cure Tuberculosis for varied reasons. Naivety among patients and continued spread of the Mycobacterium bacilli contribute to the emergence of drug resistant strains. The emergence of multidrug resistant TB (MDR-TB), is of great concern because it requires the use of second-line drugs that are difficult to procure, much more toxic and expensive than First Line Drugs (FLDs).[10]

Thus, the detection and treatment of drug susceptible or

single drug resistant TB is an important strategy for preventing the emergence of MDR-TB.[19] Extensively drug-resistant (XDR-TB) which are MDR strains that are resistant to *isoniazid rifampicin* and any *fluoroquinolones*, and to at least one of the 3 second-line anti-tuberculosis injectable drugs—i.e., *capreomycin*, *kanamycin*, and *amikacin* have also been reported.[8]

Kenya has adopted the current WHO recommendations on treatment and care for drug-resistant TB which stipulates that in patients with confirmed rifampicin-susceptible, isoniazid-resistant tuberculosis (Hr-TB), treatment with *rifampicin*, *ethambutol*, *pyrazinamide* and *levofloxacin* is recommended for a duration of 6 months while in patients with confirmed rifampicin-susceptible, isoniazid-resistant tuberculosis, it is not recommended to add *streptomycin* or other injectable agents to the treatment regimen.[11] A shorter all-oral *bedaquiline*-containing regimen of 9 to 12 months' duration is recommended in eligible patients with confirmed multidrug- or rifampicin-resistant tuberculosis (MDR/RR-TB) who have not been exposed to treatment with second-line TB medicines used in this regimen for more than 1 month, and in whom resistance to *fluoroquinolones* has been excluded.[21] Patients who are at high risk of DR TB are identified and are prioritised in receiving further Drug Susceptibility Testing (DST) beyond GeneXpert in the TB reference laboratories. This test includes first line (FL) and second line (SL) probe assay (LPA), culture and phenotypic DST.

Mycobacteria Tuberculosis Drug Resistance and HIV

Drug-resistant Tuberculosis is considered a potential obstacle for elimination of TB globally. Surveillance reports show that 12 million people living with HIV are co-infected with TB. Sub Saharan Africa bears the global burden with 70% of all the cases.[5] Co-infection of HIV and MDR-TB complicates Tuberculosis control and management. A number of studies have documented increased mortality among patients co-infected with MDR-TB and HIV. This co-infection has been equally responsible for Extensively drug-resistant TB (XDR-TB) outbreaks.[5] Better outcomes with decreased mortality have been described with concomitant treatment for both anti TB and anti HIV drugs.[5]

The extent of global problem of DR-TB and HIV co-infection has not been well-defined. Some of the reasons for the lack of these data are; HIV testing and TB drug resistance testing are not adequately assessed through surveillance. Surveillance from different studies from different geographical settings have shown discordant associations due to heterogeneity in setting, demographic profile, methodology and analysis of data.[22] According to WHO, 24 countries reported data on MDR-TB stratified by HIV status. The findings show that there was heterogeneity in geographic distribution with the majority confined to high-risk groups, even in countries showing a high prevalence of MDR-TB along with an emerging HIV epidemic. Only 11 countries, majority from Eastern European and Central Asian regions reported strong associations between HIV and drug resistance.[16] There are several epidemiological reasons that M/XDR-TB may be associated with HIV. The reasons suggested are rapid progression of disease due to harbouring of DR strains, particularly in the immune compromised compared to - immunocompetent state; drug mal absorption of anti-TB drugs, such as *Randethambutol* (E), leading to drug resistance and treatment failure; early reactivation of an infection due to increased vulnerability in an immune compromised state acquired from community or institutional transmission; direct contact with DR-TB cases, suggesting primary or transmitted resistance.[23] Resistance that has been acquired can be reduced by adhering to optimised therapy, whereas the control and management of primary resistance requires interventions to block the dynamics of transmission.[24] Understanding the importance of attained and primary resistance is important in implementing TB control policies especially in HIV-endemic settings, where high incidence of primary resistance have been reported.[18] Infection with HIV can influence tuberculosis drug-resistant in many ways, including the length and magnitude of infectiousness, the duration of exposure, and the vulnerability of the exposed population.[25,26]

Kenya is among the 14 countries globally that are in all the three lists of high burdened countries for TB, TB/HIV and MDR-TB and the fifth highest burdened in Africa.[22] According to the 2019 report from Kenya National Tuberculosis, Leprosy and Lung Disease program, Kisumu County was among counties with the highest TB co-infection rate in Kenya (59%) after, Homabay (64%) and Siaya (63%) which is way above the National co infection rate of 28%.[11] The report further states that the TB prevalence rate in Kisumu is at 379 out of 100,000 people, this is higher than the average National TB prevalence of 223 per 100,000 people. Although the development of drug resistant TB strains and subsequent treatment failure is a common clinical scenario in Kenya, information about TB drug resistance among HIV infected population is scanty, especially in HIV predominant regions like Kisumu County.[8] Different studies in Kenya have shown that people living with HIV are more associated with tuberculosis, however, data on the relationship between tuberculosis drug resistance and HIV status are heterogeneous nationally. As such and given the existing gap, there was need to determine the association between tuberculosis drug resistance and HIV status in HIV endemic region of Kisumu County, Western Kenya.

MATERIALS AND METHODS

Study Site

This study was carried out in Kisumu County, located in Western Kenya. The County lies between latitude 0^0 20'S and 0^0 50'S and longitudes 33^0 20'E and 35^0 20'E. It is bordered by various counties as follows; Kericho lies to the East, Nandi to the North East, Homa Bay to the South, Vihiga to the North West, Siaya County to the West, and delimited by Lake Victoria, the second largest freshwater lake in the World. Kisumu covers approximately 567km^2 on water and 2086km^2 of land area, representing 0.36% of the total land area of Kenya's 580,367km^2.[27]

The County has a total population of 1,153,343; 489,392 between 0 to 15 years and 663,951 being 15 years or above.[28] Administratively, the County has 7 Sub-counties namely: Kisumu East, Kisumu West, Kisumu Central, Nyando, Muhoroni, Nyakach, and Seme. The health care tier system in Kisumu County consists of level 1 (commu-

nity facilities), level 2 (Dispensaries), level 3 (Health centres), level 4 (county hospitals) and level 5 (County referral hospital). This study recruited patient from all health facilities handling Tuberculosis patient within the county. The HIV prevalence between 15 to 49 years is 16.3% (male 15%, female 17%) with an average prevalence of 18.6% against the national prevalence of 4.5%. Malaria remains a major health problem with a prevalence estimated at 27%.[27] The TB prevalence rate in Kisumu is 379 out of 100,000 people which is higher than the average National TB prevalence of 223 and TB-HIV co-infection rate of 59%.[28]

Study Design

Hospital and laboratory based descriptive cross sectional study design was done on Tuberculosis patients attending TB clinics and hospital facilities within Kisumu County. The study included all clinically suspected TB patients. This study was conducted between November 2020 and October 2021 to understand the magnitude of first line Tuberculosis drug resistance burden among HIV cases from Kisumu County, Kenya.

Sampling Technique

This study employed 100 percent sample of all clinically suspected Tuberculosis patients attending various health facilities within Kisumu County. Tuberculosis clinical suspects were recruited into the study and classified as HIV positive or negative based on their clinical data. Sputum samples from Tuberculosis clinical suspects were subjected to fluorescent microscopy, BACTEC™ MGIT™ 960 system (MGIT) (Becton Dickinson (BD) Bioscience, Erebodegem, Belgium) and Geno Type MTBDR*plus* (Hain Life Science GmbH, Nehren, Germany). Saturated sampling was preferred in this study because TB Clinics and Hospital facilities within Kisumu County were quite few.

Inclusion Criteria

The patients enrolled in the study had to be clinically presenting as a TB case as per the Kenyan Ministry of health case definition for Tuberculosis, and capable of expectorating sputum for study purposes. Informed consent (or parental permission), after demonstrating their understanding formed the basis for recruitment. For children under age 12, parental consent was sought, and both assent and parental consent was sought for participants ≥ 12 to <18 years of age, assent required signing of a consent form.

Exclusion Criteria

1. New patients who had started TB treatment more than one week before the study were excluded from enrolment. This is because patients who submit sputum samples after starting treatment, and in whom a positive sputum smear is observed, are more likely to be harbouring drug resistant strains, thus introducing bias. Additionally, a significant proportion of cultures would fail to grow in patients on treatment.

2. Patients who were unable to provide adequate sputum specimen for testing.

Sample Size

The sample size was calculated based on a sampling method recommended by WHO for drug resistance survey in tuberculosis.[29] The sample was determined by taking the prevalence of rifampicin resistance of 1.3% from previous year, desired precision of 0.5%, a 95% Confidence Interval and non-response rate of 15%. The calculated sample size needs to be increased by 15 to 20% to account for expected losses. Losses include patients diagnosed as smear positive who do not return to the diagnostic centres or do not produce an adequate sample for the survey and patients whose susceptibility testing does not give interpretable results,[29] (WHO Guidelines for drug resistance Survey).

$$n = N * z^2 * (1 - g) / (N - 1) * d^2g + z2 * (1 - g)$$

Where:

N = total number of new sputum smear positive pulmonary patients registered in the selected sentinel sites during one year;

z = z-value (from the standard normal distribution) that corresponds to the desired Confidence Level (narrowing the Confidence Interval from 95% to 90% will result in some reductions in sample size;

if Confidence Interval =90%, z= 1.65);

d = absolute precision (as a decimal, e.g. 0.01 or 0.02 meaning to err within 1 or 2% of the true proportion);

g = previous estimate of proportion of new cases with rifampicin resistance * (1 + anticipated change of previous estimate). The anticipated change can be considered as the change that the sentinel system should be able to detect. This change is expressed as a decimal, with a negative sign if a decrease is anticipated or a positive sign if an increase is anticipated. For example, a 40% decrease from the previous estimate would be expressed as an anticipated change of -0.4; thus g = earlier estimate * (1 - 0.4) = previous estimate * 0.6

The sample size hence was =256

Where, N=223 per 100,000, d= 0.05%, Z=1.96, P=1.3%

Sample Collection Transport and Storage

Study participants who met the minimum inclusion criteria were recruited into the study. They were then given sputum cups by the clinician or laboratory personnel in the recruiting facility to have their sputum samples taken. A pipette drop from the sample was used to perform bacteriology so as to confirm the sample for acid fast bacilli at the facility and an aliquot of the sample was then parked in screw cups with double biohazard bags inside a cooler box and transported to Kenya Medical Research Institute (KEMRI) Microbiology reference Laboratory in Kisian for further confirmatory staining, culturing and molecular drug resistance testing. Local specimen shipment was done according to regulation provided by the International Air Transport Association (http://www.iata.org/ ads/issa/htm).

At Kenya Medical Research Institute Microbiology reference laboratory, sputum samples together with the laboratory request form were received from health facilities within the County and checked for completeness in filling the laboratory request form, correct sample tube labelling and leakage. Those meeting the acceptance criteria were given laboratory study number and refrigera-

ted at minus four (- 4°C) awaiting processing.

Sample Decontamination and Microscopy

Decontamination of sputum specimen was done using the *N*-acetyl-l-cysteine-sodium citrate-NaOH (NALC-NaOH) method.[29] Samples were then decanted following centrifugation at 3000 g for 15 min, and the pellets re-suspended to make 3 ml using phosphate buffer solution. Four aliquots of 1.0 ml were made from the stock sample, 1 aliquot was used for florescent microscopy, another for phenotypic DST, Line Probe Assay and the remaining stored at – 80 °C as back up. Staining and microscopy was done as follows; Carbol Fuschin was used to flood heat-fixed sputum sample smears. The slide that was flooded was flame heated, after 10 minutes it was washed with deionised water, decolourised with 3% acid alcohol, flooded with malachite green and left for 2 minutes to stain. This stain was then washed with water and smear air dried and later observed microscopically using X100 oil immersion objective.[30] Microscopy was done to all 256 Sputum samples.

Phenotypic Testing

Phenotypic drug resistance testing for *M. Tuberculosis* was done for first line anti tuberculosis drugs using BACTEC™ MGIT™ 960 system (MGIT) (Becton Dickinson (BD) Bioscience, Erebodegem, Belgium) system in the KEMRI Tuberculosis Microbiology Laboratory. After decantation of sediments to be cultured, a vial of Mycobacteria Growth Indicator Tube (MGIT) containing a lyophilised mixture of antimicrobials was reconstituted with 15.0 ml of Mycobacteria Growth indicator supplement. A micropipette was then used to transfer, 0.8 ml of the mixture to each MGIT tube to be inoculated with specimen including both negative and positive controls.

Using a sterile pipette, 0.5ml of the sample was added to labelled MGIT tubes that were closely tightened and inverted a couple of times to get a uniform constitution. The MGIT tubes were then inserted into the BACTEC machine after scanning each tube.[30] The instrument was maintained at a temperature of 37°C + or - 1°C, which was the optimum growth temperature for *M. tuberculosis*.

Mycobacteria Growth Indicator tubes were incubated until flagged positive by the instrument, negative tubes were flagged negative after a maximum period of 6 weeks when no growth occurred. The positive flags were removed and scanned on the instrument which was followed by visual observation of the tube.

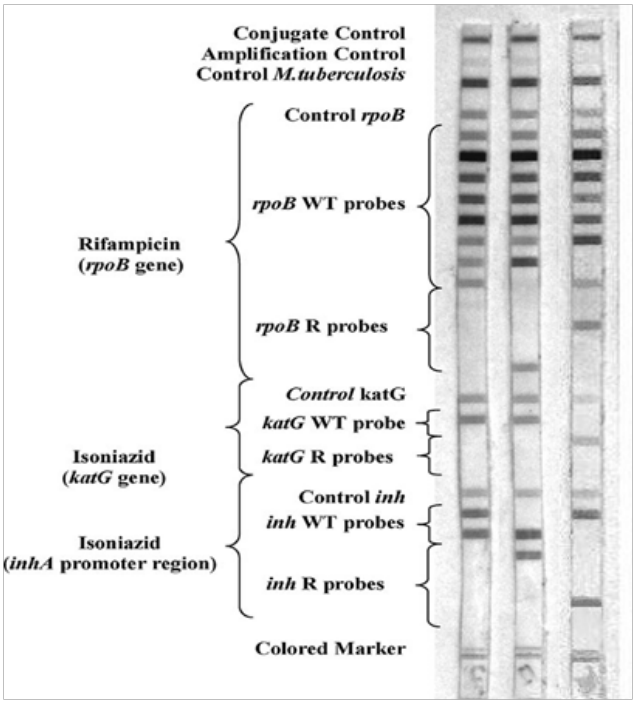
Line Probe Assay

GenoType® MTBDR*plus* assay for detection of first line resistance was performed according to the manufacturer's recommendations (Hain Life Science GmbH, Nehren, Germany). Using multiplex PCR, GenoType® MTBDR*plus* assay was used to target specific mutations in the Rif-resistance determining region (RRD) of the rpoB gene (from codon 505 to 533) to detect rifampicin resistance and mutations in the *inhA* promoter (from -16 to – nucleotides upstream) and *katG* (Codon 315) regions for isoniazid resistance. The genes responsible for first line drug resistance such as *katG*, *inhA*, *rpoB* were amplified and the resulting biotin-labelled amplicons were hybridised to DNA probes bound to membrane probe. For amplification 35µl of a primer nucleotide mixture, buffer for amplification containing 5µl mM MgCl2, 2.5µl of deionised water, 2.5µl Taq polymerase (ROCHE, Mannheim, Germany), and five microlitre of DNA to make a final volume of 50µl. The protocol for amplification consisted of denaturation of 15 min at 95°C then followed by 10 cycles of 30 seconds at 95°C and 2 minutes at 58°C, an additional 20 cycles comprising 25s at 95°C then at 53°C to 70°C of 40 seconds each, and finally extended at 70°C for 8 minutes. Binding of the single stranded amplicons to probes bounded on membrane strips followed by addition of conjugate, then substrate to detect band patterns that are visible on the strip. Then strips were allowed to dry and interpreted according to the instructions provided by the manufacturer. For each gene, GenoType MTBDRplus assay detects the presence of mutant and wild type probes.

Interpretation

Each strip of Line Probe assay had 27 reaction zones and these included 6 controls bands, namely; conjugate band, M. tuberculosis complex, amplification, *rpoB*, inhA and katG, 8 *rpoB* wild type (*WT1–WT8*) and 4 mutant probes (*rpoB MUT D516V, rpoB MUT H526Y, rpoB MUTS531 L and rpoB MUT H526D*), one katG wild type, 2 mutant and 2 *inhA* wild type and 4 mutant probes and *inhA MUT3B T8A* (Figure 1).

FIGURE 1. GenoType MTBDRplus Bands



Source: GenoType MTBDRplus test (Hain Life science GmbH, Nehren, Germany

Either missing wild type band or the presence of mutant band was taken as a symbol of a resistant strain. To prov-

ide a consistent result, all the 6 expected control bands had to appear correctly, otherwise, the result was considered invalid.

Data Management
Data Collection and Storage
The study employed questionnaires, clinical reports and laboratory test reports as the tools for collecting data. Study Participants who met the inclusion criteria were explained to the purpose of the study, possible risk and benefits and those who agreed to participate in the study were duly informed, consented and enrolled into the study.

This study had both paper and electronic study forms for each patient, linked by a unique study Identification (ID) number given to each participant. The list linking study ID numbers with specific individuals (face sheet) was stored separately and securely, i.e., in a different physical location, in a locked Good Clinical Practice (GCP) compliant cabinet at Kenya Medical Research Institute, Centre for Global Health(KEMRI-CGHR)-Kisumu.

The first form contained all demographic data and clinical data for the participants. The second form was a laboratory request form and contained all laboratory test results such as mycobacteriology data, smear microscopy, DST and LPA test results and dates of all tests. All study form data were kept in locked Good Clinical Practice complaint file cabinets in the clinics and laboratories at KEMRI-CGHR -Kisumu. Electronic files in the electronic database were stored in the password-protected computers. Confidentiality was assured by ensuring that all data-containing study forms and specimens were identified using study ID numbers unique to each participant.

All laboratory information was communicated directly to the clinicians and collated in the patients' charts and on the laboratory study forms. Only clinical and study staff had access to information collected or generated as part of this study.

Data Analysis
Data was collected onto paper and electronic forms and then entered into Laboratory Information management system database. The study database had quality check codes built in and was also checked against primary sources from clinicians or laboratory technicians. Monitoring of the study site was conducted every month. Statistical Package for the Social Sciences (SPSS) v23 (SPSS Software | IBM) was used for data analysis and it merged the clinical and the laboratory databases prior to analysis.

Demographic data such as Sex, Age were analysed using Descriptive Analysis. Mean was used to determine the mean Age among sample Tuberculosis cases while mode was used to determine the modal sex mostly affected by Tuberculosis drug resistance. Frequency tables and bar charts were used to present this data. Inferential statistics was used to analyse categorical test results such as New and previous TB cases, HIV status, bacteriological smear and Culture MGIT Test results. Multiple response LPA drug resistance results had the variables defined and presented in frequency tables. Chi square test was used to assess the association between HIV status and drug resistance conferring mutations in Kisumu County.

Cross tabulation was used to explore First line drug resistance mutation patterns among new and previously treated cases and factors associated with drug resistance. Associations were considered statistically significant when *p-value* was less than or equal to *.05*.

Ethical Considerations
Linking of identifying information such as patient name and birth date to the study identification number appeared only on a cover sheet of the patient's data form. These data forms were kept in a locked GCP-compliant cabinet at Kenya Medical Research Institute, Centre for Global Health Research, Kisumu and were only accessible by the investigation team. Sputum samples were labelled with the date of collection and patient's study identification number. After the end of the study, the cover sheet was destroyed, unlinking the study identification number and de-identifying the data. Data entry was performed on site by the local investigators on a password protected computers and only the study investigators and data staff had access to this data.

Direct LPA which is not a routine practice was used to shorten laboratory turnaround time. Ethical approval was provided by Kenya Medical Research Institute, Scientific Ethical Review Unit (KEMRI/SERU/CGHR/002-02-330/4079) and National Commission for Science, Technology & Innovation (NACOSTI/P/21/10981).

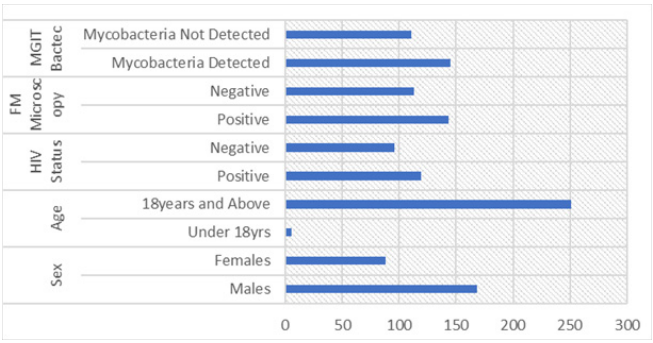
RESULTS
General Characteristics of the Study Population
A total of 256 sputum samples from Tuberculosis clinical suspected cases from Kisumu County, Kenya for period of 12 months, November 2020 to October 2021 were included in the study. The samples received were classified as new TB cases and previously treated cases as per the WHO guidelines for surveillance of drug resistance in tuberculosis.

Socio-Demographic Characteristics of the Respondents
Out of 256 study participants, 216 had their HIV status known while 40 did not respond (none response) to the HIV status question. Of the 216 respondents with known HIV status, 37 (17.1%) were new cases, while 179 (82.9%) were retreatment cases. Out of the 216, 137(63.4%) were male while the remaining 79 (36.6%) were female. Aged below 18 years were 5(2.3%), while aged 18 years and above were 211(97.7%). The ages showed a normal distribution with a mean age of 40 years, a Standard Deviation of ± 12.9 and a range of 13 to 77 years. Data on drug resistance stratified by age group and sex provides insight into risk groups and effectiveness of specific TB control activities. Additionally, the magnitude of drug resistance among younger age group is more likely to be indicative of recent transmissions than among older age group, who may be harbouring older infections. From the sample, HIV positive cases were 119(55.1%) and negative were 97 (44.9%). None response for HIV status were 41 participants. (Figure 2)

Characteristic of TB Confirmed Cases
Out of a total of 145 Mycobacteria confirmed cases on MGIT BACTEC from Tuberculosis suspected cases, 32 (22

%) were from new TB cases and 113(78%) from retreatment cases. Males were 112 (77.2%) while females were 33(22.8%) for bacilli confirmed cases. Aged below 18 years were 5(2.3%), while aged 18 years and above were 211(97.7%), 119(55.1%) were positive while 97 (44.9%) were negative. (Figure 3).

FIGURE 2. Socio-Demographic Characteristics of the Respondents



Factors Associated with HIV Status Outcome

Out of 37 Tuberculosis new cases, 15(40.5%) were positive for HIV while positivity among on treatment cases was 104(58.1%). Chi square test for TB cases and HIV Status showed (χ2=3.822, df=1, *P=.051*), (OR=0.49,95%CI (0.23-1.01). Out of 121 samples that showed mycobacteria detected from BACTEC Culture, 75(62%) were positive for HIV while 46(38%) were HIV negative. Chi square test for BACTEC culture results and HIV status outcome was (χ2=5.28, df=1, p=0.022), (OR=1.89,95%CI:(1.09-3.20).

Out of 84 samples that were positive on FM AFB, 46(54.8%) were positive for HIV, while 38(45.2%) were HIV negative. Chi square test for FM AFB and HIV status was (χ2=0.006, df=1, p=0.938), (OR=0.98, 95%CI: (0.71-1.38). Out of 87 samples that showed mycobacteria detected from First line LPA, 53(60.9%) were positive for HIV while 34(39.1%) were negative. Chi- square test for FL LPA and HIV Status was (χ2=1.99, df=1, *p=.157*), (OR=1.27, 95%CI: (0.91-1.78). (Table 1)

Phenotypic and Molecular Drug Resistance among HIV Cases

First line phenotypic drug resistance for Isoniazid showed a total of 11(5.1%) out of which 8 (6.7%) were HIV positive and 3(3.2%) were HIV negative cases. Chi-square test of association between FL DST for isoniazid resistance and HIV status showed that (χ2=1.457, df=1, *p=.186*), (OR=2.17,95%CI :0.59-7.97)).

First line phenotypic rifampicin resistance showed that 10(4.6%) were resistance detected, out of which 8 (6.7%) were HIV positive and 2(2.1%) HIV negative. Chi- square test of association between rifampicin resistance and HIV status showed (χ2=2.62, df=1, *p=.095*), (OR=3.3,95%CI (0.71-14.9). First line LPA drug resistance for isoniazid showed that out of 9(4.2%) that were resistance detected, 6(5.0%) were HIV positive while 3(3.1%) were HIV negative. Chi- square test of association between FL LPA drug resistance for isoniazid and HIV Status showed (χ2=0.508, df=1, *p=.36*), (OR=1.63,95%CI :0.42-6.35). First line LPA drug resistance for rifampicin showed that out of 10(4.6%) that were resistance detected, 8 (6.7%) were HIV positive while 2(2.1%) were HIV negative. Chi-square test of association between FL LPA drug resistance for rifampicin and HIV status showed (Chi-Square=2.742, df=1, *p=.36*), (OR=4.89,95%CI: 0.59-39.94). (Table 2).

Mutant and Wild Type Gene Probes

The study found out that mutant probes among the HIV positive were *inhA MUT1* 1(0.7%), katG *MUT1* 4(2.6%), *roB MUT2A* 3(2.1%), *roB MUT3* 1(0.7%), *roB MUT3/katG MUT1* 1(0. 7%). Mutant probes among the HIV negative were *inhA MUT1* 1(0.7%), *katG MUT1* 1(0.7%) and *roB MUT2A* 1(0.7%). Wild Type gene deletion among the HIV positive cases were observed in probes *katG WT* 3(2.1%), *roB WT7*, *katG WT* 1(0.7%). Wild Type gene deletion among the HIV negative cases were *inhA WT1* 1(0.7%), *inhA WT1/inhAWT2* 1(0.7%), *katG WT* 1(0.7%). (Figure 4)

Codon and Amino Acid Change among HIV Outcome

Codons analysed among the HIV positive participants were; codon-*15* 1(0.7), codon *315* 4(2.8%), codon *526* to *529* 4(2.8%), codon *530* to *533* 2(0.9%). Codons analysed among the HIV negative participants were; codon-*15* 2(1.4%), codon *315* 1(0.7%), codon *526* to *529* 1(0.7%). Amino acid changes among the HIV positive cases were; *C15T* 1(0.7%), *H526R*, *S315T1* 1(0.7%), *H526Y* 3(2%), *S315T1* 3(2%), *S531L* 1(0.7%), *S531L*, *S315T1* 1(0. 7%). Among the HIV negative cases were; *C15T* 2(0.9%), *H526Y* 1(0.5%), *S315T1* 1(0.5%). Out of 9 (4.2%) that were INH resistance on Line Probe assay, 6(5.0%) were from HIV positive cases while 3(3.1%) were from HIV negative cases. Additionally, out of 10(4.6%) that were rifampicin resistance, 8(6.7%) were from HIV positive cases while 2(2.1%) from HIV negative cases. All the 2 MDR cases arose from positive participants representing (0.02%) of the total HIV positive participants. (Figure 5)

DISCUSSIONS

Out of 256 study participants, 216 had their HIV status known while 40 did not respond (none response) to the HIV status question. This study found out that out from the response of 216, there were more males 137(63.4%) compared to females 79 (36.6%). This is in agreement with the WHO report that indicate that relatively more males than females are exposed to Tuberculosis and this could be attributed to the difference between the two sex groups in biological, societal role and access to health facilities.[8] Majority of participants were aged 18 years and above 211(97.7%) while the remaining 5(2.3%) were aged under 18 years.

All the patients had a mean age of 40 years with a Standard Deviation of ± 12.9 and a range of 13 to 77 years. The current study is in good agreement with a study reporting that the 31 to 40 years' age group was the most predominant group for isolation of DR-TB and that male population was at the highest risk.[31] According to Ahmed et al in a study that was conducted in India which is one of the high burden Tuberculosis countries, it was found that 17.2% of collected samples were from

TABLE 1: Factors Associated with HIV Status Outcome

		No of Patient (%) n=216	HIV Positive	HIV Negative	P-Value	OR (95%CI)
TB Case	Retreatment	179(82.9)	104(58.1)	75(41.9)	0.51	1.40(1.00-19)
	New TB Case	37(17.1)	15(40.4)	22(59.5)		
MGIT_ BACTEC	Mycobacteria Detected	121(56)	75(62)	6(47.4)	0.022	1.89(1.09-3.20)
	Mycobacteria Not Detected	95(44)	44(46.3)	51(52.6)		
FM_AFB	Positive	119(55.1)	46(38.7)	38(39.2)	0.938	0.98(0.71-1.38)
	Negative	97(44.9)	73(61.3)	59(60.8)		
LPA Tuberculosis	Mycobacteria Detected	87(40.3)	53(44.5)	34(35.1)	0.157	1.27(0.91-1.78)
	Mycobacteria Not Detected	129(59.7)	66(55.5)	63(64.9)		

TABLE 2: Phenotypic and Molecular Drug Resistance among HIV Cases

	HIV Positive N(%)	HIV Negative N(%)	Total Resistance N(%)	P Value	OR (95%CI)
DST Isoniazid	8(6.7)	3(3.2)	11(5.1)	.227	2.17(0.59-7.97)
DST Rifampicin	8(6.7)	2(2.1)	10(4.6)	.105	3.3(0.71-14.9)
LPA Isoniazid	6(5.0)	3(3.1)	9(4.2)	.476	1.63(0.42-6.35)
LPA Rifampicin	8(6.7)	2(2.1)	10(4.6)	.98	4.89(0.59-39.94)

FIGURE 3. Characteristic of TB Confirmed Cases

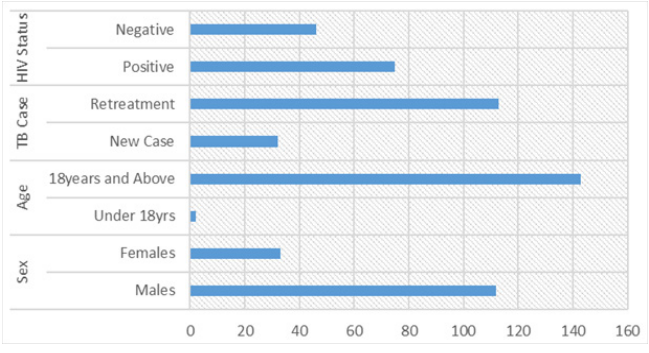
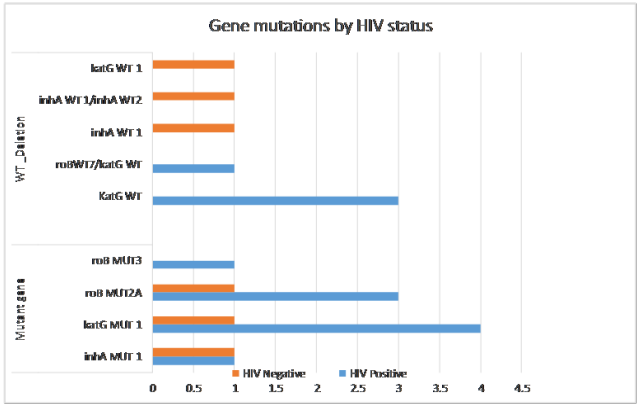


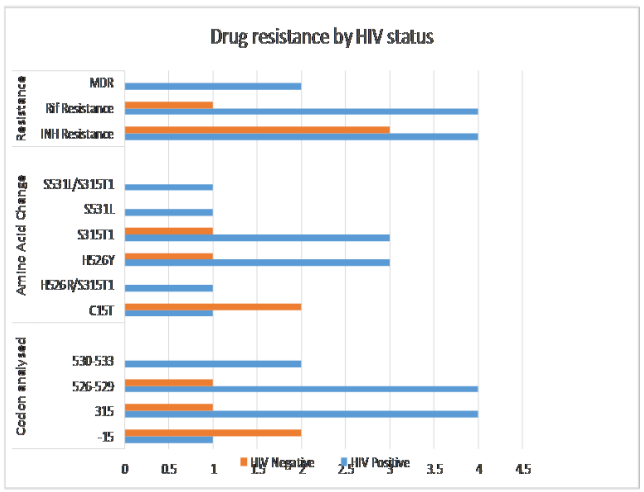
FIGURE 4. Chart of Mutant and Wild Type Gene Probes



new cases and 82.8% were from previously treated cases.[32] These findings are consistent with the current study that found out that majority of TB cases were retreatment cases 179(82.9%) while new Tuberculosis cases were 37 (17.1%). Children under the age of 18 years were more likely to be associated with isoniazid resistance (OR 5.02,95%CI:0.785-32.095) and resistance to rifampicin (OR 5.58,95%CI:0.862-36.072) compared to adults of 18 years and above. Additionally, the study showed that females were more likely to develop isoniazid resistance, Odds Ratio (OR 1.59,95%CI:0.499-5.067) and rifampicin resistance (OR 2.86,95%CI:0.83-9.882) compared to males.

General isoniazid resistance was 11(5.1%) in all the cases while rifampicin was 10(4.6%) across all the TB cases.

FIGURE 5. Chart of Mutant and Wild Type Gene Probes



This study showed that, out of a total 145 that confirmed mycobacteria detected, there was anon response of 24 for the variable HIV status. Out of a total of 121 confirmed Tuberculosis cases that responded to the HIV status variable, 75(62.0%) were HIV positive while 46(38.0%) were HIV negative. Positivity among the new cases was 15(40.4%) while positivity among the on-treatment cases was 104 (58.1%). According to a study conducted among drug naïve patients in Nairobi Kenya, HIV increased the incidence of TB and the risk of TB infection by 16 to 27 times in PLHIV than in the general population.[12]

Sing et al also estimated that PLHIV, especially with fewer than 200/cm3 CD4 count show a 19 (15 to 22) -fold increased risk of developing active TB compared with those who are HIV negative.[5] These findings are consistent with the current study that found out that HIV status and Tuberculosis cases were significantly associated at $p<.05$. Specifically, HIV positive cases were more likely associated with retreatment cases (OR 2.0,95%CI:1.0-4.3) compared to new cases. First line Phenotypic drug resistance for isoniazid showed a total of 11(5.1%) out of which 8 (6.7%) were HIV positive and 3(3.2%) HIV negative, while rifampicin resistance showed that 10(4.6%) were resistance detected, out of which 8 (6.7%) were HIV positive and 2(2.1%) HIV negative. First line LPA showed that out of 9 (4.2%) that were INH resistance, 6(5.0%) were from HIV positive cases while 3(3.1%) were from HIV negative cases.

Additionally, out of 10(4.6%) that were rifampicin resistance, 8(6.7%) were from HIV positive cases while 2(2.1%) from HIV negative cases. Of all the MDR cases, 2 were from HIV positive participants which was an indicator of poor treatment outcome for HIV cases.

The Odds ratio for rifampicin resistance among the HIV positive cases was higher for both DST rifampicin (OR 3.3,95%CI:0.71-14.9) and LPA rifampicin (OR 4.89,95%CI:0.59-39.94) compared to isoniazid DST isoniazid (OR 2.17,95% CI:0.59-7.97) and LPA isoniazid (OR 1.63,95%CI:0.42-6.35).

Chi square test showed that there was no significant relationship between Tuberculosis drug resistance and HIV for phenotypic Isoniazid resistance, ($\chi2=1.457$, df=1, $P=.227$) and rifampicin resistance, ($\chi2=2.629$, df=1, $P=.105$) and molecular Isoniazid resistance ($\chi2=0.508$, df=1, $P=.476$) and rifampicin resistance ($\chi2=2.742$, df=1, $P=.98$). These findings are consistent with findings from Khan et al that showed that there was no evidence that HIV infection modifies the fitness of drug-resistant strains.[26,33,34]

The study found out that HIV positive clients had high INH mutations in the promoter region of inhA gene at codon *-15* with amino acid change of *S315T1*, while low INH resistant strains had mutations in the *katG* gene at codons *315*. Additionally, HIV positive clients experienced mutations at codons *526* to *529* and *530* to *533* in the *rpoB* genes with amino acid changes *H526Y* and *S531L*. All the MDR strains were from HIV positive cases and had mutations in the *rpoB* and *katG* genes. This is consistent with a systematic review from Sultana et al, that found out that the odds of developing MDR-TB in HIV infected patients was 42% higher than those of HIV negative individuals.[35] In other studies conducted in regions of HIV prevalence, there was growing evidence to suggest that infection with more than one strain occurred.[36,37] The r*poB* gene displayed mutations at codons *530* to *533* with amino acid changes of *S531L* and *S315T1*, while *katG* had mutations at codon *526* to *529* and *315* with amino acid changes of *H526R* and *S315T1*. HIV negative clients experienced mutations in the inhA, katG and rpoB genes.

The study found out that the frequent mutant probes among the HIV positive was *katG MUT1* 4(2.8%), while common mutant probes among the HIV negative was *katG MUT1* 1(0.7%) and *roB MUT2A* 1(0.7%). Wild Type gene deletion among the HIV positive cases were observed highest at probes *katG WT* 3, whereas Wild Type gene deletion among the HIV negative cases were associated mostly with h probes *inhA WT1* 1(0.7%), *katG WT* 1(0.7%). Greater variability and unknown mutations was observed in mutant probes from HIV positive cases than in HIV negative cases.

CONCLUSION

This study showed that there was no significant relationship between Tuberculosis drug resistance and HIV status. This could be attributed to lack of comprehensive data stratification on HIV testing and TB drug resistance testing. Surveillance data from different studies in different geographical settings have attributed discordant associations due to heterogeneity in setting, demographic profile, methodology and analysis of data. Children under the age of 18 were more associated with isoniazid and rifampicin resistance compared to adults. The magnitude of drug resistance among younger age groups is more likely to be indicative of recent transmission from older age groups, who may be harbouring older infections. The County and National government should strengthen Tuberculosis drug resistance surveillance among children especially in HIV high burden regions like Western Kenya. Additionally, a greater variability in mutations and presence of unknown mutations were observed in HIV positive participants compared to HIV negative patie-

nts. This could be an indicator of poor outcomes for Tuberculosis patients who are co-infected with HIV. Understanding Tuberculosis molecular epidemiology and its variability among the HIV prevalent populations emphasizes the need for research in HIV-endemic settings to develop appropriate regional specific interventions for drug resistant tuberculosis. Greater changes in amino acid sequences among retreatment cases compared to new cases that were observed may be an indication that such mutations might be acquired during treatment courses by repeated administration of the same anti-TB drugs. Further population-based studies are required to guide policies on transmission of drug-resistant tuberculosis strains in HIV endemic settings like Kisumu County, Western Kenya.

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ORIGINAL ARTICLE

Prevalence, Clinical Presentation and Factors Associated with Uterine Fibroids Among Women Attending the Gynecology Outpatient Department at a Large Referral Hospital in Southwestern Uganda

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ABSTRACT

Background: Uterine fibroids are the most common benign female gynecologic tumors. There are multiple risk factors, including age and reduced fertility. There is however a paucity of data on disease burden and risk factors among African populations.

Objective: We determined the prevalence, clinical presentation and factors associated with uterine fibroids among women at Mbarara hospital gynecology clinic, Uganda.

Methods: We conducted a cross sectional study from November 2018 to February 2019 on 319 women attending gynecology clinic. An abdomino-pelvic ultrasound scan was performed on each participant and data analyzed using Stata Version 13. Multivariable logistic regression was used to determine association between selected characteristics and uterine fibroid appearance. P value of less than 0.05 was interpreted as significant.

Results: The number of women with fibroids was 90 out of 319, representing a prevalence of uterine fibroids of 28.2%. About 67 (74.4%) of the participants with fibroids were symptomatic having pelvic pain 65 (72.2%), menorrhagia 57 (63.3%), pelvic mass 20 (22.2%) and failure to conceive 9 (10%). Women in age group of 31 – 50 years (adjusted OR 4.2; 95% CI,2.0 to 8.5), those separated from their spouses (adjusted OR 4.4; 95% CI,1.8 to 10.5), overweight (adjusted OR 4.9; 95% CI, 2.6 to 9.6), obesity (adjusted OR 4.1; 95% CI,1.6 to 10.5) were more likely to be diagnosed with uterine fibroids while delayed menarche (adjusted OR 0.4; 95% CI, 0.1 to 0.8) was protective.

Conclusion: The study found the prevalence of uterine fibroids to be high. Majority of patients were symptomatic at presentation with pelvic pain, menorrhagia, irregular menses and pelvic mass. Uterine fibroids cause significant morbidity among reproductive age women. The identified risk factors included overweight and age group of 31 to 50 years. We recommend Ultrasound scan in women of reproductive age attending gynecology clinic to detect uterine fibroids early in order to manage them promptly so as to prevent the associated complications.

Background

Uterine fibroids, also known as uterine myomas or leiomyomas, are benign, monoclonal tumors of the smooth muscle cells of the uterus.[1] Uterine fibroids are composed of smooth muscle cells, vascular smooth-muscle cells, fibroblasts and extracellular matrix (ECM).[2] They are non-cancerous tumors which are myometrial in origin.[3] It is uncommon for *fibroids* to develop into cancer (leiomyosarcoma) and this occurs in<0.1 % of cases.[4] They are the most common benign tumors in women of reproductive age and are asymptomatic in at least 50% of afflicted women.[5]

Transvaginal and abdominal ultrasound scanning is the golg standard method for diagnosis of uterine *fibroids* in terms of accuracy and availability, with magnetic resonance imaging being a close second option in complex clinical circumstances.[6,7] The management of uterine fibroids can be approached medically, surgically, and even by minimal access techniques.[6] According to a 2010 World Health Organization report, fibroids affect between 20 to 25% of women, and close to 235 million women representing 6.6% of global women population are estimated to be affected worldwide.[8,9] Etiology of uterine *fibroids* is unknown and advances have been made in understanding the hormonal factors, genetic factors, growth factors, and molecular biology of these benign tumors.[10] Uterine *fibroids* decrease the quality of life by causing significant morbidity among women of reproductive age and the clinical effects of

of these tumors are related to their local mass effect, resulting in pressure upon adjacent organs, pelvic pain or problems related to pregnancy, including infertility and repetitive pregnancy loss.[11] Some of the clinical manifestations are heavy and prolonged menstrual bleeding, anemia secondary to bleeding,[12] increased urinary frequency, and bowel disturbance.[13] As a consequence, uterine *fibroids* are ranked as the major reason for hysterectomy.[14]

The Previous studies suggest multiple risk factors for developing *fibroids* but the exact etiology of *fibroids* is unknown. Uterine *fibroids* have been found to be commonly associated with age within the reproductive age group (35 to 49) but the rate of increase slows at older ages suggesting older premenopausal uterus is less susceptible to fibroid development.[15] Other risk factors for *fibroids* include parity which has been and still is inversely associated with a risk of fibroid development and it's postulated that during postpartum uterine remodeling, there could be selective apoptosis of small lesions and ischemia during parturition has been proposed as a mechanism.[16,17]

Coffee and caffeine consumption are associated with increased levels of early follicular phase E_2, independent of alcohol or tobacco use[18] and may enhance sex steroid production. Other factors associated with uterine *fibroids* include early menarche, caffeine intake, reduced fertility, obesity, consumption of red meat, hypertension, diabetes mellitus.[19,20] However, there is still a paucity of data on disease burden and risk factors among African populations. This study was therefore undertaken to determine the burden, clinical presentation and factors associated with uterine *fibroids* in women attending gynecology outpatient department at Mbarara regional referral hospital in south western Uganda.

METHODOLOGY

Study design and population

We conducted across-sectional study among 319 women seeking services at the gynecology outpatient department at Mbarara regional referral hospital (MRRH) between November 2018 and February 2019. Mbarara Hospital is a public regional referral hospital that serves as a teaching hospital for Mbarara University of Science and Technology as well. The hospital serves as a referral for the south western region of Uganda for a population of about 3 million.

Mbarara regional referral hospital has different departments including radiology department where our study participants had their ultrasound scans were done. There's also a Gynecological Outpatient Department (GOPD) where these women were recruited from. Women are screened at the general Outpatient Department (OPD) and based on their symptoms are sent to the GOPD, but some women will present with referral notes directly having attended another health facility before MRRH.

Sample Size Calculation

We determined the sample size by using the Keish and Leslie (1995) formula as below;

$n= Z^2pq/d^2$ Where n=sample size; Z=1.96 (the Z score value corresponding to 95% level of confidence interval); p=percentage frequency of outcome factor in the population. For this study, we used the prevalence of uterine fibroids among women attending the gynaecology outpatient clinic in Nigeria.[21]

q=1 – p, d=0.05 which is the margin of error. Thus, the calculated sample size was 319.

Sampling Procedure

Women were told about the study by a research assistant, after their consultation was completed and invited to participate. Only those who provided voluntary informed written consent were eligible to participate in the study. They were consecutively recruited and underwent the study procedures, including ultrasound scan to ascertain presence or absence of fibroids.

Data Collection Procedure

The research assistants and principle investigator administered interviews translated into the local language of Runyankore. We collected data on basic demographics, medical factors, gynecological factors, obstetrical factors and lifestyle factor. The interviews were conducted after the patient consultation was completed to prevent interference from the routine clinical care.

Assessment for uterine *fibroids*: The primary outcome for this study was presence of uterine fibroids. The abdomino-pelvic ultrasound scan reports confirmed the diagnosis of uterine *fibroids* when there was presence of well-defined solid hypoechogenic or hyperechogenic mass in the uterus with or without calcifications. A specialist radiologist at MRRH conducted the ultrasound scan. The results were relayed back to the clinician for patient management and the same information was collected as part of the study variables.

Inclusion and Exclusion Criteria

We included women who were aged at least 18 years seeking health care at the GOPD at MRRH, regardless of the symptoms. Women who were below 18 years of age and those who did not consent to participate in the study were excluded from the study.

Statistical Analysis

The data was collected using the questionnaires and entered into REDCap then exported to the software Stata Version 13. Summary statistics were used to characterize the participants. The categorical variables like age group, marital status, age category at menarche, parity, history of use of oral contraceptives, and smoking, were summarized using percentages or proportions. We compared potential factors associated with uterine *fibroids* such as the socio-demographics characteristics for women with and those without uterine *fibroids*. Chi-squared test analysis for categorical variables was performed. We considered *P* values <.05 to be statistically significant. We used multivariable logistic regression analysis to identify factors independently associated with uterine fibroids. Backwards stepwise elimination was used to create the final model. Data from all participants enrolled into the study was included in the final analysis.

Ethical consideration

We obtained ethics approval from the Faculty of Medicine

Research Committee, Mbarara University, The Mbarara University Institutional Ethical Research Committee and administrative approval was sought from the office of the Executive Director at MRRH before the process of data collection started. We obtained final approval from the Uganda National Council of Science and Technology (Reference number is HS384ES). Informed consent was obtained from all respondents. Access to data was limited to those directly involved in the study by providing specific passwords and usernames to the Principle Investigator and the Research Assistants. The PI and Research Assistants had different levels of database access. The PI had rights to enter, export data and analyze the entered data while the Research Assistants had rights to enter data and save the data. The consent form was translated into the local language (Runyankole). Confidentiality was ascertained by interviewing the participants in a room where no other patients were. This helped the participants to have their information volunteered with ease knowing that no one else was listening through except the research assistants doing the interviews.

RESULTS

Three hundred nineteen (319) study participants were enrolled into the study, 90 (28.2%) of these had uterine fibroids. There were significant differences in age groups, marital status, body mass index and age of menarche between women with and those without uterine fibroids as summarised in Table 1.

Women with fibroids were older than those without and were mostly in the 31 to 50 age category. Majority of women with fibroids were separated from their husbands or were obese. Of the 90 cases with uterine fibroids 67 (74.4%) were symptomatic while 23 (25.6%) were asymptomatic and the Majority respondents with uterine fibroids had lower abdominal pain 72.2%, menorrhagia were 63.3%, irregular menses 50%, pelvic mass 22.2%, failure to conceive 10%, recurrent miscarriage 4.4% and urine retention 2.2% was the least common of the clinical presenting features (Table 2).

In multivariate logistic regression, Age group between 31 to 50 years (OR 4.2; 95% CI, 2.0 to 8.5, *P≤.001*), separation from spouse (OR 4.4; 95% CI, 1.8 to 10.5; *P<.002*), overweight (25 to 29.9kg/m2) (OR 4.9; 95% CI, 2.6 to 9.6; *P≤.001*) and obesity (>30kg/m2) (OR 4.1; 95% CI, 1.6 to10.5; *P<.004*), while late age of menarche (OR 0.3; 95% CI, 0.1 to 0.8; *P<.015*) maintained their significant association with uterine fibroids (Table 3).

DISCUSSION

The prevalence of uterine *fibroids* at Mbarara Regional Referral Hospital (MRRH) was 28.2%. This is comparable to findings from the studies elsewhere in sub Saharan Africa, notably in Nigeria[22] where the prevalence was 29.3% and Kenya prevalence of uterine fibroids ranged from 10 to 20% whereas in Ghana, it was 36 %.[3] The participants in all these studies share common features in the socio demographic characteristics and the studies were all conducted at tertiary hospital settings, similar to our study setting.

Our study found that majority of the study participants had symptomatic uterine fibroids at presentation.

TABLE 2: Clinical Presentation of Uterine Fibroids

Clinical presentation of uterine fibroids	Frequency n=90	Percentage (%)
Pelvic pain	65	72.2
Menorrhagia	57	63.3
Irregular menses	45	50
Pelvic mass	20	22.2
Failure to conceive	9	10
Recurrent miscarriage	4	4.4
Urine retention	2	2.2

The commonest clinical presenting symptoms included: pelvic pain, menorrhagia and irregular menses. Other clinical features included pelvic mass, failure to conceive, recurrent miscarriage and urine retention. These findings are in agreement with a study done in Ghana where the majority of these women had menstrual irregularities.[3] The study done in Nigeria found that pelvic mass was the commonest clinical presentation unlike in our study.[22] This is because study in Nigeria included only women with pelvic masses. Our study found majority of our participants were symptomatic unlike other studies that showed uterine fibroids were asymptomatic.[23-25] One potential explanation for this finding is that our study was hospital based where by patients presented with symptoms as the reason for attending while other studies screened community-based participants where the majority had no symptoms.

Our study found that women in the age group of 31 to 50 years were more likely to have uterine fibroids compared to other age groups, younger or older. This is similar to findings of other studies such as in Israel where uterine fibroids were more common among women between age of 31 and 50 years,[26] with similar findings in Nigeria and Ghana.[3] This could be explained by the exposure to high steroid hormones namely estrogen and progesterone during this period.[27] In our study setting, most women complete their family about 35 years and therefore they have the long fertile period without pregnancy which is a potential risk factor. We also found out that being separated from a spouse was significantly associated with having uterine fibroids. These findings are in agreement with those from a study done by Laughlin and group.[15] This finding may be explained by the long pregnancy free intervals characterized by the absence of ovarian activity which is the risk factor for uterine fibroids.

Our study found out that being overweight and obese were highly associated with having uterine fibroid which is similar to the other studies which showed high body mass index to be associated with fibroids.[3,28,29] This is because fat tissue converts testosterone into estrogen, and obesity can lead to decreased levels of a protein called sex hormone binding globulin that binds to estrogen and progesterone, resulting in more unbound hormones.[27]

These combined effects result in more estrogen and progesterone within the uterus, which may lead to fibroid development. The study also found late age of

TABLE 1: DemographicCharacteristics of Study Participants

Characteristics	All N=319	With uterine fibroids n=90	Without uterine fibroids n=229	Pvalue
Age in years				
<30	151(47.3)	20(22.2)	131 (57.2)	≤.001
31 – 50	142(44.5)	63(70.0)	79 (34.5)	
51 – 70	26(8.2)	7(7.8)	19 (8.3)	
Marital status				
Married	180(56.4)	21(23.3)	159 (57.2)	≤.001
Separated	66(20.7)	51(56.7)	15 (6.6)	
Single	42(13.2)	6(6.7)	36 (15.8)	
Widow	31(9.8)	12(13.3)	19 (8.3)	
Educational level				
None	20(6.3)	7(7.8)	13 (5.7)	.108
Primary	149(46.7)	43(47.8)	106 (46.3)	
Secondary	91(28.5)	18(20.0)	73 (31.9)	
Tertiary	59(18.5)	22(24.4)	37 (16.2)	
Body mass index (BMI)				
Under weight (<18.5)	11(3.5)	1(1.1)	10 (4.4)	≤.001
Normal (18.5-24.9)	147 (46.1)	19(21.1)	128 (55.9)	
Overweight (25-29.99)	127 (39.8)	55(61.1)	72 (31.5)	
Obese (>30)	34 (10.7)	15(16.7)	19 (8.3)	
Gynecological factors, n (%)				
Parity				
Nullipara (0)	45(14.1)	18(20)	27(11.8)	.172
Multipara(1-5)	202(63.3)	52(57.8)	150 (65.5)	
Grand multipara (>6)	72(22.6)	20(22.2)	52(22.7)	
Menarche				
Early (<12)	16(5.02)	4(4.4)	12(5.2)	.049
Normal (13-15)	267(83.7)	81(90)	186 (81.2)	
Late (>=16)	36 (11.3)	5(5.6)	31(13.5)	
Family planning , n(%)				
Oral contraceptive pills				
Yes	**33(20.8)**	**8(24.2)**	**25(19.8)**	**.580**
Medical factors , n(%)				
Hypertension				
Yes	21(6.6)	9(10)	12(5.2)	.129
Life style factors, n(%)				
Smoking				
Yes	16(5.0)	4(4.4)	12(5.2)	.770
Alcohol use				
Yes	67(21.0)	23(25.6)	44(19.2)	.212

n=frequency; %=Percentage

menarche (>16 years) was protective factor of uterine fibroid similar to findings of other studies.[30] This is because of delayed exposure to steroid hormones that predispose to fibroids.

Our study had some strengths: First, it was conducted in southwestern Uganda, making it one of the few studies to investigate this subject in resource limited settings. Secondly, the study involved a sizeable number of participants hence making our findings more generalizable to the population despite this being a hospital-based study.

However, our study had a limitation. The definitive diagnosis of uterine fibroids was not absolute since we were not able to conduct histology. We were making our conclusions based on the features of uterine fibroids on ultrasound and clinical examination.

TABLE 3: Factors Associated WithUterine Fibroids

Characteristics	Univariate OR (95% CI)	P-value	Multivariate OR(95%CI)	P-value
Age in years				
<30	Ref			
31-50	5.2(2.9 - 9.3)	.000	4.2(2.0 - 8.5)	≤.001
51-70	2.4(0.9 - 6.5)	.080	2.5(0.9 - 8.6)	.140
Body Mass Index				
Normal (18.5-24.9)	Ref			
Underweight (<18.5)	0.7(0.1 - 5.6)	0.714	0.6 (0.1 - 5.8)	.677
Overweight (25-29.9)	5.1(2.8 - 9.3)	0.000	4.9 (2.6 - 9.6)	≤.001
Obese (>30)	5.3(2.3 - 12.2)	0.000	4.1 (1.6 - 10.5)	.003
Marital status				
Married	Ref			
Separated	4.4(2.0 - 9.0)	0.000	4.4 (1.8 - 10.5)	.001
Single	0.5(0.2 - 1.3)	0.163	1.5 (0.5 - 4.6)	.443
Widow	1.9(0.8 - 4.3)	0.092	2.2 (0.8 - 5.8)	.124
Menarche				
Normal (13-15)	Ref			
Early (<12)	0.8(0.24 - 2.4)	0.852	0.9 (0.2 - 3.8)	.878
Late (>16)	0.4(0.2 - 0.9)	0.047	0.3 (0.1 - 0.8)	.014

CI confidence interval, OR odd ratio

CONCLUSION

Our study found the prevalence of uterine fibroids to be high. Majority of patients were symptomatic at presentation with pelvic pain, menorrhagia, irregular menses and pelvic mass. The factors associated with Uterine fibroids included; age group 31 to 50 years, high BMI, having separated from the spouse while late age at menarche was protective. We recommend routine ultrasound scanning in women of reproductive age attending gynecology clinic to detect uterine fibroids early in order to manage them promptly so as to prevent the associated complications. This will reduce on the morbidity associated with uterine fibroids. Weight reduction campaigns should be encouraged among women of reproductive age.

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EAHRC
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ORIGINAL ARTICLE

Aetiology and Antimicrobial Susceptibility Pattern of Bacteria Pathogens from Hospitalised Adult Patients at a Tertiary Care Hospital in North Eastern Tanzania

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ABSTRACT

Background: Antimicrobial resistance (AMR) is a dynamic and a rapidly increasing health concern worldwide. However, it is unevenly distributed with limited data from the developing countries. In Tanzania, it is estimated that there is a higher prevalence of AMR pathogens among hospitalised patients in tertiary care hospitals than in lower level health facilities. This is associated with longer hospitalisation, increased health care costs and higher mortality rates. The aim of this study was to determine the aetiology and AMR pattern of bacteria isolates from adult patients admitted at Kilimanjaro Christian Medical Centre.

Methodology: A total of 487 participants were enrolled in a cross sectional study conducted from April 2018 to March 2019. Bacteria isolates were from blood 262 (52.4%), urine 147 (29.4%) and wounds 91 (18.2%). Conventional methods were used to determine bacteria species while antimicrobial susceptibility was determined by using the disc diffusion method.

Results: The isolates were predominantly Gram-negative bacteria with Escherichia coli, the most common pathogen in blood 55 (21%) and urine 45 (30.6%) while Pseudomonas aureginosa18 (19.8%) was the most common isolate from wounds. There was 100% resistance to Ampicilin among E.coli, Klebsiella spp and Proteus spp.. Gentamycin resistance was high in E.coli 57/90 (56.7%), Klebsiella spp 27/58 (46.6%) and P. aureginosa 24/54 (44.4%) while resistance to Amikacin was low.There was high resistance to Ceftriaxone in E.coli 44/62 (70.9%) and Klebsiella spp21/36 (58.3%) and resistance to Ciprofloxacin was 67/92 (72.8%) and 26/55 (47.3%) in E.coli and Klebsiella spp respectively. A relatively lower Carbapenem resistance was observed.

Conclusion: There is an alarming high AMR to commonly used antibiotics, leaving a few available options, which are more expensive and not easily available. Therefore there is an urgent need to strengthen efforts to curb AMR in this setting while focusing treatment on the local culture and sensitivity pattern.

INTRODUCTION

Antimicrobial Resistance (AMR) is a rapidly increasing health problem worldwide. In September 2016, the UN declared AMR 'the greatest and most urgent global risk'[1]. Antibiotics have extensively been misused in both humans and food-producing animals and this accelerated the selection and spread of resistant bacteria.[2,3] The misuse of antibiotics includes; using antibiotics to treat non-bacterial infections, self-medication and using incorrect doses.[3] The Global Health Security Agenda assessment concluded that Tanzania has high levels of inappropriate use of antimicrobials both in humans and animals without proper systems in place to collect data on the prevalence of antibiotic resistance in common pathogens.[4] The prevalence of self-medication was at 58% and 76.3% in community-based studies conducted in rural and urban settings in north-eastern Tanzania.[5,6]

Currently, AMR causes over 700,000 deaths per year worldwide. In USA alone, 35,000 people die each year due to AMR while in the European Union AMR causes 25,000 deaths annually.[7,8,9] It is estimated that by 2050, 10 million deaths will be attributed to AMR every year.[7] In Africa, the available data indicates that the region shares the worldwide trend; however information concerning the extent of the problem is limited because effective surveillance of drug resistance is carried out in only a few areas.[2] A recent systematic review on Africa found that AMR data is not available for more than 40% of the countries.[10]

Although increasing AMR affects both developed and developing countries, these settings face different challenges.[3] Sub-Saharan Africa (SSA) including Tanzania has a higher burden of infectious diseases with less effective active surveillance systems.[11] Moreover, the resistance pattern in these settings may not be comparable to developed countries and -

hence the need for local data.[3] This may be due to lack of appropriate functioning drug regulatory mechanisms, limited diagnostic facilities, unauthorised sale of antimicrobials, inappropriate prescriptions and lack of patient education in developing countries as compared to developed countries.[3]

In a systematic review of antimicrobial resistance in Africa, *Streptococcus pneumoniae* resistance to Penicillin was reported in 14 out of 144 studies with a Median Resistance (MR) of 26.7%. Amoxicillin resistance in *Haemophilus* influenza isolates was 18 out of 53 (34.0%) while MR of *Escherichia coli* to amoxicillin and *gentamycin* was 88.1% and 29.8% respectively. Although *Carbapenem* resistance was uncommon in *Enterobacteriaceae* it was common in *Acinetobacter* and *Pseudomonas aureginosa*.[10]

Ideally, the choice of antibiotic should be guided by AMR surveillance data and treatment guidelines. Contrary to this, patients with bacterial infections in developing countries are mainly treated empirically. This underscores the need for timely and regular updates of the constantly changing drug resistance patterns.[11] Failure to address this poses several detrimental effects in terms of increasing health-care costs, length of hospital stay, as well as increasing morbidity and mortality rate and further likelihood of accelerating development of resistance.[7,12,13] In Tanzania, there is a recommended/ standard guideline for treatment of different bacterial infections. However, several studies have reported different AMR patterns across the country.[14, 15, 16]

This study aimed to determine the current aetiological agents with their antimicrobial susceptibility patterns among hospitalised adult patients to guide antimicrobial stewardship as well as infection prevention and control programs, and improve patients' care. The study also aimed to heighten the awareness of policy-makers, health care workers, and the general population on the extent of AMR in this setting.

MATERIALS AND METHODS

Study Area and Population

Across sectional record based study which included patients admitted at Kilimanjaro Christian Medical Centre (KCMC) between April 2018 to March 2019 was conducted. KCMC is a zonal referral hospital (level 3 health facility) in North-eastern Tanzania with an official bed capacity of 630 serving over 15 million people. In Tanzania, it is estimated that there is a higher prevalence of AMR pathogens among hospitalised patients in tertiary care hospitals than in lower level health facilities.[11] The inclusion criteria was; all hospitalised patients aged 14 years and above who had their blood, urine or wound swab culture taken during the study period. These included patients admitted in the Medical, General Surgery, Urology and Orthopedics wards. It also included patients admitted in Medical and Surgical intensive care units. Patients whose cultures had no growth or yielded growth of contaminants were excluded from the study.

Sample Size and Sampling Procedures

Purposeful sampling method was used where patients whose culture grew bacteria isolates from blood, urine and wound samples (positive culture results) were identified from the laboratory registry.

Data Collection Tools and Data Collection Procedures

Data was collected from the KCMC microbiology laboratory registry. Selected patients with positive culture results and susceptibility patterns were then reviewed and extracted as the primary data source. These were linked with patients' information in the patients' files from medical records through a data extraction sheet. Species were identified using selective culture media and biochemical identification methods. Antimicrobial susceptibility testing was performed using disc diffusion on Muller–Hinton II Agar (MHA) according to Clinical Laboratory Standards Institute (CLSI, 2013) guidelines. Gram-Negative (GN) bacteria isolates were tested for various antibiotics examples, i.e., Ampicillin, Amoxicillin-clavulanic, Ceftriaxone, Ciprofloxacin and Gentamicin. Gram-Positive (GP) bacterial isolates were tested for drugs such as Ceftriaxone, Trimethoprim-sulfamethoxazole, Erythromycin and Vancomycin. The choice of antibiotic agents varied depending on the range of antibiotics available to the laboratory.

Data Analysis

The data was entered and analysed using Statistical Package for Social Sciences (SPSS) version 23 developed by International Business Machine Corporation (IBM). Categorical data was summarised in percentages. Continuous variables were summarised using median with their respective measures of dispersion. Descriptive statistics was used to determine patterns of antibiotic resistant isolates among patients with positive blood, urine and wound swabs culture.

Ethical Clearance

Ethical clearance was obtained from the Institution Review Board at KCMU-College Tumaini University, Ethical clearance certificate number: 2368. Privacy and confidentiality was adhered to by using code numbers as opposed to using patients' names.

RESULTS

Socio-Demographic and Clinical Characteristics of Study Participants

A total of 2,934 cultures from wound, blood and urine specimen were done at KCMC, and out of these 717 (24%) cultures had bacteria growth. A total of 487 patients with positive culture results met the inclusion criteria and therefore were enrolled in the study. 13 specimens had 2 isolates each; making a total of 500 bacteria isolates that were analysed. (Figure 1)

Participants' age ranged from 14 to 110 years with median age (IQR) of 60 (41-73) years. Most of the patients 341 (70%) were males and majority were admitted in Urology 223 (45.8%) and Medical wards 104 (21.4%). The most prevalent disease condition was urinary tract infections (UTIs) 143 (29.4 %), while the main comorbidity was Diabetes Mellitus 89 (18.3%) (Table 1)

Aetiology of Infections

The distribution of pathogens from blood, urine and wounds specimens was 262 (52.4%), 147 (29.4%) and 91 (18.2%) respectively. The majority, 436 (87.2%) were Gram-negative (GN) with *Escherichia Coli* being the most frequent GN 113 (22.6%) while *Staphylococcusaureu s*41 (8.2%) was the most predominant Gram-positive (GP)

isolate. The *E.coli* was the most common isolate from both blood and urine samples; 55 (21%) and 45 (30.6%) respectively. *Pseudomonas aeruginosa* was the predominant isolate from wounds 18 (19.8%) (Table 2)

TABLE 1: Socio-Demographics and Clinical Characteristics of the Study Participants (N=487)

Characteristics	Frequency	Percentage (%)
Median age in years (IQR)	60 (41-73)	
Age(Years)		
14-34	103	21.1
35-54	108	22.1
55-64	67	13.9
65+	209	42.9
Sex		
Male	341	70.0
Female	146	30.0
Ward		
Surgical	91	18.7
Medical	104	21.4
ICU	47	9.6
Burn	18	3.7
Urology	223	45.8
Obstetric	4	0.8
Referral		
Dispensary	14	2.9
Health centre	35	7.2
District hospital	101	20.7
Regional hospital	76	15.6
Zonal hospital	2	0.4
Self referral	259	53.2
Disease condition		
Wounds *	120	24.6
UTI	143	29.4
Pneumonia	18	3.7
URTI	9	1.8
Intestinal obstruction	10	2.1
Peritonitis	6	1.2
Comorbidities		
Diabetes mellitus	89	18.3
Renal Failure	49	10.1
Cancer	32	6.6
Stroke	18	3.7
Obstructive Uropathy	72	14.8

IQR - Interquartile range, ICU- Intensive Care Unit (Medical and Surgical) URTI - Upper Respiratory Tract Infection, UTI - Urinary Tract Infection, * Wounds - Diabetic wounds, traumatic wounds, bed sores, chronic ulcerative wounds, burn wound, cellulitis, post-surgical wounds, Obstructive Uropathy - Prostate enlargement and urethral stricture.

Antimicrobial Susceptibility
Gram-Negative Bacteria
Penicillin resistance was high with 100% resistance to - ampicillin among *E. coli*, *Klebsiella Spp* and *Proteus Spp* isolates tested. A relatively high resistance rate to Gentamicin was noted across the spectra of the GN; *E. coli* 57/90(56.7%), *Klebsiella Spp* 27/58(46.6%) and *Pseudomonas Spp* 24/54 (44.4%) in contrast to Amikacin which showed low rates of resistance (17.6%) (Table 3).

High AMR was also observed to Fluoroquinolones in *E. Coli* 67/92 (72.8%) and *Klebsiella Spp* 26/55(47.3%) showing resistance to Ciprofloxacin. Resistance to third-generation Cephalosporins was also high across all commonly isolated GN bacteria. *E. Coli* were resistant to Ceftriaxone, Cefotaxime and Ceftazidime by 44/62 (70.9%), 34/49 (69.4%) and 9/13 (69.2%) respectively. Relatively high sensitivity was observed to Carbapenems (Table 3).

Gram-Positive Bacteria
There were lower AMR rates in GP as compared to GN bacteria. *S. aureus* demonstrated high resistance to Erythromycin 21/35 (60.0%), Clindamycin 13/25 (52.0%), Ciprofloxacin 11/24 (45.8%) and Meropenem 3/7 (42.9 %). (Table 4)

DISCUSSION
Socio-Demographic Characteristics
The study was conducted at a tertiary care hospital in the North-eastern part of Tanzania, from April 2018 to March 2019 on bacterial isolates from blood, wound and urine cultures among hospitalised patients. The study showed high AMR pattern to commonly isolated pathogens. This is consistent with similar studies conducted in other developing countries.[17,18,19,20]

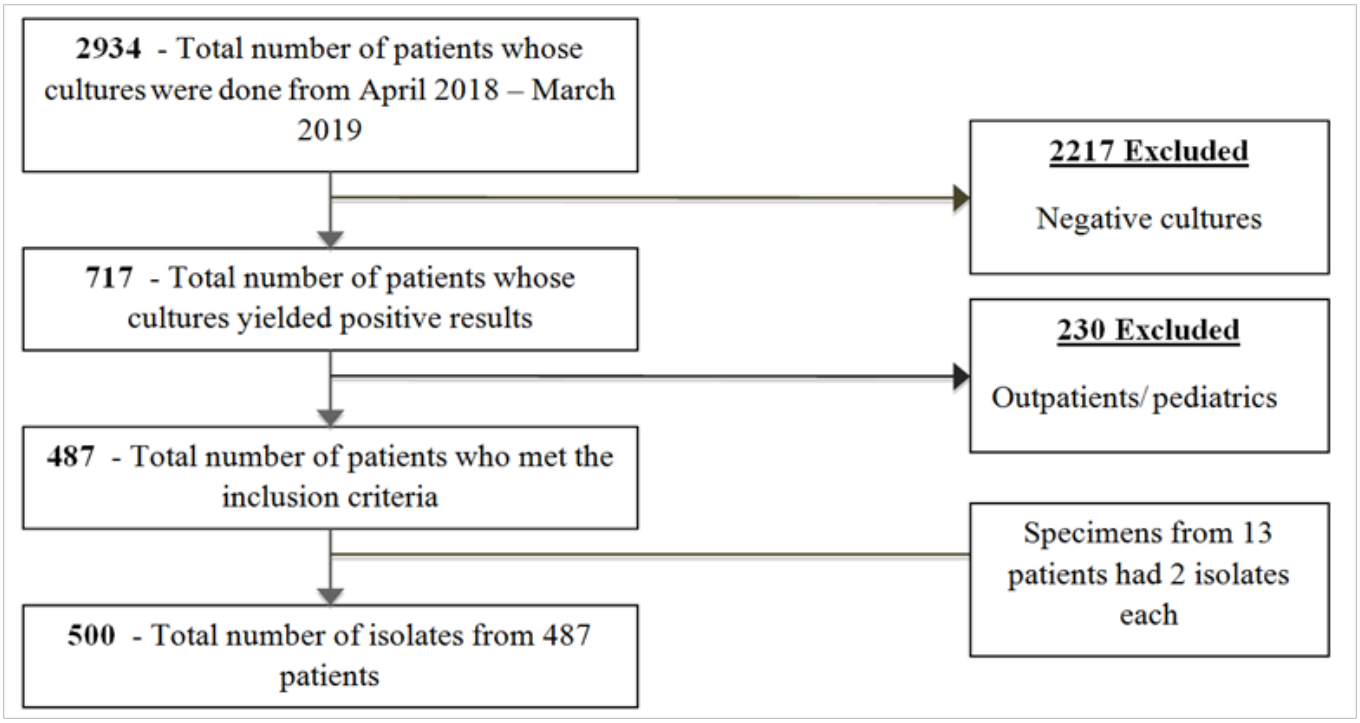
The majority of the participants were males. This is similar to reports from other studies within Sub-Saharan Africa (SSA), countries like; Tanzania, Ethiopia and Rwanda which registered 61%, 51.3% and 54.3% male participants respectively.[14,17,21] A relatively higher proportion of male participant in this study can be due to the fact that a significant number of patients 223 (45.8%) where from the Urology ward, which admits more male than female patients.

A similar study that was conducted in Rwanda among hospitalised adult patients reported a significant proportion of participants being above 65 years of age.[21] The possible reason for this finding could be due to the association between comorbidities and old age. More than a third of participants in this study had either Diabetes Mellitus, Renal failure, Cancer or Stroke. These comorbidities, together with old age cause immune suppression thus affecting the body's ability to fight infections.[22,23] The study also shows the double burden of communicable and non-communicable diseases in this setting.

Aetiology of Infections:
The dominant pathogens were GN bacteria. This is in agreement with other studies conducted in hospital settings in Tanzania, Ghana, Lebanon and Ethiopia.[14,18,24,25]

This can be contributed to the fact that majority of these isolates are normal flora on skin and in the gastro intestinal system of healthy individuals, hence they can easily be disseminated to other areas to cause infection.

FIGURE 1: Flowchart for Study Participants (487) and Culture Isolates (500)



Some can also be found in the hospital environment, in instruments such as ventilators, linens and can easily be transmitted through the hands of healthcare workers. As a result, GN bacteria have emerged as a hospital acquired pathogens.[8]

In a systematic review on antimicrobial resistance in West Africa, *E Coli* was found to be the most commonly reported bacterium (60.4%).[18] It was a predominant isolate in both blood and urine cultures. Kumburu et al and Musicha et al, found *E.coli* as the most common cause of blood stream infections by 28.4% and 8.8% in Tanzania and Malawi respectively.[14,26] Furthermore, findings from other hospital based studies in Tanzania, some European countries, Gabon, and India reported that *E. coli* was the most common isolate in UTIs by 26.2%, 52%, 36.3% and 52% respectively.[15,19,27,28] GN facultative anaerobe, a colonizer of the gastrointestinal tract is well known to be the most frequent cause of community and hospital acquired UTI and pyelonephritis.[13] On the other hand, *Pseudomonas Spp* which was the the predominant isolates from wounds in this study was also a predominant isolate from surgical site infections at Bugando Medical Centre (BMC) 14 (15.6%) and Muhimbili National Hospital 24 (16.3%) in Tanzania as well as isolates from burn wounds in a Ghanaian hospital 26 (30.2%).[15,16, 29] It was also the most frequent isolate from wounds of patients with Diabetes Mellitus[30]. Contrarily, some studies in Tanzania, Ghana and Bangladesh have reported *S. aureus* as the most common isolate from wound infections by 16 %, 46.3%, and 40.4% respectively.[14,31,32] The hospital environment might have contributed to the observed difference. It is possible that there was cross contamination among admitted patients since *S. aureus* are prominent nosocomial pathogens commonly found in the hospital environment.[8]

Klebsiella Spp were the most common isolates among patients with Stroke. This is in agreement with a study conducted in Taiwani (23.5%).[33] Stroke patients have stroke-induced immune suppression with prolonged hospital stay making them susceptible to infections. *Klebsiella Spp* are one of the major causes of nosocomial infections.[34]

Antimicrobial Susceptibility

There was a higher AMR rate among GN bacteria as compared to GP bacteria. We also found alarming and high rates of AMR among common isolates against commonly used antibiotics, higher than findings from a previous study done at KCMC 5 years prior. However, unlike in our study, the previous study considered both adult and paediatric -patients and samples collected included; blood, wound swabs, sputum and stool cultures.[14] A good example is on resistance to penicillin whereby; *E.coli* resistance to Ampicilin was 13/19 (68.4%) while in *Klebsiella Spp* resistance was 24/26 (92.3%).[14] Similarly for Cephalosporins, *E. coli* resistance to Ceftriaxone was 8/19 (42.1%), while *P. aureginosa* resistance to Ceftazidime was 4/22 (18.2%).[14] High AMR

TABLE 2: Bacteria Isolates Obtained from all Culture Positive Specimens (N500)

Bacterial isolated	Type of clinical specimens			
	Blood n (%)	Wound n (%)	Urine n (%)	Total N (%)
Gram-positive				
S. aureus	31(11.8)	9(9.9)	1(0.6)	41 (8.2)
S. pyogenes	10(3.8)	1(1.1)	0(0.0)	11 (2.2)
Enterococcus pp	5(1.9)	0(0.0)	5(3.4)	10 (2)
Bacillus spp	0(0.0)	0(0.0)	2(1.4)	2 (0.4)
Gram-negative				
E. coli	55(21.0)	13(14.3)	45(30.6)	113 (22.6)
Coliform spp	36(13.7)	12(13.2)	23(15.6)	71 (14.2)
Klebsiella spp	32(12.2)	12(13.2)	26(17.7)	70 (14)
P. aeruginosa	34(13.0)	18(19.8)	16(10.9)	68 (13.6)
Proteu spp	22(8.4)	16(17.6)	7(4.8)	45 (9)
Citrobacter spp	15(5.7)	4(4.4)	14(9.5)	33 (6.6)
Enterobacter spp	6(2.3)	1(1.1)	6(4.1)	13 (2.6)
Acinetobacter spp	9(3.4)	3(3.3)	0(0.0)	12 (2.4)
Serratia spp	2(0.8)	1(1.1)	2(1.4)	5 (1)
Morganella spp	3(1.1)	0(0.0)	0(0.0)	3 (0.6)
Providencia spp	1(0.4)	1(1.1)	0(0.0)	2 (0.4)
Shigella spp	1(0.4)	0(0.0)	0(0.0)	1 (0.2)
Total	**262 (100)**	**91(100)**	**147(100)**	**500 (100)**

rates were also observed for other most commonly used and easily available antibiotics including Gentamycin and Ciprofloxacin.[14]

These high rates of resistance are in agreement with other similar reports, which showed 90% and 96% *E.coli* resistance to Ampicilin in Tanzania and Rwanda respectively.[11,21] Moreover, at BMC, *E.coli* resistance to 3rd generation Cephalosporins and Ciprofloxacin was found to be as high as 12/19 (63%) and 16/24 (66.7%) respectively with even a higher resistance of *P. aureginosa* to third generation Cephalosporins 14/16 (87.5%).[27] This observation might be contributed by the irrational use of antibiotics. Antibiotics are widely used in the community either without prescription or guidance of culture and sensitivity results. Mboya et al., found that the use of most antibiotics 135 (88.8%) bought from the community pharmacies in the municipality where KCMC is located were irrational. The most bought antibiotics were; Ampicilin-cloxacilin 41 (27%), Amoxycylin 29 (18.4%), Metronidazole 14 (8.7%) and Ciprofloxacin 13 (8.1%).[31] Injectables, especially Ceftriaxone are commonly used in hospital settings as shown at BMC.[15] The emergency of AMR as a result of selection pressure can explain the phenomenon observed.[35] Due to lack of resources, empiric treatment without adequate antimicrobial susceptibility evidence is usually given in limited resources settings, which may be entirely ineffective or foster further resistance.[36, 37]

On the other hand, overall lower AMR rates were observed against Amikacin and the lowest against Carbapenems. In Rwanda, *E.coli* sensitivity to Imepenem was exactly the same as reported in this study at 92% while at BMC, *Klebsiella Spp* were 100% sensitive to Carbapenems.[14,27] Amikacin and Carbapenems are relatively less used antibiotics but are increasingly becoming the preferred treatment options.

CONCLUSION

There is an alarming and increasing AMR from isolates among patients admitted at KCMC. AMR was more observed among GN bacteria with resistance to commonly used antibiotics. This is of a major concern in a region that is highly burdened with both communicable and non-communicable diseases, with limited supply of more expensive and more effective drugs.

We recommend the study findings to be used by the antimicrobial stewardship programs to assist clinicians in selecting appropriate antibiotics against various infections in different disease conditions. The local health authorities should also be prompted to step up infection control programs in health facilities. Efforts to curb AMR should also be directed towards the community as witnessed by the fact that most of the patients were self referral from the community, however they presented with high levels of antimicrobial resistant pathogens at the time of hospital admission.

Study Limitations:

Some patients' record files had missing data and thus some detailed information on patient profiles was not available. It was also difficult to distinguish community from hospital-associated infections for some patients due inadequate documentation. Some samples were taken after starting antibiotic treatment, which may have led to

TABLE 3: Antimicrobial Resistance Pattern of Common Gram Negative Isolates to Commonly Used Antibiotics

Antimicrobials	E. coli		Coliform spp		Klebsiella spp		Pseudomonas spp		Proteus spp	
	T	R(%)	T	R(%)	T	R(%)	T	R(%)	T	R(%)
*Amoxy-clav	105	61(58.1)	62	37(59.7)	62	12(19.4)	9	7(77.8)	39	18(46.2)
Amikacin	74	10(13.5)	53	8(15.1)	55	1(1.8)	51	9(17.6)	31	5(16.1)
Ampicillin	19	19(100.0)	13	11(84.6)	8	8(100.0)	2	1(50.0)	2	2(100.0)
Ceftriaxone	62	44(70.9)	39	24(61.5)	36	21(58.3)	20	16(80.0)	30	13(43.3)
Ceftazidime	13	9(69.2)	8	6(75.0)	13	8(61.5)	33	11(33.3)	5	2(40.0)
Ciprofloxacin	92	67(72.8)	65	27(41.5)	55	26(47.3)	63	21(33.3)	38	3(7.9)
Cefotaxime	49	34(69.4)	16	10(62.5)	24	16(66.7)	6	5(83.3)	16	3(18.8)
Gentamycin	90	57(56.7)	61	28(45.9)	58	27(46.6)	54	24(44.4)	39	18(46.2)
Nitrofurantoin	61	18(29.5)	30	13(43.3)	41	22(56.7)	3	2(66.7)	16	11(68.8)
Meropenem	38	2(5.2)	27	5(18.5)	0	0(0.0)	19	4(26.7)	9	1(11.1)
Tetracycline	7	5(85.7)	1	1(100.0)	5	4(80.0)	1	1(100.0)	0	0(0.0)
*Trime-sulf	7	5(71.4)	1	1(100.0)	4	1(25.0)	1	1(100.0)	2	1(50.0)
Piperacillin	5	0(0.0)	6	2(33.3)	5	0(0.0)	50	7(14.0)	3	1(33.3)
Imepenem	37	3(8.1)	16	1(6.3)	19	0(0.0)	38	3(7.9)	7	0(0.0)

T - total number of isolates tested against antimicrobial agent, R - percentage of isolate resistance to antimicrobial agent, *Amoxy-clav: Amoxicillin–Clavulanic Acid, *Trime-sulf: Trimethoprim-sulfamethoxazole (co-trimoxazole)

selection and over-representation of resistant isolates.

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ORIGINAL ARTICLE

Urogenital Schistosomiasis Knowledge, Attitudes, and Practices among the Community Members in Lindi, Tanzania: A Qualitative Study

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ABSTRACT

Background: Urogenital schistosomiasis caused by *Schistosoma haematobium* (*S. haematobium*), remains a public health problem in Lindi region. Despite twelve rounds of praziquantel preventive chemotherapy. There is a scarcity of information on the factors perpetuating the transmission of *S. haematobium* in Lindi. Therefore, this study aimed to explore the urogenital schistosomiasis knowledge, attitudes, and practices among the community members in Mtama district in the Lindi region of Tanzania.

Methodology: A cross-sectional study employing a qualitative approach was conducted in Mtama, Lindi in May 2021. The respondents were purposively sampled, and a total of 6 Focus Group Discussions (FGDs), 2 in each village were conducted. The FGDs were audio-recorded, transcribed verbatim, and analysed thematically to identify emerging themes.

Results: Majority of respondents were aware of the endemicity of *S. haematobium* and the ongoing distribution of praziquantel preventive chemotherapy. Respondents had inadequate knowledge of the disease causation and the role of snails in disease transmission. Also, misconception on the modes of disease transmission was observed. Respondents had undesirable attitudes. They were against regular screening of urogenital schistosomiasis and were into the use of traditional ways of treatment to dodge screening and treatment costs. Respondents exhibited inappropriate water, sanitation, and hygienic practices (WaSH), thus perpetuating disease transmission.

Conclusion: Despite the community being aware and knowledgeable of urogenital schistosomiasis, there is inadequate understanding of how the disease is transmitted, the roles of snails in *S. haematobium* transmission, coupled with undesirable attitudes and inappropriate practices. These potentially compromise the ongoing Government efforts to control the disease in Lindi region. Therefore, there is need to initiate a community-based health education programme targeting behaviour change.

INTRODUCTION

Urogenital schistosomiasis (UGS) is among the serious neglected tropical diseases accountable for significant morbidities in tropical and sub-tropical regions.[1] Transmission of urogenital schistosomiasis involves an intermediate host (*Bulinus* snails) and a definitive host (human).[2] Adult *S. haematobium* worms inhabit the vesicular and pelvic venous plexus of the bladder and produce eggs which are eliminated with urine. When the eggs are released in water under optimal conditions, the eggs hatch and release miracidia which swim and penetrate *Bulinus* snails. Within the *Bulinus* snail, the miracidia develop into *sporocysts* which later develop into infective *cercariae*. When the infective *cercariae* are released from the snail into the water stream, they swim, penetrate the skin of the human host during water contact activities, and shed their forked tails, thus becoming *schistosomulae*. The *schistosomulae* migrate via venous circulation to the lungs, then to the heart, and then develop in the liver, exiting the liver via the portal vein system when mature and reside in the vesicular and pelvic venous plexus of the bladder.[3]

The most commonly affected individuals in endemic areas are the children (pre-schoolers and school-aged children) and people engaged in water-related occupations.[1] Urogenital schistosomiasis in infected individuals leads to dysuria, haematuria, nutrition deficiencies, iron deficiency anaemia, bladder lesions, hydronephrosis and bladder squamous cell carcinoma.[4] Also, in children, it causes growth retardation, cognitive dysfunction, malnutrition, and reduces their ability to learn in school.[5] Globally in 2020, it was estimated that 436 million people live in 78 endemic countries, so they are at risk of getting urogenital schistosomiasis, and over 112 million people were infected.[1] Among the infected people, over 76 millions suffered minor bladder morbidity, 24

million suffered major bladder morbidity, 19 million had kidney problems, 9.6 million had major hydronephrosis due to infection, and 0.162 mortality due to kidney and bladder squamous cell carcinoma.[1] Africa, particularly sub-Saharan Africa, carries the highest burden of the disease (more than 90%) when compared to other regions. This is due to limited access to clean water, inadequate sanitation provision, and poor hygienic practices.[1,6,7]

In Africa, Tanzania is amongst countries with a high burden of urogenital schistosomiasis. Its distribution and prevalence vary across the region.[8] Studies have reported the prevalence of urogenital schistosomiasis to ranging from 1% to 88%, reaching 100% in some regions.[9–12] Like other urogenital schistosomiasis endemic countries, a higher prevalence has been reported among school-aged children below 15 years of age compared to other at risk populations (pre-schoolers and people engaged in water-related activities).[8] In the Lindi region where this study was conducted, there is history of high prevalence of *S. haematobium* since 1987.[13] Recent studies have reported the prevalence of *S. haematobium* to be ranging from 23% to 52.7%, indicating ongoing transmission of the disease.[7,14]

The control of urogenital schistosomiasis is mainly through the distribution of praziquantel preventive chemotherapy. However, if water contact is continued, re-infection can occur after a short period of time (6 months) because praziquantel does not prevent subsequent infection.[15] Other control interventions to complement praziquantel preventive chemotherapy include; snail control to kill the intermediate host (*Bulinus spp*), improvement of Water, Sanitation, and Hygiene (WASH), and health education for behaviour change.[16]

The high burden of urogenital schistosomiasis in Tanzania sparked the initiation of a mass praziquantel preventive chemotherapy in 2005 among school-aged children in 11 regions.[17] The praziquantel preventive chemotherapy is distributed once per year in primary schools targeting children who attend school only, out of school children are left untreated.[17]

Lindi region was among the first 11 regions where praziquantel distribution was implemented. However, despite the use of praziquantel for more than a decade (12 rounds)[17], the prevalence of urogenital schistosomiasis has remained high,[7,14] conceivably indicating persistent transmission, and, there is limited information on the factors contributing to the ongoing transmission of *S. haematobium* in the area.

Hence, there is need to establish urogenital schistosomiasis related knowledge, attitudes and practices among the community members to determine the causes of urogenital schistosomiasis persistent transmission. The study results will contribute towards the sustainable control of urogenital schistosomiasis in the region. Therefore, this study explored the urogenital schistosomiasis knowledge, attitudes, and practices among community members in Mtama district, Lindi region. The findings might be used to modify the existing control programme by including health education and promotion to accelerate the control of urogenital schistosomiasis.

METHODS

Study Design

A community-based cross-sectional study employing a qualitative method of data collection was carried out in the Mtama district in May 2021 to explore the urogenital schistosomiasis knowledge, attitudes, and practices among community members in the Mtama district. This study was part of a large study conducted in the Lindi region to investigate the current burden of *S. haematobium* among children and the factors associated with the persistency of transmission.

Study Setting

This study was conducted in the Mtama district, previously known as the Lindi district council. Mtama district is among the 6 districts of Lindi region located in the South-East of Tanzania's mainland. The district has an area of 5975 km² divided into 31 wards. According to the Tanzania 2012 National Census, the district has an approximate population of 194,143 (females are 102,496 (52.8%)).[18] The tropical climatic conditions of Mtama district, the annual rainfall of 910 mm with an average temperature of 26.3°C favour the breeding and survival of the intermediate host of the *S. haematobium* known as *Bulinus* snails (*Bulinus globosus* and *Bulinus nasutus*).[14]

The ongoing economic activities in Mtama district include; agriculture, livestock keeping, and fishing. Some of these activities such as rice farming and fishing predispose the community to the risk of transmitting and acquiring urogenital schistosomiasis. During farming and fishing activities, farmers and fishermen are exposed to the infested water and hence cercaria penetration through their skin. Also, the lack of toilet facilities around the water sources perpetuates unhygienic activities such as open urination in and around the water sources. Incase, one has urogenital schistosomiasis, such acts would lead to contamination of the water sources with *S. haematobium* eggs. Mtama district has 117 primary schools, one hospital, 6 health centres, and 42 dispensaries with parasitic diseases (urogenital schistosomiasis, malaria, soil-transmitted helminths, and filariasis) affecting the majority.[17,19]

Study Population, Inclusion and Exclusion Criteria

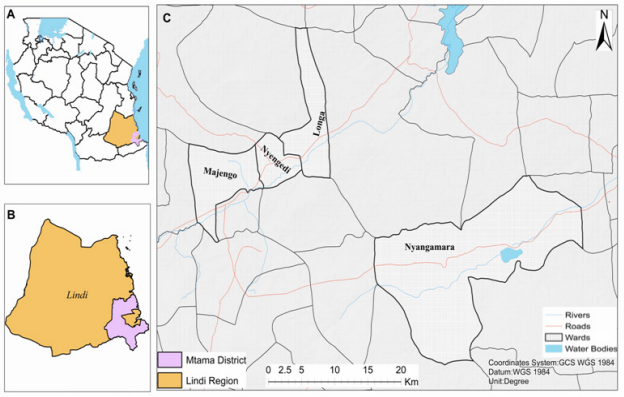
The study population included community members aged 18 years and above living in Mtama district. Study participants were selected among the high risk groups of people whose occupations (farming and irrigation) and domestic tasks involve contact with cercariae-infested waters. Also, these members visit water bodies with their younger children (below 5 years) or school-aged children who assisted them in agricultural or domestic activities. Hence, they were at risk of being exposed to cercariae-infested water. The study included only permanent residents of the Mtama district who agreed to participate in the study and signed the informed consent. Participants who were unable to communicate due to medical conditions were excluded.

Sampling

Purposive sampling was employed to select the study respondents. First, 3 wards (Longa, Nyengedi, and Nyangamara) were purposively sampled from the list of 31 *S. haematobium* endemic wards (Figure 1). Then, one

village per ward was purposively selected, whereby three villages (Mtua longa, Nyengedi A, and Nyangamara from Longa, Nyengedi, and Nyangamara wards, respectively) were selected. In each of the selected villages, a total of 8 to 12 community members were purposively sampled for each of the 2 Focus Group Discussions (FGDs) conducted. The possiblity of selection bias of the respondents was mitigated by defining the study population, setting the inclusion and exclusion criteria, and ensuring the selected participants matched the desired criteria.

FIGURE 1. Map of Mtama District Showing Wards Sampled



(The map was designed based on the data collected using Geographic Coordinate System (GPS) and mapped under GPS WGS 1984)

Data Collection

Focus Group Discussions were held with community members to explore their knowledge, attitudes, and practices on urogenital schistosomiasis and WaSH using a FGD topic guide. The FGD topic guide consisted of a series of open-ended questions and each question had probes for further exploration of the concepts. A total of 6 FGDs were held at the village offices, 2 FGDs for each of the selected villages. To ensure freedom of expression, the first FGD was held with women only and the other with men. All FGDs were audio-taped.

Data Analysis

The qualitative data from FGDs was analysed using a thematic framework approach. The collected audio data was transcribed verbatim and translated from Kiswahili to English to obtain its textual format. For familiarisation, the transcripts were read and reviewed by the investigators several times. Inductive coding of the information from the transcript followed. Coding was done by highlighting phrases or sentences describing the content. After codes creation, the patterns among the codes were identified to form categories. The categories were compared with the data set to ensure that the generated categories were significant and thoroughly represented the actual data. Finally, the categories were integrated until the major themes solidified.

Ethical Consideration

Ethical approval for the study was provided by the Ethical Review Board of the Muhimbili University of Health and Allied Sciences, registration number: MUHAS-REC-12-2020-457. Permission to conduct the study in the Mtama district was obtained from the administrative units of Lindi region, from ward to village level. Verbal informed consent (for respondents who could not read and write) and written informed consent (for those who knew how to read and write) was obtained from the study participants after thorough explanation of the study aims, procedures for the study and their rights to withdraw from participation. Numbers were given to participants as opposed to using participants' names so as to maintain confidentiality. Collected data was transferred to the principal investigator's laptop and secured with a password so that only the authorised personnel could access the data.

RESULTS

Socio-Demographic Characteristics of the Study Respondents

A total of 57 community members participated in the 6 focus group discussions conducted at the Mtama district. Their age ranged from 18 to 57 years, with 29 (50.9%) of the respondents being females. The petty business was the main source of income for 29 (50.9%) of the respondents (Table 1).

Theme one: Awareness of urogenital Schistosomiasis as a Public Health Problem in the Mtama District

Endemicity of Urogenital Schistosomiasis and the Sources of Information on the Disease in Mtama

Most of the respondents reported that urogenital schistosomiasis was among the diseases that affected people of the Mtama community, especially among children. The majority of the respondents reported that despite urogenital schistosomiasis being highly endemic, they did not receive any information concerning the disease. However, some respondents mentioned schools and school-aged children as the source of information on the distribution of schistosomiasis preventive chemotherapy in school-aged children.

One of the respondents noted;

"We do not receive information about urogenital schistosomiasis in the community. However, when it comes to the treatment of school-aged children, children are usually told to inform their parents." (Nyangamara FGD, Female 006, 37 years)

Awareness of the ongoing efforts to address the urogenital schistosomiasis in Mtama

The majority of the respondents were aware of the distribution of the preventive chemotherapy to school-aged children as the intervention for the control of urogenital schistosomiasis. One of the respondents mentioned the manual picking of snails twice a year to be among the intervention used to control the transmission of the disease in Mtama:

"Apart from the drugs distributed to school-aged children, we usually handpick the snails at least twice a year to clean the water sources. This helps to minimize the transmission of urogenital schistosomiasis." (Longa FGD, Female 008, 32 years)

Theme two: Knowledge on urogenital schistosomiasis among community members of Mtama

Understanding of urogenital schistosomiasis (causative agent, transmission, roles of snails, symptoms, treatment, control and prevention)

Most of the respondents defined urogenital schistosomiasis as urinating blood or bleeding at the end of peeing, while few respondents defined it as genitalia itching and stomach pain when urinating. None of the respondents was able to state the correct causative of urogenital schistosomiasis. However, majority of the participants knew the mode of transmission. The most mentioned mode of *S. haematobium* transmission was contacting infested water during agricultural activities, swimming, and domestic chores such as laundry. Also, there were some misconceptions on the mode of transmission, such as drinking unboiled water or sharing the toilet with the infected individuals, as pointed out by one of the respondents:

"The transmission of S. haematobium occurs when sharing latrines with infected individuals not observing proper hygiene." (Nyangamara FGD, Male 004, 28 years)

None of the respondents was aware of the role of snails in the transmission of urogenital schistosomiasis. The symptoms of urogenital schistosomiasis were well known in this community conceivably due to the high endemicity of the disease. Symptoms participants mentioned included; blood in the urine, vaginal discharge, abdominal pain, pelvic pain, genital itching, blood in semen, and dysuria. Regarding the population at higher risk of urogenital schistosomiasis, participants mentioned school-aged children, adults working in the rice paddy fields and women doing domestic chores in the infested water. The respondents did not mention the under-fives as a population at risk of disease acquisition.

Treatment for Urogenital Schistosomiasis

All of the participants in the 6 conducted FGDs reported urogenital schistosomiasis as a treatable disease, with the majority preferring to the modern (hospital) way of treatment while some preferred to traditional treatment. Some of the respondents noted;

"Both traditional and modern treatments are used to treat the disease in this community. However, traditional treatments relieve the symptoms shortly, and the symptoms tend to recur." (Nyengedi FGD, Female 007, 26 years)

"They will tell you that they prefer modern treatment. However, most of us end up using traditional treatment due to the cost implications. The traditional treatment is affordable compared to hospital treatment where you have to pay to see the doctor, diagnosis and treatment." (Longa FGD, Male 005, 31 years)

Interventions to Mitigate Urogenital Schistosomiasis in the Community

Regarding government's intervention programs on urogenital schistosomiasis prevention and control in Mtama area, the respondents mentioned the ongoing distribution of preventive chemotherapy for treating and preventing the disease among school-aged children. The respondents further mentioned several preventive measures employed at individual and community level to prevent urogenital schistosomiasis. These measures included snail collection once or twice per year and wearing of gumboots at the rice paddy fields and in shallow water.

TABLE 1: Socio-Demographic Characteristics of Study Respondents (n=57)

Characteristics	n(%)
Gender	
Male	28(49.1)
Female	29(50.9)
Age group (years)	
18-37	41(71.9)
38-57	16(28.1)
Marital status	
Single	24(42.1)
Married/cohabiting	30(52.6)
Divorced/separated	03(5.3)
Level of Education	
No formal education	12(21.1)
Primary school education	27(47.4)
Secondary education	12(21.1)
College	06(10.5)
Main source of income	
Agriculture	13(22.8)
Animal husbandry	09(15.8)
Employment	06(10.5)
Petty business	29(50.9)
Duration of residence (years)	
1-19	13(22.8)
20-38	35(61.4)
39-57	09(15.8)
Villages of residency	
Mtua longa	20(35.1)
Nyengedi A	17(29.8)
Nyangamara	20(35.1)

Participation in Preventive Chemotherapy

Regarding parents allowing their children to participate in the preventive chemotherapy program, most of the parents mentioned that the drug is beneficial for treatment and prevention of urogenital schistosomiasis among school-aged children. For the parent who did not allow their children to participate in the program, there mainly reason for refusal was due to the side effects experienced after intake of the drugs and inadequate information regarding the disease and it's treatment as reported by one of the respondent:

"Some community members do not allow their children to take the drugs because of the side effects experienced." (Nyengedi FGD, Male 008, 26 years)

Theme three: Attitudes on Urogenital Schistosomiasis among Community Members

Discrimination of the infected individuals

Respondents didn't report any form of discrimination or stigmatisation of the infected individuals. They reported

that infected individuals were treated the same as uninfected individuals as confirmed by one respondent:

"We do not discriminate against each other, instead we emphasis the sick to go to the hospital for treatment." (Nyangamara FGD, Female 010, 18 years)

Local Beliefs and Related Misconceptions

Majority of the respondents believed that urogenital schistosomiasis was not associated with local superstitious beliefs. However, few of the respondents reported association between urogenital schistosomiasis and superstition in the Mtama district as reported by one respondent:

"Yes, some people do believe. Sometimes the symptoms and the experience with recurrent infection, you might think you have been bewitched." (Longa FGD, Female 006, 47 years)

The Severity of the Disease in the Mtama Community

With respect to severity of the disease, all of the respondents believed urogenital schistosomiasis was a serious disease due to morbidities associated with the disease. Respondents mentioned bladder destruction, sterility, bladder cancer, and erectile dysfunction as some of the complications associated with the disease.

Screening for Urogenital Schistosomiasis

With regard to screening of urogenital schistosomiasis, few respondents (13, 23%) believed regular screening was important for knowing their infection status, being treated earlier, and avoiding morbidity. Majority of the respondents were against regular screening unless they were severely sick, the reason being it's expensive to screen.

The following were some of the responses:

People of this community don't screen for S. haematobium because of the costs. It's better to buy drugs at the pharmacies when sick rather than screening." (Longa FGD, Female 008, 32 years)

"No one in my family has ever screened for urogenital schistosomiasis. When they experienced symptoms of the disease, we bought medicines at the pharmacy. Imagine seeing a doctor, you have to pay 2500Tsh, diagnosis is 3000Tsh, and treatment is 1000Tsh per drug." (Nyengedi FGD, Male 008, 26 years)

Theme four: Water, Sanitation, and Hygiene Practices toward Urogenital Schistosomiasis among Community Members of Mtama

Water availability and ongoing activities in or near water sources

Majority of the respondents reported that Mtama district was surrounded by several water sources, which are unclean and unsafe for consumption and use. The water sources used by the community include; taps, rivers (Mnongo, Nyengedi, and Lukuledi), spring (Nahimba), stream (Kitumba), short and deep wells, dam (Mbawe), ponds, and Nyengedi irrigational scheme. Regarding the ongoing activities in and near water sources, majority of the respondents reported being engaged in activities such as agricultural (rice cultivation), fishing, and domestic chores that predispose them to infested water as reported by one of the respondents:

"Yes, agricultural activities, this community is involved in vegetable cultivation, sugarcane, and rice farming. Also, all the women do most of their domestic chores at water sources." (Longa FGD, Male 001, 21 years)

Exposure of Children (Under-Fives) to Water Sources

Majority of the respondents reported that women move with under-five children to water sources. These children play from the infested waters thus being exposed to the disease at an early age.

One of the respondents noted;

"To children, water is like a nanny. Once you place the child in water, the child will play without disturbing me when I am working. This habit starts very early, around 2 years in this community."(Nyengedi FGD, Female 007, 28 years)

The Availability and use of Latrines among the Community Members

All respondents from Longa and Nyengedi wards reported that there were no latrines at the water sources. Respondents from Nyangamara reported the presence of a pit latrine that is full, and that there was no initiative to build another one. It was also reported that the lack of sanitation facilities near water sources perpetuated unhygienic practices such as open urination and defecation in or near water sources, as reported by one of the respondents:

"Why waste time urinating and defecating outside the water while no one can see you when doing it in water. Also, defecating and urinating in water is advantageous because of enough water to wipe" (Nyangamara FGD, Male 006, 25 years)

Clearly, majority of the respondents (46, 81%) did not know if open urination caused transmission of urogenital schistosomiasis. Only a few respondents (11, 19.3%) reported that open urination contaminate the water sources and contribute to the transmission of diseases.

The habit of wearing protective gear (shoes) when in water sources/ crossing water sources

The study revealed that majority of the respondents (44, 77.2%) didn't wear protective shoes (gumboots) while working in water or crossing the water sources. This is because majority of the respondents reported not being able to afford the gumboots. Also, only a few respondents (13, 23%) were aware that walking barefooted could cause the acquisition of the disease, but they did not correctly know-how.

Treatment of Water

A few respondents (16, 28.1%) reported treating the water, and the majority did not treat the water due to economic reasons. The methods used for water treatment were; boiling, filtration and use of chemicals. This was clearly reported by one of the respondents who stated:

"In the past when water guard was provided free of charge, we used to treat water but not anymore because now we need to buy." (Longa FGD, Female 003, 44 years)

Most of the respondents knew the importance of water treatment despite not doing it. They mentioned 2 benefits; killing of microorganisms causing the diseases and the purification of water to make it clean and safe for human consumption. Majority of the respondents reported not boiling water for bathing the children due to the cost of

buying charcoal or fire woods (35, 85.3%). Instead, they reported that water is exposed to the sunlight for warming so as to prevent the children from acquiring cold and pneumonia. When the respondents were asked to state if boiling water before bathing the child was helpful in killing the parasite that cause urogenital schistosomiasis, most of them (49, 86%) were not aware. However, a few responded it could help.

DISCUSSION

A successful and sustainable control of urogenital schistosomiasis in endemic areas depends on adequate knowledge, positive attitudes, and appropriate preventive practices regarding the disease. The study explored the community knowledge, attitudes and practices on urogenital schistosomiasis. The study findings revealed that the community of Mtama district was aware of urogenital schistosomiasis as among the endemic disease affecting people in the area. This was probably because the disease has been endemic with a high prevalence for more than 3 decades in the Mtama district.[13] This is in line with the findings of a systematic review which showed high awareness of urogenital schistosomiasis in sub-Saharan Africa.[20]

Mtama community members reported that they did not receive any information regarding the disease due to lack of community health education campaigns. However, due to an ongoing distribution of preventive chemotherapy with praziquantel campaign, school-aged children are given health education about the disease and are regarded as a source of information about the disease to the community. Schools and school-aged children were reported among the sources of information in the communities by other urogenital schistosomiasis studies in endemic countries.[21–25]

Regarding the awareness of the Government's ongoing urogenital schistosomiasis control efforts, majority of the respondents were aware of the distribution of preventive chemotherapy because this intervention has been going on in Mtama for more than a decade. This observation is similar to reports from other studies conducted in Tanzania showing high awareness of the use of praziquantel preventive chemotherapy as the control intervention due to ongoing school campaigns.[10,23]

Only one respondent was aware that manual picking of snails could help to interrupt disease transmission; this was due to lack of awareness and inadequate knowledge on the role of snails in disease transmission. Knowledge on *S. haematobium* can generate effective change in the endemic communities by reducing risk behavioural practices and increasing the community's participation in the control interventions.[26] Most of the respondents were able to describe urogenital schistosomiasis and symptoms, reasonably reflecting their long experiences with these symptoms. These findings are consistent with observations from studies conducted in Tanzania, Ghana, and Cameroon.[23,26–28] However, none of the respondents knew the causative agent of the disease and the exact role of the snails in the transmission of the disease, despite one mentioning the manual picking of snails as a control intervention. This finding is in line with reports from studies conducted in Cameroon and Yemen.[27,29] Despite majority of respondents (43, 75.4%) knowing the correct mode of disease transmission, some misconceptions were also observed, which might influence the increase or decrease in disease transmission as reported in other sub-Saharan countries.[20,21,30]

The findings revealed that there is use of traditional treatment for urogenital schistosomiasis due to inability to pay for hospital costs. Similar to observations reported in Nigeria, Ghana, and Mali.[21,27,30,31] The use of traditional remedies for urogenital schistosomiasis treatment is a growing public health concern in sub-Saharan Africa, requiring urgent intervention.

The improvement of WaSH was not mentioned among the interventions for the sustainable control of urogenital schistosomiasis, which could be due to inadequate knowledge of the role of poor WaSH on the transmission of urogenital schistosomiasis. The World Health Assembly (WHA) recommends promotion of WaSH as an integrated urogenital schistosomiasis control component and elimination strategy as a means of reducing the contamination of water bodies with *S. haematobium* eggs and human contact with cercariae infested water.[7] Despite WHA recommendation, promotion of WaSH has been lagging in low- and middle-income countries where urogenital schistosomiasis is endemic due to limited resources.[32]

The use of praziquantel preventive chemotherapy was viewed by community members as beneficial to school-age children. However, some reported that praziquantel was not beneficial due to its side effects. There is a possibility that the group that believes that praziquantel is not beneficial restrain their children from participating in the ongoing intervention campaigns. Thus, those children will serve as the reservoir of the infection and compromise the Government's ongoing control efforts.[33] Studies conducted elsewhere in Africa reported that fear of praziquantel side effects has discouraged parents and guardians from allowing their children to take praziquantel when distributed in schools.[32,34,35]

Community attitudes towards urogenital schistosomiasis and its control interventions have major implications for causing persistent transmission of the disease and affecting the uptake of the required interventions. The study findings revealed an absence of discrimination of patients suffering from urogenital schistosomiasis in the community. However, there was a belief that urogenital schistosomiasis is associated with superstition. Such beliefs lead to delayed diagnosis and prompt treatment hence resulting in morbidity.[20,21,36] All respondents believed that urogenital schistosomiasis is a serious disease due to the morbidities associated with it. This finding is in agreement with other studies conducted elsewhere.[27,29,37] Despite being aware of the morbidities associated with urogenital schistosomiasis, majority of the community members do not regularly screen for *S. haematobium* so as to avoid the costs involved. This calls for the need for free screening and treatment of *S. haematobium* at health facilities. Inadequate WaSH conditions have a role in the transmission of urogenital schistosomiasis.[7,38] Mtama community is surrounded by several water bodies with several ongoing human activities such as agriculture, fishing and domestic chores. The mentioned activities are known to increase the odds of acquiring the infection.[6,38]

In Mtama, mothers have a common practice of visiting the water sources with their under-five children, these children play in the water. This results in early exposure to infection from water bodies contaminated by infected school-age children and adults and this contributes to the reservoir of continuity of transmission.[39,40] There were no toilets at water sources or near water sources. The lack of toilets results in contamination of water with faeces, urine excreta and increases the odds of disease transmission.[7] The respondents reported unhygienic practices, such as open urination, which contaminate directly the water sources, and walking barefooted while working in water, or crossing the water sources, which facilitate the acquisition of the disease via the cercariae penetration.[41] The respondents were not aware if bathing a child with boiled water could lower the odds of acquiring the infection. It was reported that boiling water made it free of cercariae and safe for domestic use.[42]

Study Limitations

The nature of some of the research questions on the interview guide required respondents to recall past experiences and information. This could have resulted in under or over-reporting of actual situations.

The study relied on information from the community members rather than direct observation in the community. Also, the study did not interview medical officers of Mtama regarding screening and treatment of urogenital schistosomiasis, this could have complimented the information provided by the respondents.

CONCLUSIONS AND RECOMMENDATIONS

Despite the community being aware and knowledgeable of urogenital schistosomiasis, the study revealed that there is inadequate understanding of the disease causative agent and transmission, including the roles of snails, accompanied by misconceptions about the disease. Also, the study revealed the existence of undesirable attitudes on screening and treatment of the disease, and inappropriate WaSH practices, and this perpetuate transmission of the disease in Mtama community. There is need for initiating a community-based health education programme to address the undesirable attitudes and inappropriate WaSH practices. This should be integrated with other ongoing urogenital schistosomiasis interventions in Mtama district. It is also pertinent for the government to ensure that there is adequate supply of water in homesteads and sanitation facilities at or near water sources so as to minimise the number of people visiting the water sources and people involved in unhygienic practices, respectively. Also, the government should provide free screening and treatment of urogenital schistosomiasis at the health centres. This will encourage regular screening and the use of modern (health facility) treatment.

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ORIGINAL ARTICLE

Effectiveness of Artemether Lumefantrine and Dihydroartemisinin Piperaquine in Clearance of Gametocytes in Uncomplicated *Plasmodium falciparum* Malaria in Tiwi Kenya

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ABSTRACT

Background: Over 80 countries worldwide have now implemented WHO recommendations to use Artemisinin-Based Combination Therapy as a first-line treatment for ***Plasmodium falciparum*** malaria. The sexual stage of ***P. falciparum*** is responsible for the transmission of malarial parasites to infectious mosquitoes. Studies on gametocytes are generally based on microscopic detection, which is not sensitive, and there is a need for more sensitive molecular techniques that can detect and quantify gametocytes at densities as low as 0.02 to 0.1 gametocytes per micro-litre. The objective of this study was to determine the clearance rates of gametocytes after AL and DHA&P in uncomplicated ***P. falciparum*** and to compare the effectiveness of microscopy and reverse transcriptase-polymerase chain reaction in gametocyte detection.

Methods: In a randomised controlled clinical trial of samples collected, gametocyte densities were quantified by microscopy by counting against 500 leukocytes in the thick smear converted to numbers of parasites per micro-litre by assuming a standard count of 800 leukocytes per micro-litre of blood after staining with 10% Giemsa stain and by reverse transcriptase-polymerase chain reaction using primers specific to the pfs25 gene.

Results: There was no significant difference between the drug's gametocyte clearance ($p<.082$). The drugs cleared gametocytes in infected patients by day 28 as detected by microscopy. There was a significant difference in the detection of gametocytes by RT-PCR and microscopy ($p<.001$).

Conclusion: This study showed that Artemether-Lumefantrine and Dihydroartemisinin piperaquine have gametocytocidal effects on ***P. falciparum*** and the study on the clearance of gametocytes using both artemether-lumefantrine and Dihydroartemisinin piperaquine may be carried out using a larger sample size for policy implementation. The reverse transcriptase-polymerase chain reaction is more effective than microscopy in detecting low levels of gametocytes and the pfs25 gene can be used in the detection of gametocytes in the field to monitor the clearance of gametocytes.

INTRODUCTION

Malaria is a global life-threatening disease in humans caused by 5 species of *Plasmodium* parasites, *Plasmodium vivax, P. ovale, P. malariae, P. falciparum,* and *P.* knowlesi.[1] Globally, there were an estimated 241 million malaria cases in 2020. This represents about 14 million more cases in 2020 compared to 2019 and 69,000 more deaths.[2] Approximately two-thirds (47,000) of these deaths were linked to disruptions in the provision of malaria prevention, diagnosis, and treatment during the pandemic.

The mortality rate due to malaria in children aged under 5 years had reduced from 87% in 2000 to 77%. In 2020, malaria deaths increased by 12% compared with 2019, to an estimated 627 000; an estimated 47 000 (68%) of the additional 69 000 deaths were due to service disruptions during the COVID-19 pandemic.[2] In Kenya, malaria remains a leading cause of morbidity and mortality, especially in young children and pregnant women. It accounts for 30% of outpatient attendances and 19% of admissions to health facilities in endemic areas.[2] The prevalence of Malaria in Kenya was at 1.1% by the year 2020 according to the 2021 world malaria report 2021.[2] The Kenya malaria indicator survey conducted by the Ministry of Health (MOH) in 2020 reported Malaria prevalence at 8%[3]. In Kenya, Artemether Lumefantrine and Dihydroartemisinin-piperaquine are used as the first and second-line drugs respectively for the treatment and management of malaria cases.

The malaria control program in Kenya has been scaling up vector control interventions, timely diagnosis, and effective treatment of malaria using Artemisinin-Based Combination Therapy (ACT), and Intermittent Preventive Treatment for Pregnant Women (IPTP). Only the asexual parasite load causes the symptomatic disease; antimalarial drugs are primarily active against this stage, although some are-

also active against developing or mature gametocytes (gametocytocidal), and some may also disrupt the development of the ookinete in the mosquito gut.[3]

Gametocytocidal activity is conventionally regarded as advantageous because it may have a public health benefit in decreasing transmission. It is hypothesized that killing the transmission stages will reduce the rate at which resistance spreads.[4] The National Malaria Strategy adopted 4 interventions which include providing the right drugs at the right time, protecting pregnant women, promoting distribution and use of insecticide-treated nets, and pre-empting epidemics.[4] The transmission of malaria depends on the presence of mature sexual stage parasites or gametocytes in human peripheral blood.

The emergence and spread of *P. falciparum* resistance to antimalarial drugs are one of the greatest challenges facing global efforts to control malaria. To prevent drug resistance, there is clear evidence that combining more than one drug can improve its efficacy without increasing toxicity.[5] The development of highly effective artemisinin derivatives offers hope for the treatment of malaria using Artemisinin-Based Combination Therapy. The Artemisinin-Based Combination Therapy prevents individual resistance to individual drugs by relying on the principle of combining2 drugs with different mechanisms of action.[6] The fast-acting artemisinin derivative rapidly clears the main parasite load within a few hours to its therapeutic levels and thus reducing subsequent gametocyte carriage.[7] The partner drug, which is generally longer lasting, clears the rest of the parasites. In this study, we used 2 different artemisinin combination drugs: Artemisinin-Lumefantrine (AL) and Dihydroartemisinin-piperaquine (DHA&P) for the detection of clearance of gametocytes using microscopy and quantitative PCR.

MATERIALS AND METHODS

Study Site

The study was conducted in Tiwi in Kwale County of Coastal Kenya. This region has continuous malaria transmission with children and pregnant women being the most vulnerable group.[8] Malaria spread in the region is largely restricted to the long rainy season (May-June) and unpredictable short rainy seasons (October-November). This study was conducted from May to June during the rainy season when parasite density rises due to the rains that provide good breeding sites for mosquito vectors. The average annual rainfall of the region is 508 mm (10 years average). The entomological inoculation rate in the region has never been estimated.

Study Design

The study was a randomised clinical trial with a minimum of 116 participants. A randomisation list of how the 2 study drugs were allocated was computer-generated by an off-site investigator. Sequentially, numbered, and sealed envelopes containing the treatment group assignments were prepared from the randomisation list.

Study Population and Sampling Procedures

The study subjects were recruited from malaria patients who visited Tiwi Health Centre. The recruitment was based on the inclusion and exclusion guidelines for the assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated *P. falciparum* malaria.[9] The study nurses assigned treatment numbers sequentially and allocated treatment by opening the envelope corresponding to the treatment number. Only the study nurse was aware of the treatment assignments. All other study personnel, including the study physicians and laboratory personnel involved in assessing outcomes, were blinded to the treatment assignments. The patients were not informed of their treatment regimen.

Ethical Consideration

The study protocol (SSC No. 1955) was approved by the Scientific Steering Committee and the Ethical Review Committee of the Kenya Medical Research Institute. Written informed consent was obtained from the patients, and for participants below 18 years, the parents or guardians consented on their behalf. Only those who consented were recruited for the study. The slide and filter paper samples were given codes to conceal the identities of the patients. Patients were explained in detail the entire study procedure including the need to voluntarily participate, the anticipated benefits and/or risks, and the duration of involvement in the study. The risk of participation in this project was minimal. The possible risks of drawing blood included infection, bruising, and bleeding.

Inclusion Criteria

Those who consented and met the inclusion criteria were recruited for the study. The study subjects were children aged 6 months to10 years with uncomplicated *P. falciparum* malaria with no other *Plasmodium* species present using light microscopy, having an initial parasite density of 500- 100,000 asexual parasites/µl, having a measured axillary temperature of ≥37.5°C ≤ 39.5°C, no history of antimalarial drug intake during the previous month, providing informed consent (by parent or guardian, where appropriate) and willingness to return for follow-up.

Exclusion Criteria

Children who reported treatment with antimalarial chemotherapy 2 weeks before recruitment, those who experienced persistent and severe malaria, and evidence of a chronic disease or an acute infection other than malarial parasites, were residing outside Tiwi were excluded.

Patient Recruitment and Follow-up

In total, 200 cases suspected of uncomplicated malaria were screened for eligibility to participate in the study during an 8-week recruitment period, in May and June 2011. A total of 84 children were excluded because they did not meet the inclusion criteria. A total of 116 patients fulfilled the inclusion criteria and were recruited of which 58 were randomly administered DHA&P and, 58 AL.

Sample Collection

Blood samples were collected via a finger prick and spotted on a slide from the study subjects and followed up on days 3, 7, 14, and 28 for microscopic examination. Subsequently, from the finger prick, a drop of blood was collected on filter paper on the days scheduled for follow-up for molecular studies. The filter papers were air-dried

and stored in a zip-lock plastic bag with desiccators for molecular analysis. Study participants received the same treatment regimen for all subsequent episodes of malaria. The reference drugs were administered according to weight-based guidelines for fractions of tablets as follows: AL was given twice daily for 3 days. One tablet of DHA&P was given once per day for 3 days. Patients were given a glass of milk or requested to be breastfed after each dose of study medication. The first daily dose of study drugs was directly observed for 30 min at the study clinic, and the dose was re-administered if vomiting occurred.

Laboratory Procedures

Finger prick blood samples (50 µl) for RT-PCR analysis were collected on Cytiva Whatman 903™ protein saver card 1, manufacturer Cytiva US filter papers, and air-dried at room temperature. Nucleic acid extraction was performed as described by Bousema et al.[10] Total RNA was isolated using a High Pure RNA isolation kit.[11] (Roche, Lewes UK) Gametocyte-infected blood obtained from an *in vitro* culture of *P. falciparum* 3D7 clone was used as the positive control. Filter papers spotted with 50µl of *Plasmodium*-negative whole blood were used as negative controls for all steps of analysis. Briefly, the columns were centrifuged at 12,000 rpm for 2 minutes at room temperature. Some of RNA was used to make cDNA while the rest was stored at -80^{0}C.

Amplification of the pfs 25 Gene

The 21 µl mix for each reaction tube was made consisting of; 3µl of 10µm dNTPs, 8µl of 1× RT buffer 1µl of anti-sense primer, 1 µl of reverse transcriptase enzyme, and 8 µl of RNA and incubated at 37^{0}c for 45 minutes in a thermocycler[12], 7 µl cDNA was added to a 23 µl master mixture containing 100 µM of each dNTP, buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$), 0.3 µl of Pfs25F (5′- atcgatATGAATAAACTTTACAGTTTGTTTCT-3′), 0.3 µl of Pfs25R, (5′-T7-CATTTACCGTTACCACAAGTTA-3′), 14.36 µl of ddH_2O and 0.24 µl of enzyme Taq polymerase. The PCR cycling conditions were for 25 cycles. Initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 30 seconds, annealing at 50°C for 35 seconds, and extension at 68°C for 2.5 minutes. 2 µl of the product of the first PCR was used as a template for a nested PCR using a set of internal primers sense 25-1, (5′-TAATGCGAAAGTTACCGTGG-3′) anti-sense 25-2(5′CCATCAACAGCTTTACA GG-3′).

Agarose Gel Electrophoresis

The amplicons of pfs 25 gene products were resolved by electrophoresis on a 2.0% agarose gel, stained with ethidium bromide, run for 30 minutes at 80 volts and the product was estimated by comparison to gel pilot mid-range ladder (100) molecular weight marker (Qiagen®) North American, the US in Germantown, Maryland, run in the adjacent lane. The presence of a 500bp band representing amplification of the Pfs25 gene was determined using ultraviolet illumination and a digital camera.

Statistical Analysis

Data was entered in an excel spreadsheet and analysed by Stata 16. The prevalence of gametocytes was calculated by the number of positive patients with gametocytes divided by the total number of patients positive with malaria parasites. The student's t-test was used to analyse the clearance rates of gametocytes by both drugs using gametocyte density while the Chi-square was used to analyse the prevalence data of gametocytes detected by both techniques from days 0, 3, 7, 14, and 28.

RESULTS

Table 1 shows the mean gametocyte density (g/µl) detected by microscopy in the different study groups in patients treated with AL and DHA&P at different time intervals. In general, both drugs cleared all gametocytes by day 28. The number of gametocytes decreased to zero by day 28 but the difference was not significant (*p<.082*).

Prevalence Rates

The presence of gametocytes in clinical samples was assessed by microscopy and RT-PCR. Table 2 shows the prevalence at different times in 2 groups detected by microscopy. On day 0, gametocyte prevalence by microscopy was 12(24.0%) in the group treated with DHA&P and 4(8.3%) in the group treated with AL (p=.036). On day 3, the gametocytes prevalence was 11(22.0%) in the group treated with DHA&P and 3(6.2%) in the group treated with AL (p=.026). On day 7, the gametocyte prevalence was 4(8.0%) in the DHA&P and 2(4.2%) with AL (p=.678) while on day 14, the gametocyte prevalence was 0(0.0%) in the DHA&P and 1(2.1%) with AL(p=.490). On day 28 gametocytes were not detected by microscopy in both groups treated with DHA&P and AL.

Table 3 Shows gametocyte prevalence detected by RT-PCR. The prevalence was 24(48.0%) in the group treated with DHA&P and 31(64.6%) in the group treated with AL (p=.098). On day 3, the prevalence was 19(39.6%) and 30(60.0%) in the AL and DHA&P groups respectively (p=.043). On day 7, the prevalence was more than twice in the group treated with DHA&P compared with 12(25.0%) in the AL group (p=.002) while on day 14 the prevalence was 10(20.8%) in the group treated with AL and 30.0% in the group treated with DHA&P (p=.298). On day 28, the prevalence was 6.3% in the group treated with AL and 10% in the group treated with DHA&P (p=.715).

Evaluation of the Effectiveness of Microscopy and RT-PCR in the Detection Of gametocytes

Table 4 shows the total gametocyte prevalence detected by microscopy and RT-PCR. Overall, RT-PCR was able to detect significantly more cases of malaria in day 0, 55(56.1%) vs. 16(16.3%); *p<.001*, day 3 49(50.0%) vs. 14(14.3%); *p<.001*, day 7 40(40.8%) vs.6(6.1%); p<.001, day 14 25(25.5%) vs. 1(1.0%); p<.001, and in day 28 8(8.2%) vs. 0.0%; *p=.003*). On average, RT-PCR detected 10 times more gametocytes compared to microscopy and the difference between the methods in the detection of gametocytes was significant.

DISCUSSION

During the period of malaria infection cases, gametocytes are responsible for the transmission of malaria from infectious female anopheles' mosquitoes to human beings.[13] In this study, the effectiveness of AL and DHA&P

TABLE 1: Mean Number of Gametocytes (g/µl) Detected by Microscopy in the Different Study Groups

Day of examination	AL		DHA&P		
	n	Mean+SE	N	Mean+SE	p-value
Day 0	4	112+21	12	92+10	0.363
Day 3	4	64+29	12	63+10	0.956
Day 7	4	24+15	12	17+8	0.696
Day 14	4	8+8	12	0+0	0.082
Day 28	4	0+0	12	0+0	N/A

TABLE 2: Gametocyte Prevalence (%) by Microscopy on the Different Follow-Up Days

Day of examination	MICROSCOPY				
	AL (n=48)		DHA&P (n=50)		
	n	%	N	%	*p- value*
Day 0	4	8.3	12	24.0	.036
Day 3	3	6.2	11	22.0	.026
Day 7	2	4.2	4	8.0	.678
Day 14	1	2.1	0	0.0	.490
Day 28	0	0.0	0	0.0	N/A

TABLE 3: Gametocyte's Prevalence (%) by RT-PCR in the Different Follow-Up Days

Day of examination	RT-PCR				
	AL (n=48)		DHA&P (n=50)		
	n	%	N	%	*p -value*
Day 0	31	64.6	24	48.0	.098
Day 3	19	39.6	30	60.0	.043
Day 7	12	25.0	28	56.0	.002
Day 14	10	20.8	15	30.0	.298
Day 28	3	6.3	5	10.0	.715

TABLE 4: Total Gametocyte Prevalence (%) Detected by Microscopy and RT-PCR

Day of examination	MICRO (n=98)		RT-PCR (n=98)		
	n	%	N	%	p-value
Day 0	16	16.3	55	56.1	<0.001
Day 3	14	14.3	49	50.0	<0.001
Day 7	6	6.1	40	40.8	<0.001
Day 14	1	1.0	25	25.5	<0.001
Day 28	0	0.0	8	8.2	<0.003

in the clearance of gametocytes of *P. falciparum*, the effectiveness of RT-PCR and microscopy in the detection of gametocytes, and the detection of the pfs25 gene of the gametocytes were evaluated.

Results showed no difference in the clearance of gametocytes by both drugs. This is because according to statistical analysis performed, there was no significant difference even though in the table results section, few gametocytes were reported in the 2 drugs that were used for the treatment of patients. The drugs cleared gametocytes in positive patients by day 28. These findings are similar to findings reported by Petra *et al.*[14] who found that clearance of gametocytes in patients treated with DHA&P had no significant difference with patients treated with AL. Both drugs rapidly clear parasitemia and fever, and demonstrate a significant gametocidal effect, even in areas of widespread parasite resistance to other antimalarial.[15]

In this study, the 2 drugs showed similarities concerning their effectiveness and clearance of gametocytes compared with other studies conducted elsewhere[16,17,18]. However, most of these studies had a follow-up of 42 days which is different from the 28 days follow-up in this study. This could have been the reason for the difference in the results observed. Despite the effectiveness of AL, there were substantial limitations to this regimen, including; twice-daily dosing and the need for administration with fatty food. However, many other studies have analysed the efficacy of AL and DHA&P in the clearance of gametocytes, and all reported very good results.[19, 20, 21, 22]

This study showed less effectiveness of DHA&P on gametocyte clearance in comparison with AL when a more sensitive RT-PCR test was used for gametocyte detection. This could limit the effectiveness of DHA&P in areas with low malaria transmission. However, this finding should be further investigated in larger studies in different study sites with different transmission intensities. Using RT-PCR, gametocytes were present in low numbers throughout the 28 days of follow-up in both study groups. Previous studies have shown that both drugs can reduce malaria transmission in the community.[19,23] However, the 90% gametocytaemia clearance cited in these studies were observed > 20 days post-treatment. In our study, both groups of patients had < 5% gametocytaemia on day 14 post-treatment. Artemisinin derivatives kill young gametocytes.[24] This may explain the persistence of gametocytes after 3- and 7-day courses of treatment in uncomplicated malaria.

Prolonged gametocytaemia has been proposed as an early sign of the emergence of antimalarial drug resistance.[25] This might be a concern given the poor gametocytocidal effects of DHA&P. However, gametocyte density remained low, and the gametocyte clearance was fast. During the 28 days of follow-up, few patients had gametocytes in this study, which reflects the good gametocytocidal properties of the Artemisinin-based combination therapy. However, artemisinin-based combination therapy has, in general, a negative effect on gametocyte development and survival and thus influences malaria transmission, at least in low transmission areas.[26, 27, 28] In this study, there was a significant difference in the detection of gametocytes with RT-PCR and microscopy. On day 28 of the follow-up period, RT-PCR detected up to 10 times more gametocytes confirming that RT-PCR was more effective than microscopy. RT-PCR detection techniques have demonstrated that gametocytes can be seriously underestimated using microscopy. In this study, RT-PCR gave estimates of gametocyte prevalence 10-fold higher than microscopy. This compares well with other studies which recorded ten times higher gametocytes than estimated by microscopy.[29] The detection of gametocytes by microscopy is insufficiently sensitive to assess potential infectivity. Gametocyte densities below the microscopic threshold for gametocyte detection (~ 5 gametocytes/µl) frequently result in mosquito infection.[30] This study showed that with sensitive detection RT-PCR, a difference in gametocyte clearance can be observed but these results should be confirmed in larger studies and in other study areas with different malaria transmission intensities.

The RT-PCR detected the *P*fs25 gene using primers specific for this gene with the presence of approximately 500 base pair bands representing the amplification of this gene. The RT-PCR detected the presence of gametocytes in positive malaria patients, and this showed it can be applied to guide case management in the control of malaria transmission. RT-PCR is reliable in determining the prevalence data of gametocyte carriage in the population, needed to know the infectious reservoir and battle the ongoing transmission of malaria.[31]

The RT-PCR was able to detect gametocytes below the threshold of microscopic detection and is highly specific for its gametocyte targets and also in the presence of a vast excess of asexual forms[32], as shown in this study where it detected gametocytes below the threshold of microscopic detection. The RT-PCR had a detection limit of 20 to 100 gametocytes/mL of blood, and the high-throughput format allows its use in the assessment of gametocyte carriers in the population, and it is critical in understanding malaria transmission dynamics in epidemiological studies.[33,34] A previous study with RT-PCR showed a very high prevalence of gametocytes in symptomatic children in Kenya.[35] The RT-PCR detection of gametocytes enables the treatment of carriers to clear parasitaemia and reduce the source of infection available to mosquitoes that emerge at the start of the rainy season. This could contribute to malaria control strategy if high coverage with effective therapy is achieved.

CONCLUSIONS

This study showed that AL and DHA&P have gametocytocidal effects on *P. falciparum.* The RT-PCR is more effective than microscopy in the detection of low levels of gametocytes. The pfs25 gene can be used in the detection of gametocytes in the field to monitor the clearance of gametocytes. The findings of this study can be used as policy guidelines in rolling out mass artemisinin-based combination therapy to reduce the gametocyte prevalence in asymptomatic and symptomatic patients to prevent and control malaria cases. It is critical in understanding malaria transmission dynamics in epidemiological studies.

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ORIGINAL ARTICLE

Concurrent Infection With Dengue and Chikungunya Viruses in Humans and Mosquitoes: A Field Survey in Lower Moshi, Tanzania

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ABSTRACT

Introduction: Dengue and Chikungunya have re-emerged as important diseases of global concern. Co-infections with Dengue virus (DENV) and Chikungunya virus (CHIKV) could have serious outcomes if not diagnosed and managed optimally. However, the key focal points for the maintenance of CHIKV and DENV infections and the extent of their co-infection remain poorly understood in many geo-ecologically distinct parts of Tanzania.

Objective: We aimed to comparatively examine the prevalence and factors for seropositivity to DENV and CHIKV and their infection rates in humans and mosquitoes

Methods: A cross-sectional study was performed in the Lower Moshi area of the Kilimanjaro region from April to July 2020. DENV and CHIKV exposure was determined by detecting IgM to the viruses using enzyme linked immunosorbent assay whereas infection was determined by real time quantitative polymerase chain reaction (RT-qPCR) assay.

Results: Insecticide Treated Bed Net (ITN) use ($\chi2=3.504$; $p< 0.05$), being ≥7 individuals living in the same household ($\chi2=4.655$; $p<0.05$) and a recent travel to an urban destination ($\chi2=3.39$; $p< 0.05$) were the only factors associated with CHIKV seropositivity. ITN use was the only factor associated with CHIKV infection ($\chi2=5.204$; $p<0.05$). A recent travel to an urban destination ($\chi2=4.401$; $p< 0.05$) was the only factor associated with DENV seropositivity. Five (1.5%) Ae. aegypti pools were positive for CHIKV whereas 1 (0.3%) was positive for DENV. Two Cx. pipiens, pools (1.9%) were positive for CHIKV. None of the Cx. pipiens mosquitoes was positive for DENV. No associations between DENV and CHIKV seropositivity was observed in humans but DENV infection was strongly associated with CHIKV infection ($\chi2 = 238.45$; $p<0.01$). CHIKV infection was observed to be consistently higher in both, humans and mosquitoes.

Conclusion: Detection of DENV and CHIKV in both humans and vector mosquitoes confirms that both viruses are actively circulating in the Lower Moshi area of Kilimanjaro region in Tanzania. Our findings point out the Lower Moshi area as a potential focal point for the maintenance of the two viruses and possibly other vector borne viruses. We call upon sustained active surveillance of arboviruses and other re-emerging infections to be better prepared for possible outbreaks by the viruses.

INTRODUCTION

Dengue and Chikungunya are vector borne diseases of public health and socioeconomic importance with shared endemic profiles and symptoms. Co-infections with Dengue virus (DENV) and Chikungunya virus (CHIKV) could have serious outcomes if not diagnosed and managed optimally.

In recent years, the spread of DENV and CHIKV has gained global concern, especially, in tropical and subtropical regions because of their recurring outbreaks[1]. Both DENV and CHIKV are spread by common mosquito vectors, mainly Aedes aegypt.i[2] Dengue is considered as the most important arbovirus disease compared to Chikungunya, mainly known from its epidemics in continental Africa and Asia. Chikungunya, on the other hand, has been prevalent in Africa and Asia for many years.[3,4] CHIKV was also detected in America in 2013, whereby more than 2 million cases have been reported.[5]

Although CHIKV and DENV belong to different genera of the Togaviridae family, ie, the alphavirus and the *flavivirus* genera respectively, both cause febrile syndromes that share many similar signs and symptoms including fever plus any two of the following: nausea, vomiting, rash and headaches that leads to a

high likelihood of misdiagnosis by clinicians.[6] A wide range of vector-borne and zoonotic pathogens exist in tropical Africa and elsewhere.[7] Most of these pathogens co-infect a significant proportion of inhabitants in a given setting.[8,9] Co-infections with both viruses may obscure clinical suspicion, as signs and symptoms for many of these pathogens overlap. In endemic areas, this becomes a particularly pressing issue that must be taken into account to ensure accurate diagnosis for optimal case management. Although, currently, there is no empirical evidence of a higher severity in these DENV-CHIKV co-infection cases, reports are available that report a more severe clinical disease in dual infection with arboviruses than mono infection.[10-12]

A recent study conducted in the same study area, reported an active transmission of Rift Valley Fever virus (RVFV) in Lower Moshi area of Kilimanjaro region, pointing to it as a potential hotspot for RVF.[13] The presence of vector mosquitoes for Dengue and Chikungunya viruses in the area,[14] prompted the design of this study to determine the prevalence of DENV and CHIKV in humans and vector mosquitoes (Aedes aegypti and Culex pipiens) in the absence of current outbreaks. Dengue and Chikungunya have re-emerged as important pathogens of global concern.[15,16] However, the key focal points for the maintenance of CHIKV and DENV infections remain poorly understood in many geo-ecologically distinct parts of Tanzania. Results from the current study will not only be useful in understanding the burden of the viruses and the extent of DENV-CHIKV co-infection in the area, but also inform health care providers and policy makers on potential unreported hotspots for DENV and CHIKV outbreaks and thus guide decision makers to implement integrated vectors interventions (IVM).

METHODOLOGY

Study Design and Site

This was a cross-sectional study conducted in lower Moshi area (37°20'E 3°21'S) of Moshi district, Kilimanjaro region of Tanzania between April and July 2020 involving 3 villages, namely Mikocheni, Chemchem, and Arusha Chini. Lower Moshi, as described previously[13], is an intensive rice irrigation area, located on the southern foothills of Mount Kilimanjaro[13] (Figure 1).

The population in the area is engaged in agriculture and livestock keeping. Two rivers, the Pangani and the Rau provide water for irrigation. The rice irrigation schemes have structured and unstructured canal networks covering an area of about 1,100 hectares. During the rainy season, temporary pools that serve as mosquito breeding sites are formed. Their persistence beyond the rains contributes to unremitting mosquito breeding. The area has two rainy seasons; the long rains which run from March to June and the short rainy season from November to January. The study was carried out during the rainy season when vector activity is at its peak in order to capture the highest possible transmission of the viruses studied.

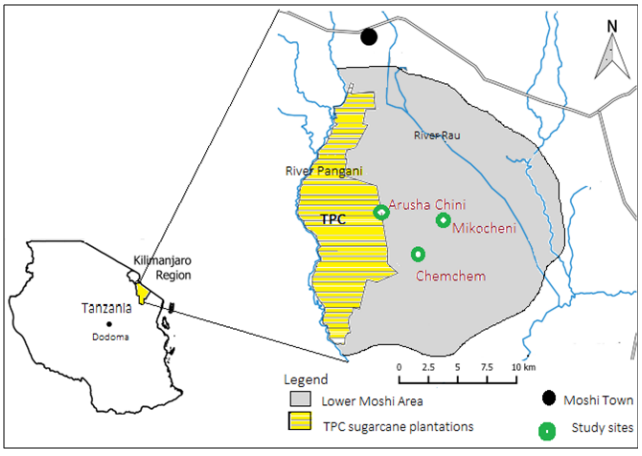
Participants and Sample Collection

Participants in this study were males and females aged between 10 and 70 years from 266 households. The main occupation of inhabitants in the area is agropastoralism. Consent to participate in the study was obtained from adults aged ≥18 years whereas parents or legal guardians for participants aged <18 years assented for participants aged 18 years or below. Sample size was estimated by "Epitools" online sample size (ss) calculator based on the formula ss= $Z^2(P)\ (1\text{-}P)/\varepsilon^2$, where, Z is the value (1.96 for 95% confidence level [CI]), P represents prevalence, and ε is the minimal tolerable error at 95% CI, expressed as a decimal (0.05). These estimations gave a minimum sample size of 183. However, in order to increase the power of the statistical analyses, the sample size was increased to 266.

Collection of Blood Samples

Blood sampling was performed by phlebotomists from the Kilimanjaro Christian Medical Center (KCMC). Three milliliters of blood were collected by venipuncture from each participant from the median cubital vein. Each sample was divided into two aliquots of 1.5 ml each, and aliquots placed into plain and EDTA vacutainer tubes, respectively. To each EDTA tube containing a sample, 4.5ml of Tri Reagent (Zymo Research, Irvine, CA, U.S.A.) were added. The mixture was gently mixed by shaking for 1 minute and immediately shipped to the KCRI biotechnology laboratory at 4°C, for RNA extraction and PCR analyses. Samples in plain tubes were allowed to clot for a maximum of 20 minutes at room temperature before they were centrifuged at 2,000 x g for 10 minutes at 4°C and serum transferred to clean sterile serum tubes. Serum samples were tested for presence of IgG/IgM to DENV and IgM to CHIKV. Blood samples that were positive by serology were subjected to PCR analysis. Demographic data from participants were collected using electronic forms designed using Open Data Kit (ODK) tools (https://opendatakit.org/) deployed in Android tablets.

FIGURE 1. Map of Tanzania Showing the Lower Moshi Area where the Study was Conducted



Mosquito Trapping

Mosquito trapping was performed from 8.00 am to 6.00 pm near sampled houses for 10 consecutive days as previously described by Kajeguka and colleagues.[17] Briefly, BG Sentinel trap (BGS) (Biogents AG, Regensburg, Germ-

any) to target outdoor host-seeking adult mosquitoes. The BGS-Trap, developed by BioGents GmbH (Regensburg, Germany), is made of an easy to transport, collapsible white bucket with gauze covering. Captured mosquitoes were immediately morphologically identified in the field and sorted according to their species using taxonomic keys.[18,19] Two key most abundant and known DENV and CHIKV vector species, Cx pipiens and Ae aegypti, were sorted for qPCR analysis of DENV and CHIKV RNA in pools of 50s.

Laboratory Procedures

DENV IgM and CHIKV IgM ELISAs

Enzyme Linked-Immunosorbent assays (ELISA) for antibodies to DENV and CHIKV were performed as previously described[20]. Briefly, serum from plain tubes was obtained by centrifugation at 2,000 rpm x g for 10 minutes and serum samples stored at −20°C until serological analyses were performed. For seropositivity of CHIKV, anti-CHIKV IgM was detected using indirect ELISA kit (SD, Gyeonggi-do, Korea and IBL international, Hamburg, Germany, respectively). Detection of DENV IgM antibodies was done using a direct ELISA kit (SDInc, Gyeonggi-do, Korea) as described by[21]. All assays were performed according to manufacturers' instructions.

Ribonucleic Acid (RNA) Isolation and Detection by PCR

For DENV and CHIKV, Blood samples kept in EDTA tubes were centrifuging at 1,000 rpm x g for 10 minutes in a refrigerated centrifuge to obtain buffy coat. Ribonucleic acid (RNA) was extracted from buffy coat samples using the Boom method[22] as described by[17]. Total RNA was extracted from 200 µl of homogenized individual Aedes and Culex mosquitoes using QIAGEN RNeasy Mini Kits according to the manufacturer's instructions. Using the real-time RT-PCR method, primers and probes[23] were followed to screen mosquito homogenates for evidence of Chikungunya and Dengue viral RNA.

RT-PCR for Detection of DENV and CHIKV in Human and Mosquito Samples

Both, blood samples and mosquito extracts were tested with the RealStar Dengue RT-PCR Kit 1.0 (Altona Diagnostics [Altona], Hamburg, Germany[15]; and the Tropical Fever Core Multiplex Real-time PCR (Fast Track Diagnostics [FTD], Luxembourg). All procedures were performed according to the manufacturer's protocols.

Data Analysis

Data analysis was performed using IBM SPSS Statistics for Windows version 26 (IBM Corp, Armonk, NY, USA). Descriptive data were presented as frequencies and percentages, means, and medians wherever it was applicable. Categorical data were reported as a tabulation of proportions and compared between humans and goats. Chi-squared statistic ($\chi 2$) was used to examine associations between seropositivity to DENV and CHIKV in humans and DENV and CHIKV infection in both humans and mosquitoes. Co infection and co- exposure data was reported as numbers and corresponding percentages. Associations between exposure and infection in humans and mosquitoes was determines by the $\chi 2$ test. In all cases, associations reaching a P value of .05 or less were considered as significant.

Ethical Issues

This study obtained approval by the College Research and Ethics Committee (CRERC) of the Kilimanjaro Christian Medical University College (KCMUCo) with approval certificate #2419. The study obtained permission from the Kilimanjaro Regional and District Administrative Secretaries, District Medical and Veterinary Officers, and local village and ward executive officers of respective villages. Participants were asked to voluntarily consent to participate in the study after an explanation about the study aims, procedures, risks and benefits was made to them.

Participants aged 18 years and above signed "informed consent" forms whereas parents and/or legal guardians of participants under 18 years and participants who could not read or write signed the "informed consent" on behalf. All authors hereby confirm that all procedures in this study were approved by CRERC and were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

RESULTS

Demographic Characteristics of Participants

A total of 266 participants were involved in the study, fifty-two percent of which were aged between 21 and 50 years whereas 56.4% were females. Majority of participants (74.4%) lived in families of 4 individuals or above in the same household. Most participants had attained primary education (63.2%) and kept livestock (72.9%) (Table 1).

TABLE 1: Demographic Characteristics of Participants

Characteristics	n	%
Age group (years)		
≤20	28	10.5
21-50	140	52.7
>50	98	36.8
(Median, IQR) years	45(30-55)	
Sex		
Male	116	43.6
Female	150	56.4
Individuals living in a household		
<4	68	25.6
≥4	198	74.4
Highest education		
No formal	51	19.2
Primary	168	63.2
Tertiary	47	17.7
Type of animals kept by participant		
Animal Keeping	194	72.9
None	72	27.1

IQR, Interquartile Range

Prevalence and Factors Associated With Chikungunya Virus Seropositivity and Infection in humans

Results show that the use of Insecticide Treated Bed Nets (ITNs) (χ^2=3.504; P<.05), being more than 7 individuals

in the same household (χ^2=4.655; P<.05) and a recent travel to an urban destination (χ^2=3.39; P<.05) were the only factors associated with CHIKV seropositivity. For CHIKV infection, ITN use was the only factor that was associated with CHIKV infection (χ^2=5.204; P<.05). We observed a higher PCR positivity rate than seropositivity to DENV (Table 2).

Prevalence and Factors Associated With Dengue Virus Seropositivity and Infection in Humans

With regards to DENV, the only factor that was associated with DENV seropositivity was a recent travel to an urban destination (χ^2=4.401; P<.05). None of the studied factors was found to be associated with DENV infection (Table 3).

CHIKV and DENV Infection in Aedes aegypti and Culex pipiens

For Ae aegypti, 333 monospecific pools of mosquitoes were tested while 106 pools of Cx pipiens were tested for both CHIKV and DENV infections. Out of these, 5 (1.5%) Ae aegypti pools were PCR positive for CHIKV, while only 1 (0.3%) was positive for DENV. One hundred and six Cx pipiens complex pools were tested, of which 2 (1.9%) were PCR positive for CHIKV. None of the Culex mosquito pools was positive for DENV.

Association Between Chikungunya and Dengue Infection in Humans and Mosquitoes

When DENV and CHIKV infection in humans and mosquitoes were tested for any independent associations, no associations were detected by statistical analyses (Table 4). When we attempted to find out whether dual infection by DENV and CHIKV and seropositivity to the viruses were associated in humans, our analyses showed no associations between DENV and CHIKV seropositivity in humans. However, DENV infection (as determined by PCR), was found to be strongly associated with CHIKV infection (χ^2 = 238.45; P<.01) (Table 5). In humans, the prevalence of antibodies to CHIKV was higher than to DENV. Likewise, a marginally higher infection rate by CHIKV was recorded in humans. CHIKV infection was observed to be consistently higher in both, humans and mosquitoes (Aedes and Culex), whereas none of the Culex mosquitoes was found to be infected by DENV.

DISCUSSION

This study investigated the concurrent circulation of DENV and CHIKV viruses in humans and their designated vector mosquitoes it terms of their risk factors and comparative seropositivity and infection rates in an area intensively used for irrigation in the Lower Moshi area of Kilimanjaro region in Tanzania. Our study highlights the active circulation of DENV and CHIKV in both, humans and vector mosquitoes in the study area. From this study, individuals within a household who did not use ITNs, individuals who were more than 7 in the same household (sleeping under the same roof), and individuals who recently traveled to an urban destination were more seropositive to CHIKV. ITN use was associated with lower CHIKV infection.

Previous studies had reported a range of factors that increase the risk for infection by Dengue and Chikungunya viruses including older age and male sex.[24]

Our current study, however, reports no association of these factors with higher CHIKV or DENV seroprevalence and infection, contrary to what some previous studies had reported[25-28]. The absence of associations between older age and gender with CHIKV and DENV infection could be explained by the nature of the main economic activities in the study area, where, almost all tested individuals, young and old were engaged in livestock keeping and irrigated sugar cane farming and had reported mosquito bites. In an environment of intense transmission of arboviruses, factors such as sex and age may not be important to predispose to infection.

Studies have reported significant seroprevalence of CHIKV antibody with the agro-pastoralist lifestyle compared to pastoralist lifestyles.[29-31] Agro-pastoralism could be associated with higher infection risk including environmental suitability for vector survival and thus virus maintenance. Further, an agro-pastoralism lifestyle is more likely to offer an intimate allow close contact between humans and DENV and CHIKV vectors. Recent travel to the urban area has been consistently associated with both DENV and CHIKV seropositivity and infection.[25,32] Millions of susceptible people moving to the cities and living in shanty towns with inadequate housing and dilapidated or no basic services such as clean water, sewer and waste management is thought to results into crowded human communities and creation of large mosquito populations leading to formation of ideal conditions for arboviruses transmission.[25,32-37]

Consistently, we observed higher seroprevalence and infection rates of CHIKV than DENV, which indicates the former to be more prevalent than the latter, Although Dengue has emerged as one of the most important re-emerging diseases[16,37] that has caused six outbreaks in Tanzania over the past 10 years including thousands of reported cases and multiple deaths,[36,38,39] its seroprevalence and infection rates have been reported to be lower compared to the endemic Chikungunya virus.[27,34,36,38-40] Notwithstanding that we could not establish any associations between DENV and CHIKV seropositivity in humans, DENV infection (by qPCR) was found to be strongly associated with CHIKV infection, suggesting the presence of common factors for the transmission of the two viruses in the area.

Our study was able to detect DENV and CHIKV in vector mosquitoes collected. Generally, Ae. aegypti higher infection rate by CHIKV compared to DENV, which underscores the importance of this mosquito species in the transmission of arboviruses. Consistent to the observations that Culex is not an important vector for DENV, none of the Culex mosquitoes were positive for DENV infection. The current study shows that Ae Aegypti mosquitoes are the main vector mosquitoes for the transmission of not only DENV and CHIKV, but also other arboviruses such as Rift Valley Fever Virus in the same area[41]. This may also mean that the residents of the studied sites are at risk of being infected by multiple arboviruses. The detection of active infections of CHIKV and DENV in both humans and vector mosquitoes during silent, inter-epidemic periods, albeit at low rates for DENV, points out to the possibility that the Lower Moshi area is a potential hot spot for future DENV and CHIKV outbreaks.

TABLE 2: Prevalence and Factors Associated With Chikungunya Virus Seropositivity and Infection in Humans

	ELISA seropositivity test				PCR for infection detection			
	Positive	Negative	All	%	Positive	Negative	All	%
Sex								
Males	8	107	115	7.0	4	10	14	28.6
Females	16	135	151	10.6	5	20	25	20.0
Total	24	242	266	9.0	9	30	39	23.1
	Chi-square = 1.09; P value=.21				**Chi-square = 0.37; P value=.41**			
Age group								
11 – 20	2	26	28	7.1	0	3	3	0.0
21-30	7	33	40	17.5	3	5	8	37.5
31-40	2	43	45	4.4	1	4	5	20.0
41-50	4	51	55	7.3	2	8	10	20.0
>50	9	89	98	9.2	3	10	13	23.1
Total	24	242	266	9.0	9	30	39	23.1
	Chi-square = 4.98; P value=.29				**Chi-square = 1.918; P value=.80**			
ITNuse ¥								
Yes	3	45	48	6.3	3	0	3	100.0
No	14	132	146	9.6	4	10	14	28.6
Total	17	177	194	8.8	7	10	17	41.2
	Chi-square = 3.5; P value=.04				**Chi-square = 5.204; P value=.05**			
Individuals per HH#								
1 - 3	4	64	68	6.3	1	4	5	20.0
4 - 6	12	138	150	8.7	2	22	24	8.3
7 and more	8	39	47	20.5	6	4	10	60.0
Total	24	241	265	10.0	9	30	39	23.1
	Chi-square = 4.66; P value=.04				**Chi-square = 10.646; P value=.16**			
Types of animal@								
None	10	62	72	13.9	4	11	15	26.7
chicken	4	78	82	4.9	2	8	10	20.0
Goats/Sheep	7	47	54	13.0	2	5	7	28.6
Cattle	3	52	55	5.5	1	6	7	14.3
Goats/Sheep/Cattle	0	3	3	0.0				
Total	24	242	266	9.0	9	30	39	23.1
	Chi-square = 5.96; P value=.14				**Chi-square = 0.586; P value=.39**			
Recent travel								
Yes	6	95	101	5.9	1	11	12	8.3
No	18	147	165	10.9	8	19	27	29.6
	24	242	266	9.0	9	30	39	23.1
	Chi square = 1.884; P value=.12				**Chi-square = 2.123; P value=.15**			
Destination								
Rural	1	39	40	2.5	0	5	5	0.0
Peri-urban	0	13	13	0.0				
urban	5	43	48	10.4	1	6	7	14.3
Total	6	95	101	5.9	1	11	12	8.3
	Chi-square = 3.39; P value=.05				**Chi-square = 0.779; P value =.59**			
Education level								
No Formal education	2	49	51	3.9	0	2	2	0.0
Primary Education	16	15	31	51.6	6	22	28	21.4
Tertiary Education	6	41	47	12.8	3	6	9	33.3
Total	24	105	129	18.6	9	30	39	23.1
	Chi-square = 2.47; P value=.09				**Chi-square = 1.176; P value=.25**			

Key: ¥ Insecticide treated bed-nets; # Household; @Types of animals kept by participants

TABLE 3: Prevalence and Factors Associated With Dengue Virus Seropositivity and Infection in Humans

Variable	ELISA				PCR			
	Positive	Negative	All	%	Positive	Negative	All	%
Sex								
Male	1	114	115	0.9	2	6	8	25.0
Female	6	145	151	4.0	4	12	16	25.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 2.48; P value=.01				**Chi-square = 0; P value=.68**			
Age								
11 – 20	0	28	28	0.0	0	2	2	0.0
21 – 30	2	38	40	5.0	3	4	7	42.9
31 – 40	2	43	45	4.4	0	2	2	0.0
41 – 50	1	54	55	1.8	1	3	4	25.0
>50	2	96	98	2.0	2	7	9	22.2
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 2.485; P value=.26				**Chi-square =2.561; P value=.47**			
ITN use ¥								
Yes	0	48	48	0.0	1	2	3	33.3
No	6	140	146	4.1	3	11	14	21.4
Total	6	188	194	3.1	4	13	17	23.5
	Chi-square = 2.036; P value= .18				**Chi-square = 0.195; p value = 0.56**			
Individuals per HH#								
1 – 3	2	66	68	2.9	1	3	4	25.0
4 – 6	4	146	150	2.7	1	11	12	8.3
>/=7	1	47	48	2.1	4	4	8	50.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 0.072; Pvalue=.51				**Chi-square = 4.444; P value=.16**			
Type of animals@								
None	3	69	72	4.2	1	9	10	10.0
chicken	2	80	82	2.4	2	2	4	50.0
Goats/Sheep	1	53	54	1.9	2	5	7	28.6
Cattle	1	54	55	1.8	1	2	3	33.3
Goats/Sheep/Cattle	0	3	3	0.0	0	0	0	0.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 1.025;P value=.24				**Chi-square = 2.692; P value=.23**			
Recent travel								
Yes	0	101	101	0.0	1	5	6	16.7
No	7	158	165	4.2	5	13	18	27.8
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 4.401; P value=.03				**Chi-square = 0.296; P value=.52**			
Destination								
Rural	0	40	40	0.0	0	1	1	0.0
Peri-urban	0	13	13	0.0	0	0	0	0.0
urban	0	48	48	0.0	1	4	5	20.0
Total	0	101	101	0.0	1	5	6	16.7
	Nil				**Chi-square = 0.24: P value=.83**			
Education level								
No education	2	49	51	3.9	0	2	2	0.0
Primary education	5	163	168	3.0	3	13	16	18.8
Tertiary education	0	47	47	0.0	3	3	6	50.0
Total	7	259	266	2.6	6	18	24	25.0
	Chi-square = 1.679; P value=.02				**Chi-square = 3; P value=.10**			

Key: ¥ Insecticide treated bed-nets; # Household; @Types of animals kept by participants

TABLE 4: Associations Between Chikungunya and Dengue Infection in Humans and Mosquitoes

		Culex-DENV-PCR		Aedes-DENV-PCR		Culex-CHIKV-PCR		Aedes-CHIKV-PCR	
		Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Human CHIKV-PCR	Positive	0(0.0)	10(3.8)	0(0)	10(3.8)	1(20.0)	9(90.0)	0(0.0)	10(100.0)
	Negative	0(0.0)	256(96.2)	1(100)	255(96.2)	4(80.0)	252(96.6)	4(100.0)	252(96.2)
Human DENV-PCR	Positive	0(0.0)	9(3.4)	0(0.0)	9(100.0)	1(11.1)	8(88.9)	0(0.0)	9(3.4)
	Negative	0(0.0)	257(96.6)	1(100.0)	256(96.6)	4(80.0)	253(96.9)	4(100.0)	253(96.6)

TABLE 5: Chikungunya and Dengue Co-exposure and Infection

	DENV-ELISA*		DENV-PCR#	
	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)
CHIKV-ELISA*				
Positive	0(0.00)§	10(100.00)		
Negative	5(2.00)	251(98.00)		
CHIKV-PCR#				
Positive			9(90.00)§	1(10.00)
Negative			0(0.00)	256(100.00)

*Chi- squared (X2) = 0.199; P value=.0824; # Chi-squared (X2) = 238.45;P value=0.0001; §Dengue-Chikungunya Co-infection

The observation that almost all IgM and PCR CHIKV and DENV positive participants had subclinical infection, and that, mosquitoes carry the viruses, implies the possibility of long-term maintenance of the viruses across seasons without being diagnosed. In order to be better prepared to control possible outbreaks caused by arboviruses, extra effort in active surveillance of arboviruses across hosts is mandatory. Our public health systems need to be more vigilant in generating more information and take steps to prevent outbreaks before they occur. Notwithstanding the strength of our study findings, we acknowledge the limitation that our study could not collect and analyze other mosquito species other than Ae Aegypti and Cx pipiens which could have provided additional data on vector abundance possible infection by DENV and CHIKV viruses.

CONCLUSION

Collected during the dry season of the year, findings of the current study show that both DENV and CHIKV are actively circulating in the Lower Moshi area of Kilimanjaro region in Tanzania. These findings are evidenced by the detection of the viruses in both humans and vector mosquitoes. Ae. Aegypti is a key vector for the two viruses especially CHIKV for the transmission and possibly maintenance of the viruses. The detection of viral infections by PCR during the dry season points out to the Lower Moshi area as a potential focal point for the maintenance of the two viruses and other vector borne viruses such as RVFV. We call upon sustained active surveillance of arboviruses and other re-emerging infections for better preparedness and response to future DENV and CHIKV outbreaks and other emerging and re-emerging pantheons.

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ORIGINAL ARTICLE

Platelets Transfusion Practice at Butaro Cancer Centre of Excellence in Rwanda

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ABSTRACT

Background: To respond to the high demand of hospitals for the lack of enough platelets, in 2015, Rwanda national centre for blood transfusion introduced apheresis to produce more platelets. The high increase of impaired bone marrow among cancer patients was declared to be the main cause of the urgent demand of transfused platelets. The aim of this study was to describe the practice of platelets transfusion at Butaro cancer centre.

Methodology: A retrospective study of 238 patients who received platelets transfusions at Butaro Cancer Centre of Excellence within a period of 24 months was carried out. Laboratory register books for blood transfusion, patients' chart files and open clinic patient information software were used to identify all patients who received platelets transfusion at BCCOE during the study period. A collection form was used to record all the required data.

Results: A sum of 209 (87.8%) of receivers of platelets transfusion were cancer patients. Majority of those cancer patients had acute lymphoblastic leukaemia. Out of 1318 platelets units requested, only 925(70.2%) were received of which 573(43.4%) were O Rhesus positive. Among diagnosed cancers, Lymphomas (Chi square =7;P=.01) was statistically significant to be associated with the increase rate of platelets after transfusion. The combination of all diagnosed cancers (Chi square=11;P=.03) were associated with the increase rate of platelets after transfusion.

Conclusion: Regardless the indication of platelets transfusion, the increase of platelet count was observed after each transfusion. Ministry of health has to ensure the availability of platelets for a good care of thrombocytopenic patients of whom cancer patients are the most.

BACKGROUND

Platelets are clotting agents present in the blood cells. They work in conjunction with cytokines, clotting factors, and growth factors to arrest blood bleeding.[1]The quantity of platelets that can be removed from one unit of whole blood is known as platelets unit.[2] Apheresis procedure can be followed for platelets collection. It involves collecting platelets from a single donor but using apheresis machine.[3] Apheresis platelets have a higher residual plasma content compared with whole blood pooled platelets. Apheresis platelets are significantly costlier than whole blood pooled platelets.[3]Thrombocytopenia is often caused by a chemotherapy treatment and has to be rectified to increase platelet count. Platelets transfusion was reported to be a vital aspect of managing thrombocytopenia.[4]

Platelets transfusion have to be used in a proper way and when needed. A threshold concentration of 10,000 platelets/µl is often utilised in haematological malignancies. In patients with solid tumours, platelets transfusion is usually administered for a few days, possibly at a higher platelet level.[5,6] Excessive limitation of platelets production may expose patients to platelets concentrate shortages.[7] Platelets transfusion is found on the WHO list of important medicines since they decline mortality in acute leukaemia patients.[8] In the UK, and abroad there has been a recent rise in platelet component demand. Currently up to 67% of all platelets are used in the management of patients with haematological malignancies.[9]

Cancer patients often need to be transfused platelets if their bone marrow is impaired and therefore is not able to make enough platelets.[10]The platelets are not supplied constantly as most of the donors are not available always for this exercise. Other challenges include the significant increasing risk of product bacterial contamination due to its storage conditions, the very short shelf life, platelets refractoriness, and the limited equipment needed to produce platelets.[11] In Rwanda, blood transfusion services started in 1976 and at this time to produce platelets, ordinary method which required at least six regular blood donors to pr-

oduce a single dose of platelets was used. In order to respond to the increasing demand of hospitals for platelets and other blood components, in 2015, Rwanda national centre for blood transfusion introduced apheresis technology to produce more platelets. With this medical technology whole blood from a donor is removed and separated into individual components so that one particular component such as platelets can be collected.

However, more efforts are still needed in terms of recruiting sufficient blood donors, especially those with rare groups as well as developing new technologies for long conservation of platelets while producing sufficient quantity of platelets so that all demands are adequately responded.[12]

In Rwanda, at Butaro cancer centre of excellence (BCCOE), the number of platelets transfusion has increased more than transfusions of other blood components and majority of patients in need of platelets transfusion are from oncology department. Therapeutic platelets transfusion (platelets transfusion given when patient bleeds) or prophylactic platelets transfusion (platelets transfusion given to prevent bleeding especially when platelet count is below a given trigger level) are often needed to avoid bleeding that can be fatal to the cancer patient.[13] However, challenges and data related to the availability of platelets for transfusion have been observed at BCCOE but not yet well reported. The description of platelets transfusion practices related to platelets transfusion at BCCOE are therefore necessary. The objective of the study was to describe the current practices of platelets transfusion at BCCOE.

METHODS

Butaro Cancer Centre of Excellence

The study was carried out at BCCOE, an outstanding facility built on top of a hill in remote Burera district in Northern Rwanda. This centre of excellence was developed by Partners in Health and Rwanda Ministry of Health, and has become home to thousands of Africans fighting deadly cancer disease. Located next to the Butaro district hospital, the Butaro Cancer Centre of Excellence offers a spectrum of diagnostic oncology and treatment services, including chemotherapy, surgery, a pathology laboratory, counselling, and palliative care.

The centre is designed to facilitate patient and staff flows, and comfortably accommodate patients and their attendants during extensive treatment regimens. Patients enter from the south wing, with its interior and exterior waiting rooms. From there, they proceed to a nearby consultation room, and then into the expansive chemotherapy infusion space. The centre is at 90 km from the capital Kigali, and 48 km from the Ruhengeri regional centre of blood transfusion.

Study Design

This was a retrospective study carried out from 1st January 2016 to 31st December 2017.

Study Population

The study population included238 patients who received platelets transfusion at BCCOE within the study period.

Inclusion and Non-inclusion Criteria

To be included in the study, a person must have been a patient who received platelets transfusion at BCCOE from January 1, 2016 to December 31, 2017. Realised platelets transfusion before and after the study period were excluded from the study.

Data Collection

Laboratory register books for blood transfusion, patients' chart files and open clinic patient information software were used to identify all patients received platelets transfusion at BCCOE during the study period. A collection form was used to record all the required data.

Ethical Consideration

Ethical approval was obtained from both INES-Ruhengeri and BCCOE ethical committees. Only data routinely collected for clinical purposes were used in this study. In addition, to ensure confidentiality of the patients included in the study, extracted data did not contain names of the study participants.

Statistical Analysis

The collected data were analysed by Statistical Package for the Social Sciences for Windows version 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was used to analyse the frequencies, percentages, medians, means and interquartile range. Chi square test was performed to test for the association between disease type and changes in platelets count after transfusion. The level of significance was α=0.05

RESULTS

Profile of Patients Who Received Platelets Transfusion at Butaro Cancer Centre of Excellence

About 238 platelets transfusion were realized. Both male and female patients were represented. The age was categorized in 2 groups (<15 and ≥15 years). More than a half of participants were less than 15 years. There was a high discrepancy between the number of requested platelets and the number of received platelets in all cases (Table 1). The median baseline platelet count was 10 × 10^3/µl with interquartile range of 4 × 10^3/µl to 20 × 10^3/µl (Figure 1). The majority of requested platelets were O Rhesus positive and the least blood type was AB Rhesus negative (Table 2).

Changes in Platelet Count After Transfusion Among Patients With Different Cancers

Table 3 signposts the association between types of cancer studied and the increase rate of platelets transfused. Lymphomas (Chi square=7; *P*=.01) was significantly associated with the increase rate of platelets after transfusion. AML (Chi square =0.03; *P*=.09), ALL (Chi square =.04; *P*=.84), Other malignancies (Chi square=2; *P*=.16), Benign (Chi square =2; *P*=.16) were not partially statistically significant to be associated with the rate increase platelets transfused. All diagnosed cancers (Chi square=11; *P*=.03) were associated with the rate increase of platelets after transfusion.

PlateletsTransfusion Requested and Received Based on Blood Types

Among 238 realised platelet transfusions, the predominant patients were the ones suffering from acute lymphoblastic

TABLE 1: Profile of Patients Who Received Platelets Transfusion at Butaro Cancer Centre of Excellence

Variables	Transfused platelet units	Platelets units requested	Average number of platelets units requested per patient	Platelets units received	Average number of platelets units received per patient
Age (year)					
< 15	125 (52.5%)	690 (52.4%)	6.1	491 (37.2%)	3.9
≥ 15	113 (47.5%)	628 (47.6)	4.8	434.5 (33%)	3.8
Sex					
M	125 (52.5%)	708 (53.7%)	5.7	494 (37.4%)	4.0
F	113 (47.5%)	610 (46.3)	5.4	431.5 (32.7%)	3.8

TABLE 2: Percent, Average and Differences of Requested, Received and Transfused Platelets Units

Blood type	Number of patients transfused (%)	Platelets units requested	Average number of requested platelets units per patient	Platelets units received	Average number of received platelet units per patient	% of received platelets units used by patients	Difference between requested & received platelets units
O Rhesus +	122 (45.0)	729 (55.3%)	6	573 (43.4%)	4.4	21.3	-156
O Rhesus -	14 (5.2)	104 (7.9%)	7.4	67 (5%)	4.8	20.9	-37
A Rhesus +	51 (18.8)	242 (18.4%)	4.7	160 (12.1%)	3.1	31.9	-82
A Rhesus -	5 (1.8	22 (1.7%)	4.4	16 (1.2%)	3.2	31.3	-6
B Rhesus +	38 (14.0)	192 (14.6%)	5.1	121 (9.1%)	3.2	31.4	-71
B Rhesus -	38 (14.0)	21 (1.6%)	4.2	16.5 (1.25%)	3.3	30.4	104
AB Rhesus +	3 (1.1)	8 (0.6%)	2.7	8 (0.6%)	2.7	37.5	0
AB Rhesus -	0 (0.0)	0 (0.0%)	0	0 (0.0%)	0	0.0	0

TABLE 3: Changes in Platelet Count After Transfusion Among Patients With Different Cancers

Cancer diagnosis	Average baseline platelet count (x103 per µl)	Average increase of platelets count after transfusion (x103 per µl)	% changes in platelet count after transfusion	Chi square	P value
ALL	13.9	17.8	12.3	0.04	.84
AML	8.8	11.5	13.3	0.03	.86
Lymphomas	29.3	16.1	-29.1	7	.01
Other malignancies	17.4	31.4	28.7	2	.16
Benign	12.2	22.7	30.1	2	.16

ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia

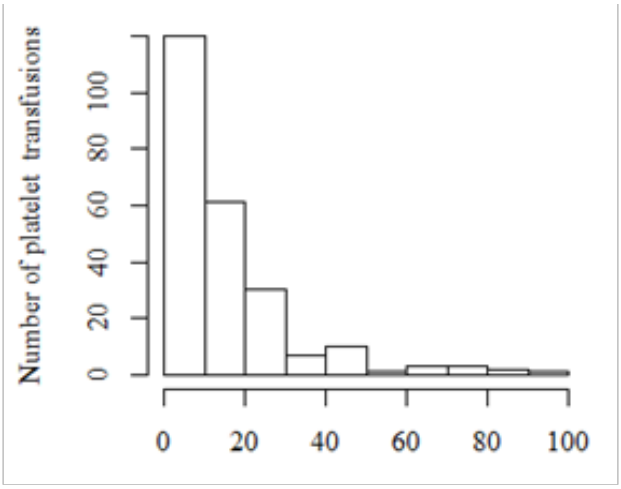
leukaemia (ALL) and were the most who received platelets transfusion. Acute myeloid leukaemia (AML) patients and lymphomas were the least likely of diagnosis to receive platelets transfusion (Table 3). Among platelets transfusion done during the study period, cancer patients (ALL, AML, lymphoma and others) received the most platelets transfusion, while non-cancer patients were the least to be transfused. There was statistical significant association (Chi square= 3.938; *P*= .05) between high platelets transfusion and cancer. The overall association

TABLE 4: Association Between Cancer Status and Platelets Transfusion Rate

	Cancer status					
	Cancer	Non-cancer	Total	X2	df	*P value*
Platelets transfusion						
High	98 (46.9)	6 (20.7)	104 (43.7)	3.938		
Low	111 (53.1)	23 (79.3)	134 (56.3)	3.1		
Total	209	29	238	7.038	1	.01

between platelets transfusion and cancer was statistically significant (Chi square= 7.038; *P*= .01) (Table 4).

FIGURE 1. Baseline Platelet Count



Threshold for platelet transfusion (x1000 cells/ul)

DISCUSSION

Platelets transfusion practice at Butaro cancer centre of excellence were assessed. Among platelets transfusion realised, the platelets transfusion was more frequent in individuals with younger age compared to older age. This could be due to the high incidence of bleeding occurring in patients with leukaemia, and also the demand for chemotherapy treatment becomes higher in this group of age.[14] The male patients were observed as an exposed group to the lack of platelets in the blood, and the demand of platelets transfusion was higher than that of female. The previous studies reported that male patients are the most affected by acute leukaemia and this type of cancer requires high platelets transfusion treatment.[15]

The transfusion of allogeneic platelet products contributes to haemostasis, however, immunisation could make it difficult to manage other complications associated with cancer.[10] The present study reported the low number of platelets transfused compared to what was requested by the patients (table 1). This could be associated with the limited storage conditions that do not allow the long storage of platelets, but also the lack of equipment to produce enough platelets can contribute to this discrepancy. Similar findings were reported by Lambert et al.[11] The median turnaround time to get the requested platelets was 1 day (interquartile range, 1 to 2).

A platelets transfusion is usually prescribed for qualitative or quantitative platelet disorders. Liebman[16]reported 150,000 to 450,000/µl as the normal range for counts of platelets. In the present study, most platelets transfusion were given using thresholds of $0 – 50 \times 10^3$/µl in critically ill thrombocytopenic cancer and non-cancer patients. A baseline platelet counts of 10×10^3/µl with interquartile range of 4×10^3/µl to 20×10^3/µl was recorded. Generally, according to Butaro cancer centre's transfusion protocol, a platelets transfusion is indicated whenever platelets count falls below 10,000/µl, or below 20,000/ µl in the presence of severe mucositis, disseminated intravascular coagulation, splenomegaly, infections, anticoagulant therapy, lumbar puncture or higher likelihood of bleeding due to local tumour invasion. Platelets are also transfused when their count falls below 50,000/µl in patients with major surgical procedure, platelets dysfunction or significant bleeding. Similarly, a number of studies such as ones conducted by Habr *et al.*[17]and Fletcher *et al*[10] suggested that platelets transfusion should be given at a threshold level which is below 20×10^3/µl.

Eight possible blood types were observed among platelets transfusion realised. Most of the requested platelets were O Rhesus positive. However, AB Rhesus negative was least blood type realised for platelets transfusion. This could be ascribed to that the most common worldwide blood type is O Rhesus positive and the rarest blood type is AB Rhesus negative.[18]

Platelets transfusion is an important parameter to manage in all types of cancer.[10] Patients with acute lymphoblastic leukaemia (ALL) received more platelets compared to other cancer types, in the present study. In addition, non-cancer patients received few platelets compared to cancer patients in this study. Therefore, there is a significant association between cancer and receiving high number of platelets transfusion. Indeed, ALL is the most common among children,[19] and generally occurs 5 times more than AML.[20] Children occupied the high percentage of the population targeted. Therefore, this is the reason why ALL is the most common diagnosis of patients who recei-

ived platelets transfusion.

Acute lymphoblastic leukaemia is also often associated with thrombocytopenia not only patients with ALL, but also patients suffering from cancer in general they undergo thrombocytopenia due to the use of myelotoxic chemotherapy regimens resulting in hypo-proliferative thrombocytopenia, and the bone marrow involvement by tumour cells.[4] Similar results were reported by Habr *et al*.[17] and they have shown that the vast majority of platelets transfusion are performed among thrombocytopenic hemato-oncological patients where 81.7% of 296 patients who received platelets transfusion had an underlying hematologic malignancy.

Platelets transfusion is found on the WHO list of important medicines since they reduce mortality in acute leukaemia patients.[8] In the present study, the average increase of platelet count (platelet increment) among patients with ALL for each platelets transfusion was greater than that of patients with lymphomas. Patients with AML had the least average increase in all hematologic malignancies. This is due to the lack of treatment for these patients at BCCOE. Regardless the number of platelets transfusion they can get, the disease keeps damaging the bone marrow and prevent it from producing new platelets in circulation.[21] However, the patients with other malignancy rather than haematological malignancy had the highest average increase of platelet count.

Cancer patients often need to be transfused platelets if their bone marrow is impaired and therefore is not able to make enough platelets.[10] The current findings describes the increase of platelets per category of diagnosis. Most of malignancies that were categorized as "other malignancy", are non-haematology ones or solid malignancies. The average increase of platelet count after transfusion in other malignancies was higher than the one in haematology malignancy. This is due to the non-interference of non-haematology malignancies with bone marrow which make platelets. This is in agreement to the study of Hassan *et al*.[22] who reported lower values for thrombocytopenia incidence among solid cancer patients compared to haematological malignancy patients. Generally, despite several conditions that can interfere with the platelet count increase after platelets transfusion, the increase of platelet count after each platelets transfusion was observed in this present study with a big difference from each category of diagnosis.

Limitations of this Study

This is a retrospective study that was carried out at Butaro cancer centre of excellence on all patients who received platelets transfusion within a period of 24 months from 1st January 2016 and 31st December 2017. Since the study was retrospective, and the accessibility of patients was not possible, the study was limited to know why the increase rate of platelets after transfusion was not the same among cancer patients and cancer type.

CONCLUSION

Platelets transfusion practice at BCCOE were studied. Unavailability of platelets was observed. Cancer patients, especially ALL patients, were the ones who received many platelets transfusion compared to non-cancer patients.

To correct thrombocytopenia, platelets were requested and most of them were O Rhesus positive. The increase of platelets count after transfusion was observed in all category of diagnosis

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ORIGINAL ARTICLE

Bacterial Cell Envelope Lysis and Hemotoxicity of Peptides Previously isolated From African Catfish, *Clarias gariepinus*

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ABSTRACT

Background: The skin mucus layer of fish is endowed with biologics including, Antimicrobial peptides (AMPs) that offer a first line of defence against pathogens. Such peptides can either inhibit bacterial growth or completely kill the bacteria and hence are regarded as a viable alternative to traditional antibiotics, in addressing the ever-increasing incidences of antimicrobial resistance. However, one of the major hurdles to AMPs use is their poor haemolytic profile. As a result, a thorough evaluation of prospective AMPs' bacterial cell membrane disruption and hemolytic potentials in the early phases of drug discovery is critical. The current study presented cell membrane destruction as well as hemo-compatibility of antimicrobial peptides previously isolated from skin mucus of African catfish, *Clarias gariepinus*.

Methods: A previously isolated antimicrobial peptide in the skin mucus of African catfish, *C. gariepinus* were profiled using 15% Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). The electrical conductivity and alkaline phosphatase assays were utilised to measure bacterial cell envelope lysis activity as a classical mode of action of the antimicrobial peptides. Afterwards, fresh Rabbit blood cells were then utilised for in vitro hemolytic assay.

Results: The peptides were found to be about 5 kDa molecular weight with, ability to damage the bacterial cell envelope causing significant leakage in periplasmic alkaline phosphatase enzyme and cytoplasmic electrolytes. Even at the highest peptide extract concentration of 100 µg/mL, no significant hemolysis was observed on the fresh rabbit blood cells [3.63%;P>.05], signifying their safety on normal mammalian cells.

Conclusion: The findings of this study pointed out that antimicrobial peptides in skin mucus of *C. gariepinus* are potentially safe source of antimicrobial drug leads; however, further studies are still required to search for possibly maximum dose that is safe to host cells but still effective against infecting bacteria.

INTRODUCTION

Pathogenic bacteria, fungi, viruses and protozoa flourish in the same aquatic ecosystem in which fish live.[1] Such habitats predispose fish to higher infection risks compared to terrestrial vertebrates,[2,3]thereby calling for a more effective natural defence mechanism. Given their underdeveloped adaptive immunity, fish mostly utilise a more complex innate defence mechanism comprising three major components: physical, phagocytic cells and chemical mediators.[4,5] Over decades, it has been demonstrated that the fish mucus layer on the skin, gills, nose and gut remains the principal first-line physical defence against infections.[6,7] The mucus layer comprises a cocktail of biologics including peptides, acute phase proteins, glycoproteins, enzymes, immunoglobulin, lectins and hemolysin that are essential in contributing potential leads in the field of drug discovery.[8,9] However, the aquatic exploration has largely been for food sourcing with far less attention to fish-derived drug leads. This has left little known about the toxicological profiles and bactericidal efficacy of fish-derived antimicrobial peptides. Yet, exploring such may be of relevance to new alternative antimicrobial drug candidates in the era of new antimicrobial drug search.[10]

To this effect, the fish skin mucus has increasingly enthralled the search for new potential bactericidal drug candidates. Natively available fish biologics are gradually gaining pursuits as potential therapeutic candidates, due to their safety, low cost of production and rapid mode action[11].

Accordingly, several studies have investigated the antimicrobial activity of different fish species, notable examples include antimicrobial activity of skin mucus extracts of *Hypophthalmichthys nobilis*,[12,13] *Clarias batrachus*,[14–16] *Heteropneustes fossilis* and *Clarias batrachus*,[17] *Channa striatus*,[18] *Catla catla*,[19] *Rutilus frisii*,[20] *Periophthalmodon schlosseri*[21] and *Anabas testudineus*[22] among others.

However, studies on the antimicrobial potentials of *Clarias gariepinus* inhabiting any of the three major lakes of Uganda have not been reported. Besides, the bacterial cell lytic activity just like hemo-compatibility of such peptide extracted from the skin mucus of *C. gariepinus* in the African region remains unknown. The current work builds on a previous study where antimicrobial peptides from the skin mucus of the African catfish, *C. gariepinus* sourced from Lake Albert, Uganda were isolated.[23] In fact, the previous study was only limited to Sephadex G-25 peptide isolation. Therefore, in the quest for potential drug leads in the era of antimicrobial resistance, the current study presents the first report on bacterial cell envelope disruption and hemolytic profiles of such peptides.

MATERIALS AND METHODS

Preparation of antimicrobial peptides

Two lyophilized archived antimicrobial peptide fractions (Peak I and Peak II) previously isolated from 24 live mature *C. gariepinus* (Figure 1) in the family *Clariidae* (mean weight 300.50±5.98g, mean length 30.60±2.11 cm) using Sephadex G-25 gel filtration chromatography,[23] were utilised in the present study. Guided by our previous study, only fractions (Peak I) with demonstrable antimicrobial activity *E. coli* [(MIC: 0.31±0.16 µg/ml) and *S. aureus* (MIC: 1.99±0.13 µg/ml)][23] and clear band upon resolving on a 15 (%) Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was considered. In addition, only the peptides extracted through Solid Phase Extraction (SPE) technique prior to Sephadex G25 bio-guided fractionation were employed in this study. This was due to the fact that cartridge's solid-phase hydrophobic matrix optimally captures the hydrophobic peptides that are later recovered through organic solvent systems.[24]

Peptide Profiling

To establish the peptide profiles, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was run as described by Laemmli with minor modifications.[25] A 15% separating gel and 4% stacking gel was used. Here, 0.01 g of lyophilized Peak I fractions and 0.01 g of lyophilized C_{18} SPE elute were dissolved in 50 µl deionized water, respectively. Thereafter, 40 µl of samples and 40 µl of sample loading buffer [12% SDS (w/v), 6% mercaptoethanol (v/v), 30% glycerol (w/v), 0.05% Coomassie blue G-250, 150 mm Tris/HCl, pH 7.0] were mixed, boiled for 5 minutes at 100 °C utilising heating block (Biobase, Shangdon, China). Then, 10 µl of the samples were then loaded into each well of the gel. Later, 8 µl of pre-stained SDS-PAGE standard markers (Thermo Fisher Scientific, Waltham, USA) were included to estimate the molecular mass of the proteins.

Electrophoresis was run at a constant voltage of 150 V for 55 minutes, until the dye front migrated to 2 cm from the bottom of the gel (Bio-Rad, Hercules, USA). Later, the gel was directly stained in 100 ml staining solution (1 g Coomassie R250, 30 ml, methanol, 65 ml deionized water, 50 ml acetic acid) for 4 hours.[26] Peptides were then directly visualized in distaining solution I (10 ml methanol, 10 ml Glacial acetic acid, and 80 ml deionized water) at 55 rpm rotation at room temperature; 25 °C (Edmund Buhler GmbH, Bodelshausen, Germany), until fairly clear bands were observed.

Alkaline Phosphatase Activity

Cell wall destruction was determined by measuring Alkaline Phosphatase Activity (ALP). An alkaline phosphatase assay kit (QuantiChrom BioAssay Systems, Hayward, USA) with *p*-nitrophenylphosphate (*p*NPP) as a substrate was used to measure the ALP activity of the cell lysate.[27] Briefly, 12 hour cultured *E. coli* were dissolved in fresh sterile medium to 106 CFU/ml. About 5 mg/ml of laboratory prepared antimicrobial peptide in 0.01 M Phosphate Buffer Solution (PBS) was added to the medium and cultured at 37°C (Heraeus, Hanau, Germany) for 30 minutes. The reaction mixture (1 ml) was then collected every hour, centrifuged at 5000 x *g* (Eppendorf, Dubai, UAE) for the next 20 hours. Thereafter, 150 µl of disodium *p*-nitrophenyl phosphate substrate buffer was added in the 50 µl supernatant and the mixture incubated in a 40 °C water bath (Grants, Cambridge-shire, UK) for 4 minutes. Absorbance was then measured at 405 nm using Microtiter plate reader (Biochrom, Cambridge, England) while 0.01 M PBS was used as a negative control. The experiment was carried out in triplicate before the Mean and Standard Error of Mean (SEM) were then calculated.

Electrical Conductivity Detection

Electrical conductivity of cytoplasmic fluid was determined as previously described by Lee et al.[28] Here, a 12 hour cultured *E. coli* was dissolved in fresh sterile medium to 106 CFU/ml. Thereafter, 0.05 mg/ml of antimicrobial peptides in 0.01 M PBS was added and cultured at 37°C (Heraeus, Hanau, Germany) for 20 minutes. The culture mixture (1 ml) was then collected every hour, centrifuged at 5000 x *g* (Eppendorf, Dubai, UAE) for the next 20 hours. The electrical conductivity was detected with Seven Go Duo SG-23 digital conductivity meter (Mettler-Toledo, Columbus, USA). The experiment was carried out in triplicate before the Mean and Standard Error of Mean (SEM) was then calculated, taking 0.01 M PBS as a negative control.

FIGURE 1. African Catfish

Clarias gariepinus (Burchell, 1822). Common name, African Catfish; Lango, Rec Lango/Twang; Luganda, Semutundu; Alur, Nyaii; Kiswahili, Kambale. A benthopelagic freshwater scaleless fish, with four pairs of slender, whisker-like sensory organs (barbel) near the mouth. An original photo by Hedmon Okella

Hemolytic Activity Testing

Hemolytic activity was assayed with a modified Rabbit blood cells method described by Lin and others.[29] Briefly,

fresh Rabbit blood cells were obtained by centrifuging whole blood from live rabbit in EDTA-coated Vacutainer (Becton & Dickinson, New Jersey, USA) in a refrigerated microfuge (Eppendorf, Dubai, UAE) at 211 x *g* for 5 min at room temperature (25 °C). Blood cells were washed three times with PBS, and then diluted with PBS to a blood cell concentration of approximately 10% (v/v).

A portion of the Rabbit blood cell suspension (500 µl) was transferred to six micro-centrifuge tubes (CellTreat, Massachusetts, USA), and mixed with 500 µL of antimicrobial peptide extract solution in 0.01 M PBS at the desired concentrations (1, 20, 40, 60, 80 and 100 µg/ml). The tubes were then incubated at 37°C (Heraeus, Hanau, Germany) to induce hemolysis. After 30 minutes of incubation, non-hemolysed blood cells were separated by centrifugation at 211 x *g* for 5 minutes at room temperature. Aliquots (100 µl) of the supernatant were transferred to a 96-well plate (Corning, New York, USA), and hemoglobin release was monitored by measuring the absorbance of the supernatant at 540 nm using microtiter plate reader (Biochrom, Cambridge, England). A blood cell solution treated with 1% Triton X-100 (to induce 100% lysis) was employed as a positive control, and an untreated blood cell suspension in 0.01 M PBS alone was used as negative control. Each assay was performed in triplicate for three independent experiments, and data were expressed as the mean and SEM. The percentage of hemolysis was calculated using the following formula[30]

Hemolysis (%)= ((Ae-An)/(Ap-An)) x 100

Where *Ae* is Absorbance of the extracts, *Ap* is the Absorbance of the positive control and *An* is the absorbance of the Negative control.

Statistical Analysis

Tableau Software v2019.4(Tableau, Seattle, U.S.A) was used to present data. All experiments were carried out in triplicate and expressed as Mean and Standard Error of Mean (SEM) using Prism 5.0 Statistical software (GraphPad, San Diego, U.S.A) and SPSS v16.0 (IBM, Chicago, U.S.A) were used to compare the means, in which a one-sample T-test was performed to determine the significance of the hemolytic activity of fish-derived antimicrobial peptides on fresh mammalian blood cells.

Ethical Consideration

Ethical approval and permission to conduct this study was obtained from the Mbarara University of Science and Technology Research Ethics Committee (22/11-18) and registered with the Uganda National Council for Science and Technology (HS449ES).

RESULTS

Study selection and Characteristics

In the present study, lyophilized Sephadex G-25 isolated antimicrobial peptides belonging to distinct peaks two peaks (Peak I and Peak II),[23] were utilised. Sephadex G-25 chromatographic resin was used and two prominent peaks (peaks I and II) were observed with absorbance measured at 280 nm. Only Peak I antimicrobial peptides demonstrated an antimicrobial activity with a MIC of 0.31 ± 0.16 and 1.99 ± 0.13 µg/ml on *E. coli* and *S. aureus*, respectively, whereas no antimicrobial activity was detected in peak II. The present study therefore, utilised only peak I archived samples of our previous study.[23]

Peptide Profiling

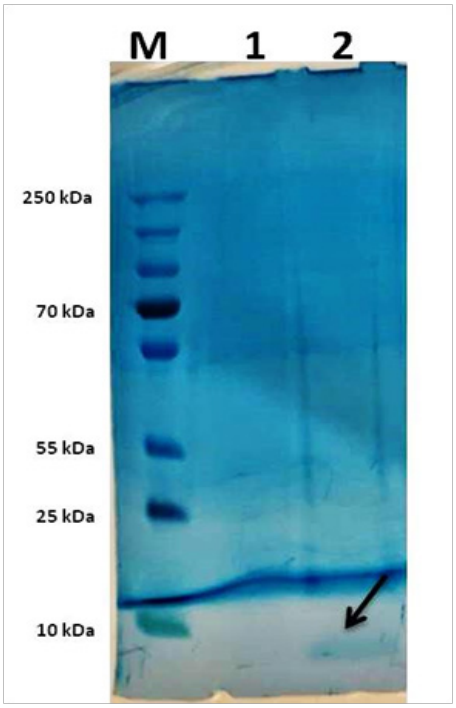
The SDS-PAGE peptide profiles of the previously extracted peptides from the skin mucus of African catfish are shown in Figure 2. Low molecular weight peptides (about 5 kDa) were observed as a clear band for the Sephadex G-25 purified extract unlike in the case of the 5 kDa-ultrafiltered C18 SPE elute.

Alkaline Phosphatase Activity and Electrical Conductivity

Throughout the culture process, the alkaline phosphatase content in the control group (0.01 M PBS), remained at a low level (below 5 iu/l), indicating the cell wall was intact (Figure 3a). In the antimicrobial peptide treated group, the alkaline phosphatase activity increased exponentially after an hour of treatment, as more alkaline phosphatase enzyme continues to leak out of the periplasm. Figure 3b showed the results of conductivity measurements.

Conductivity of the control group (0.01 M PBS) remained relatively stable at low level throughout the culture process. On the other hand, just after an hour of treatment with the antimicrobial peptides, the electrical conductivity was elevated significantly when compared to the control group, as the large volumes of the electrolyte continues to leak out of the cytoplasm during the culturing process.

FIGURE 2. A 15 (%) SDS-PAGE of peptides extracted from African catfish



The SDS-PAGE molecular marker lane (M), 5 kDa-ultrafiltered C18 SPE elute (1), C18 SPE and Sephadex G-25 isolated peptides (2). The extracted peptides were about 5 kDa.

Hemolytic activity of Antimicrobial Peptides

When the peptide extracts of *C. gariepinus* skin mucus were incubated with fresh rabbit blood cells, the percentage hemolysis increased with increase in peptide concentration (Figure 4). However, the increase in hemolytic activity was not significant even at the maximum concentration (100 µg/ml; $P>.05$) at a zero percent hemolysis test value. This signifies the safety of the fish-derived antimicrobial peptides (tested concentrations) on the Rabbit blood cells.

DISCUSSION

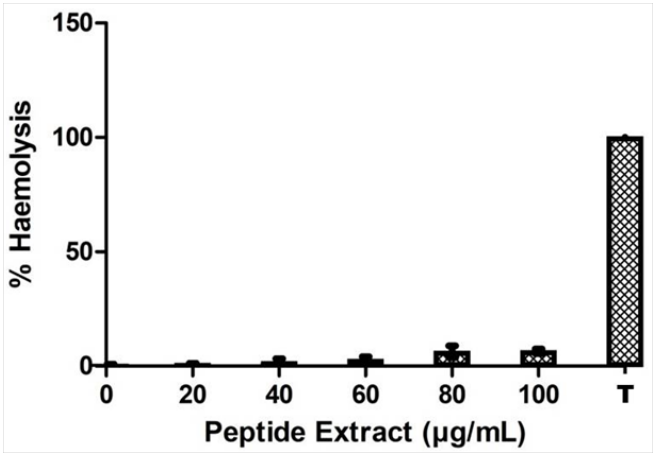
The present study demonstrated the capability of antimicrobial peptides extracted from *C. gariepinus* skin mucus in disrupting the bacterial cell wall and cell membrane. The previous study employed Solid Phase Extraction (SPE) of the antimicrobial peptides optimized the isolation of hydrophobic peptides. This is because solid phase of the SPE cartridge is a hydrophobic matrix that captures hydrophobic peptides that are later recovered by organic solvents after washing. Hydrophobicity is essential in bacterial cell membrane non-polar interactions.[31] Increased hydrophobicity up to the optimal threshold leads to enhanced ability of the peptides to associate with cellular hydrophobic lipid bilayer,[32] and most importantly, allows such peptide easily cross the membrane.[33,34] Much as, the present study was able to explore the action of these peptides on bacterial cell envelope (Figure 4), archived samples were utilized. Such samples are subject to deterioration. To mitigate this limitation, only lyophilized samples stored at 4 °C with clear band profiles on the SDS-PAGE and detected antimicrobial activity were considered for this study.

To gain insight into the mode of action of peptides, the bacterial cell envelope lysis of the *E. coli* was investigated by detecting the content of the extracellular alkaline phosphatase and electrolytes. The choice was guided by the fact that cell envelope damage is the commonest killing mechanism of peptides with antimicrobial or host defence potentials.[35] Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate esters in an alkaline environment, resulting in the formation of an organic radical and inorganic phosphate. In this study, a rapid increase in the alkaline phosphatase activity in the *E. coli* culture medium within an hour of peptide treatment was observed, this persisted till the 13th hour of treatment. This hourly trend monitoring is hinged on the fact that, antimicrobial peptides firstly targets the cell membrane,[36] and later interact electrostatically interact with negatively charged microbial cell membrane, prior to membrane destruction. Subsequently, the antimicrobial peptides may align parallel to the cell membrane like a carpet where it destroys the cell membrane in a 'detergent-like' manner,[37] or they penetrate the bilayer of the cell membrane as described in the pole model where their either form Toroidal pore/Wormhole[38] or aggregates into channel forming multimers.[39] Such sequence of events requires interval monitoring and an hour monitoring has been reported as ideal.[40]

Given that, alkaline phosphatase is localised between the cell membranes and cell walls of the bacteria[41] and and any disruption of the cell wall penetrability, leads to its discharge out of the bacteria. Therefore, *C. gariepinus* skin mucus peptides were capable of destroying the cell wall.

Similar increase in ALP activity, have previously reported for *Porphyra yezoensis* peptides[40] and Ornidazole (ORZ),[42] Linalool against *Pseudomonas fluorescens*,[43] Dihydromyricetin against Food-Borne Bacteria[44] and in AIEgen-Peptide Conjugate[45] among others, signifying bacterial cell wall destruction[46] and their potential application as far as sourcing of new antimicrobial drug candidates in the era of antimicrobial drug resistance is concerned.

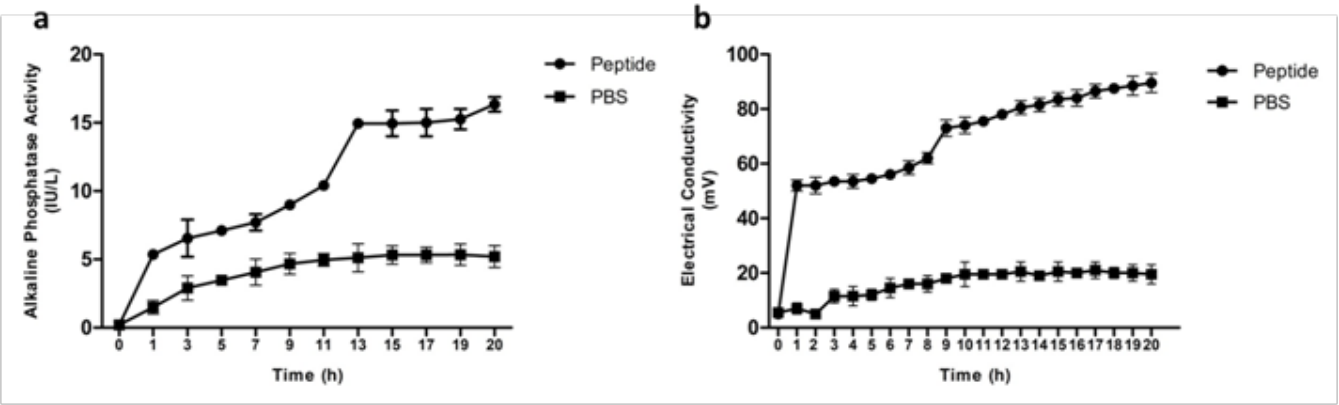
FIGURE 4. Percentage hemolysis of Rabbit blood cells by peptide extracts of C. gariepinus.



T-1% Triton X-100. The concentration of 1-100 µg/ml did not hemolyze the Rabbit blood cells. The data are expressed as Mean± SEM

Besides, cell wall distraction increases the cell membrane permeability resulting to cytoplasmic outflow and hence, a rise in extracellular electrolytes.[47,48] Monitoring of the electrical conductivity of the extracellular medium can therefore be employed as a measure of the bacterial cell membrane disruption.[49] In this study, within an hour of peptide treatment, the electrical conductivity of *E. coli* culture medium significantly increased suggesting the cell wall and cell membrane breakage amounting to cytoplasmic fluid outflow, cell collapse and death. The gradual increase in conductivity after an increase, is possibly due to the fact that majority of the cytoplasmic electrolytes are lost within the first hour of membrane shock. This in line with studies on OVTp12 peptide on *E.coli* and *S. aureus;*[50] Metallic oxide powders on *Candida albicans* NBRC1060, *Saccharomyces cerevisiae* NBRC1950, *Aspergillus niger* NBRC4067 and *Rhizopus stolonifer* NBRC4781;[51] *Porphyra yezoensis* peptides on *S. aureus;*[40] Black Paper Essential Oils (BPEO) on meat-borne *E.coli*[52] and antibiotics like Penicillin G, Nalidixic acid, Rifampicin on *Escherichia coli* 745 and *Staphylococcus aureus* 9779.[53] Moreover, cell damage as well ignites transient pore formation and freight of peptides into the cell for intracellular target interactions.[54] Much as, it would be essential to explore the interactions of the isolated peptides with the intracellular targets, it was beyond the scope of this study to explore peptide-intercellular target

FIGURE 3: Effect of Antimicrobial Peptide Treatment on Cell Wall and Cell Membrane Integrity



(a) Alkaline phosphatase activity in the culture medium, (b) Electrical conductivity in culture medium. PBS-0.01 M Phosphate Buffer Solution. Both the Alkaline phosphatase activity and Electrical conductivity were measured each hour for 20 hours. The data are expressed as Mean±SEM.

interactions as dictated by the facility constrains.

Toxicity against mammalian cells is the chief drawback that impedes most peptides from penetrating the pharmaceutical market.[55,56] To this effect, preliminary assessment of any potential lead biologic or their sources for hemo-compatibility is worthy. In this study, *C. gariepinus* peptide extracts was found to be non-hemolytic even at the highest concentration assayed (100 µg/ml), signifying their potential safety towards mammalian red blood cells and hence, relevance in sourcing of novel antimicrobial drug leads. The maximum concentration assayed (100 µg/ml), was empirically arrived at based on the previous studies.[57,58] Non-hemotoxic findings have as well been demonstrated in the skin mucus extract of Striped Dwarf Catfish, *Mystus vittatus;*[57] *and* Marine Catfish, *Tachysurus Dussumieri.*[59]

To the contrary, the mucus extracts of Spotted sea catfish, *Arius maculatus;*[58]and venomous fish such as Stonefish, *Synanceia verrucosa;*[60] Lionfish, *Pterois volitans;*[61,62] Scorpionfish, *Scorpaena plumieri;*[63] Pufferfish, *Akifugu rubripes*[64] have been reported as hemotoxic and lethal. The biologics in the hemotoxic extracts penetrates the deeper hydrophobic core of the mammalian cell membrane, once their hydrophobicity exceeds the optimal threshold.[65] Therefore, the antimicrobial peptides of *C. gariepinus* were below the hydrophobicity threshold for the studied concentrations. However, due to facility limitations, we were unable to determine the optimal threshold hydrophobicity for the extracted peptides.

CONCLUSION

The present study for the first time unveiled the toxicity profiles and possible mode of action of the previously isolated African catfish antimicrobial peptides. The peptides demonstrated outstanding lytic activity on the bacterial cell envelope. Furthermore, they were non-hemolytic to normal mammalian blood cells. The current study therefore, fronts the novel source of safe and efficacious antimicrobial drug leads of potential applications to food, medicinal and pharmaceutical industries. However, we recommend the search for potentially maximum dose that is safe to the host cells but still effective against bacteria.

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ORIGINAL ARTICLE

Soil Mineral Status, Plant Ionome and Agro-Morphological Traits of *Schkuhria Pinnata* (L.), An Antimalarial Herb: Implications for Cultivation

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ABSTRACT

Background: *Schkuhria pinnata L.*, is an antimalarial plant that is highly threatened by the destructive harvesting methods and its collection largely relies on wild sources, that are also exposed to over-exploitation and habitat destruction.
Aim of the study: The study aimed at figuring out where *S. pinnata* grows best and what its growth requirements are; in order to promote the informed cultivation practices and soil selection as a viable alternative to wild harvesting. The agronomical soil nutrient status of *S. pinnata*, and how it relates to the agro-morphological traits and plant ionome, clues on fertiliser formulations for soils where *S. pinnata* does not shrive were reported.
Methods: A randomised complete block design was employed in agronomical experimental plots in different agro-ecological zones that host Bushenyi, Ntungamo and Kasese districts. Standard procedures for soil and plant analysis were used to analyse soil physicochemical and plant ionome parameters while agro-morphological traits were physically evaluated.
Results: Results demonstrated that soil physicochemical characteristics differed significantly across the study sites ($p \leq .05$). *S. pinnata* significantly performed better in slightly acidic to neutral soils (pH between 5.87-7.25) in Kasese than in other sites. *S. pinnata* harvested from Kasese had the largest total leaf area (mean = 31.43 ± 2.41cm^2) and the highest plant biomass (mean = 7.65 ± 0.64 g).
Conclusion: The study concluded that *S. pinnata* grew best in slightly acidic to neutral, sandy loam, non-saline soils of Kasese in Western Medium-High Farmland

INTRODUCTION

Cultivation of medicinal plants is a conservation strategy that provides a sustainable supply of medicinal materials.[1] Increased demand for medicinal plant ingredients has exerted pressure on scientific research for medicinal plants' growth requirements like soil nutrients. This provides alternative sources of medicinal stocks other than natural wild sources.[2] Soil plays a vital role in plant nutrition as it reserves nutrients and water in which plants grow and develop.[3] Soil nutrient requirements by plants vary depending on quantity needed, thus, soil nutrients are categorised as macro and trace nutrients.[4] Potassium (K), phosphorus (P), calcium (Ca), nitrogen (N), and magnesium (Mg) are categorised under macronutrients, whereas manganese (Mn), copper (Cu), zinc (Zn) and iron (Fe) among others are considered trace nutrients.[4] Previous studies showed that parent bedrock undergoes weathering through physicochemical processes to provide soil nutrients. This key source is supplemented by mining, ores, industrial wastes and atmospheric deposits.[3,5] Nutrients in soil exist in a solid state under optimal soil pH (potential of hydrogen), moisture content, temperature, and soil electrical conductivity.[6]

The nutrients are released into the soil solution by desorption and dissolution processes.[7] Plants primarily obtain nutrients from the soil solution in the ionic state.[8] Under the influence of soil-plant interaction, dissolved nutrients reach the root *mycorrhizal* surface by mass flow and diffusion mechanisms.[8] Soil nutrients are later translocated through apoplastic and symplastic pathways to the aerial parts of the plant.[9] Although the uptake of a particular nutrient mainly depends on its presence in the growing media[8] and buffer capacity[9], the translocation of plant ionome is influenced by other underlying aspects such as soil temperature, soil pH[10], soil moisture[11], root architecture and activity, humus, elemental toxicity, salinity levels[6] and plant genotype.

[8,12] Plant ionome and agro-morphological traits form a fundamental tool for evaluating the efficient utilisation of soil nutrient available in the soil.[4] Additionally, the analysis of the plant ionome (i.e., the elemental composition of plant tissue) is based on the theory that the concentration of elemental nutrients in a plant is an indicator for soil capacity to provide that nutrient.[13] Basing on the variability of each nutrient in the soil, the plant ionome is best expressed as a translocation ratio that accurately provides the translocation efficiency of a given nutrient.[14]

Schkuhria pinnata (Lam.) Kuntze ex Thell, family Asteraceae[15], is a South American native plant.[16] It is normally distributed in dry areas at an elevation of ≤ 3000 metres above sea level.[17] *S. pinnata* has also been reported to be grown in African countries including Kenya, where it is being used to treat malaria among the Kikuyu community[18] and in Cegere sub-county, Apac district in Northern Uganda. However, the literature on how this plant reached African countries and Uganda in particular is scarce. According to plant database[15], *S. pinnata* grows to a height of 30 to 70 Centimetres (cm) and has deep finely divided leaves. The upper leaf surface is normally grooved and both leaf surfaces are pitted with numerous small glands.

Currently, collection of *S. pinnata* medicinal materials for traditional use largely relies on wild sources.[19] It mostly involves uprooting the whole plant[20,21] at any developmental stage from wild habitats.[19] Unfortunately, the wild source is exposed to; over-exploitation, habitat destruction and harsh climatic conditions. These have led to a high genetic pool depletion rate[22,23], minimal yields and unreliable quantity and quality supply.[24] Therefore, a sustainable production practice through cultivation is a forecasted promising approach for increasing the plant's medicinal stock.[25]

Cultivation and domestication of *S. pinnata* were reported[19] in Apac district, northern Uganda where 2 traditional practitioners were found to have cultivated *S. pinnata* plants in their home backyards. However, information concerning the soil under which *S. pinnata* was grown remains unknown. Such scenario, therefore, warranted the evaluation of soil nutrient status required for *ex-situ* growth and development of *S. pinnata*. In this study therefore, we evaluated the soil nutrient status and correlated the soil physicochemical characteristics with plant ionome and agro-morphological traits, to generate significant information on ecological plant growth requirements for cultivation and domestication of *S. pinnata.*

MATERIALS AND METHODS

Study Sites and Design

Agronomical experiments were conducted between October 2019 and April 2020 in agro-ecological zones of South Western Highlands in Ntungamo District and South Western Medium–High farmlands in Bushenyi and Kasese districts. Although, Kasese and Bushenyi study sites are located in the same agro-ecological zones, they greatly differ in weather conditions and altitudes (Table 1), thus influence plant growth performance differently.

Seed Collection and field *S. pinnata* Plant Growth

Agronomical experimental plots in triplicates were designed in randomised complete blocks.[27,28] At each site, measured experimental plots (12 x 15 metres) were set up at one metre apart. Only mature black achenes were harvested from health and mature plants growing at the National Agricultural Research Organization in Uganda at 0^0 25′ 14.0″ N, 32^0 32′ 26.0″E, 1300 m above sea level.

They were identified and authenticated with accession number 50926 at Makerere University herbarium. About 500 grammes (g) of *S. pinnata* achenes were air-dried from until the moisture content was 9.3%. The dried achenes were later stored in airtight container for 1 month[29] before sowing. In each plot, 300 seeds were sown at a depth of 1 to 3 millimetres (mm) in 20 rows, at a spacing of 30 Centimetres (cm). The rows were 60cm apart. The plant's growth was monitored under natural environmental conditions, weeding was done after every 2 weeks. The germination dates for the plants were recorded. On day 43 from the date of germination, at 9.00 a.m., the plants were harvested for agro-morphological and plant ionome studies.

Field Soil and Plant Tissue Sampling

Soil sampling was conducted using a whole-plot composite method in a zig-zag pattern.[14,27] At 1 to 15 cm deep, about 10 subsoil samples were collected, mixed thoroughly to form a composite sample.[14] This was repeated for each plot.

For plant ionome studies, a sack of fresh aerial parts i.e., leaves, stems and flowers at flowering stage was harvested. The study targeted the flowering stage because *S. pinnata* methanol extracts at this stage demonstrated the maximum antimalarial activity against chloroquine-sensitive *Plasmodium berghei* on Swiss albino mice.[30]

Soil and Plant Tissue Sample Preparation

Soil samples were air-dried at 25°C, physically pulverised and sieved through a 2 mm mesh to obtain fine powdered clean soil samples weighing 500g, which were later repackaged and clearly labelled. The plants' aerial parts were oven dried at 45°C to 9.3% moisture content, pulverised with an electric blender, sieved and 500g packed and labelled. The dry soil and plant samples were transported to the Makerere University, Department of Agriculture, and Analytical laboratory for water, soil and plant analysis.

Determination of Soil Physicochemical Characteristics and Plant Ionome

A broad spectrum of agronomical physicochemical characteristics of soil were analysed as described in the working manual for laboratory methods of soil and plant analysis.[31] Thirteen (13) soil physicochemical characteristics i.e., soil pH, organic matter, nitrogen, available phosphorus, potassium, calcium, magnesium, sodium, electrical conductivity, iron, manganese, zinc, copper and soil texture were analysed. For plant ionome, 10 nutrients i.e., nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc and copper were analysed.

Determination of Soil pH and Electrical Conductivity

Finely grounded soil (20.0g) was added to 50 mL of deionised water and the mixture was then poured into a

plastic bottle, mixed thoroughly using a high-speed electric shaker. The mixture was allowed to stand for 30 minutes, then mixed again for 2 minutes, after which measurements of soil pH were taken on soil-water solution at 1:2.5 w/v with a pH metre (JENWAY 3310; France, Paris). The mixture's electrical conductivity readings were taken using the electrical conductivity digital meter (JENWAY 4310; France, Paris).

Determination of Soil Texture by Hydrometer Method

Accurately weighed 50 g of air-dried soil of each sample was put into a 500 mL plastic beaker. The soil was saturate with 200 mL of distilled water. To the saturated soil suspension, 10 mL of 10 % Calgon solution was added and allowed to settle for 10 minutes. The suspension was made to the mark with distilled water and transferred into a plastic bottle and mixed for 12 hours with an electric shaker. The suspension was then transferred into 2000 mL graduated cylinder and a hydrometer inserted. More water was added to 1130 mL, and the hydrometer removed. The cylinder was covered tightly with a rubber bung and the suspension mixed further by inverting the cylinder carefully 10 times and the time noted. 3 drops of amyl alcohol were added to the soil suspension to remove the froth. The hydrometer was gently placed into the column and after 40 seconds, hydrometer reading was taken and the temperature of the suspension measured.

The mixing of the suspension by inversion of the cylinder was repeated for more 10 times and later allowed the suspension to stand for 2 hours and after which, both hydrometer and temperature readings were taken. Thus, the soil particle size was analysed by dispersion of soil particles into fractions of sand (2.00 - 0.05 mm), silt (0.05 -0.002 mm) and clay (< 0.002 mm), and later estimated as percentage sand, silt and clay contents, and assigned the soil textural category according to Marshall's textural triangle and their respective textural classes[31] generated.

Determination of Soil Carbon Content in Organic Matter

This was conducted according to Walkley and Black method[32], following wet oxidation using concentrated sulphuric acid (Analytical grade, Sigma-Aldrich, USA) and potassium dichromate (Analytical grade, Sigma-Aldrich, USA). For each soil sample, 0.6g of soil was added to 10 mL of 0.2M potassium dichromate and 5 mL of 98% sulphuric acid. The mixture was put in a pre-heated block maintained at 145^0C for 30 minutes and cooled to room temperature. The digest was transferred to a 100 mL conical flask, and 0.3 mL of the indicator solution was added and the resultant mixture thoroughly mixed using a magnetic rod. The resultant mixture was then titrated with 0.2M ferrous ammonium sulphate solution (Analytical grade, Sigma-Aldrich, USA). The end-point was achieved when the colour changed from greenish to brown. The titre values were recorded and corrected for the mean of 2 reagent blanks (T), and organic carbon was calculated according to the equation (1) below;

$$\text{Organic carbon \%} = \frac{T \times 0.2 \times 0.3}{\text{Sample Size}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where T is the titration volume.

Extraction of Soil Exchangeable Cations

To extract exchangeable cations i.e., potassium, calcium, sodium, magnesium into soil solution, 5.0 g of finely grounded air-dried soil sample was added to 100 mL of IM ammonium acetate (Analytical grade, Sigma-Aldrich, USA) at pH 7.0. The mixture was shaken vigorously using electric shaker for 30 minutes, filtered using a filter paper to obtain a clear soil solution.

Extraction of Soil Available Phosphorous, Nitrogen and Trace Nutrients by Digestion Process.

Dry powder of soil (0.5g) was digested in digestion mixture of 5mL salicylic acid (Analytical grade, Sigma-Aldrich, USA) and selenium-sulphuric acid (Analytical grade, Sigma-Aldrich, USA), at room temperature. The mixture was heated at 110°C for 1 hour and cooled to room temperature. 3 drops of 30% hydrogen peroxide (Analytical grade, Sigma-Aldrich, USA) were added, each at an interval of 10 seconds. Hydrogen peroxide acts as an anti-foam by oxidising the organic matter while Selenium powder lowers the boiling point and acts as a catalyst for the process. Concentrated sulphuric acid (Analytical grade, Sigma-Aldrich, USA) completes the digestion at elevated temperatures. After complete digestion at 330°C, the digest was cooled to 25°C, diluted to 50 mL using de-ionised water and filtered through Whatman filter paper to obtain a digest solution, on which available phosphorus, nitrogen and trace elements were analysed.

Extraction of Plant Ionome

Plant ionome extraction was conducted according to standard routine procedures.[31] Accurately weighed 0.5g of dry powder of plant tissue was digested in a digestion mixture (5mL) of salicylic acid and selenium sulphuric acid (selenium 3.5g: 1L sulphuric acid) at room temperature. The mixture was heated at 110°C for 1 hour and cooled to room temperature. 3 drops of 30% hydrogen peroxide were added, each at an interval of 10 seconds. After complete digestion at 330°C, the digest was cooled to 25°C and diluted with deionised water.

Analysis of Soil Physicochemical Characteristics and Plant Ionome

Analysis of Exchangeable Cations in the Soil and Plant Tissue. Analysis of potassium, calcium and sodium concentrations was conducted by adding 5.0 mL of either soil solution or plant digest to 1.0 mL of 26.8% lanthanum chloride (Analytical grade, Sigma-Aldrich, USA) in a 50 mL volumetric flask, and made to the mark point with 1M ammonium acetate solution. The calcium, potassium and sodium concentrations in the resultant samples were analysed using a flame photometer (JENWAY PFP 7; France, Paris), at wavelength of 622 nm, 766 nm and 589 nm respectively.

Magnesium concentration was determined by adding 2 mL of either soil solution or plant digest to 5 mL of 15.21g/L of strontium chloride (Analytical grade, Sigma-Aldrich, USA) in a 50 mL volumetric flask and made to the mark point with 1M ammonium chloride solution (Analytical grade, Sigma-Aldrich, USA).Either soil solution or plant digest was sprayed into the flame of atomic absorption spectrophotometer (Agilant Technologies, GTA 120; California, USA), at 285.2 nm.

Analysis of Phosphorous in either Soil or Plant Digest

2 drops of 0.5% p-nitrophenol indicator solution (Analytical grade, Sigma-Aldrich, USA) were added to digest solution (10 mL) in a 50 mL volumetric flask. Then, 5mL of ammonium molybdate/ ammonium vanadate mixed reagent (Analytical grade, Sigma-Aldrich, USA) was added and made to 50 mL with distilled water and shaken to mix. The resultant solution was allowed to stand for 30 minutes and the absorption of the solution was finally determined using Ultraviolet/Visible spectrophotometer (JENWAY 6405; France, Paris) at 882 nm.

Analysis of Nitrogen either in soil or Plant Digest

Colorimetrically, nitrogen was determined using concentrated sulphuric acid, selenium powder plus salicylic acid i.e., Kjeldahl method (Carolina *et al*., 1991). Either soil or plant digest (0.5 mL) was added to 5 mL of N1. NI was made by dissolving 3.4 g of sodium salicylate (Sigma, Aldrich, USA), 2.5 g of sodium nitrate and 2.5 g of sodium tartrate (Sigma, Aldrich, USA) in 75mL of distilled water and mixture vortexed. Later, 5ml of N2, which was made by dissolving 30g of sodium hydroxide (Sigma, Aldrich, USA) in 10 mL of sodium hypochlorite and made to litre with distilled water was added. Finally, the absorption of the complexed analyte was determined using Ultraviolet/ Visible spectrophotometer (JENWAY 6405; France, Paris) at a wave length of 655 nm.

Analysis of Trace Elements in either soil or Plant Digest

The trace elements i.e., zinc, manganese, copper and iron in the digest were analysed using the flame atomic absorption spectrophotometer(Agilant Technologies, GTA 120; California, USA), on ethylene diamine tetra acetic acid (EDTA) (Sigma, Aldrich, USA), chelating agent extracts at a wavelength of 213.9, 324.7, 248.2, 248.3 nm, respectively.

Notably, Flame Atomic Absorption Spectrophotometer (FAAS), ,which uses acetylene gas is used to analyse almost all the soil nutrients including Ca, Mg, Zn, Mn, Cu, Fe, K and Na. However, in the present study, the use of FAAS was limited to analysis of Mg, Mn, Cu, Fe and Zn due to high costs associated with acetylene gas compared to butene gas, which is used in other apparatus such as flame photometer. Also, the flame photometer that was used, only had filters for Ca, Na, K and Li. Thus, not used to analyse all the nutrients studied despite its cheaper operating costs. Extraction and analysis of available phosphorus and nitrogen involve the use of coloured reagents which complex with the analyte, thus, are perfectly detected by the Ultraviolet/ Visible spectrophotometer. Quantitatively, the elemental values obtained were expressed as the translocation ratio[14] below.

Translocation ratio=Concentration of plant nutrient A in plant tissue/Concentration of nutrient A in soil solution

Determination of Agro-Morphological Traits

Agro-morphological traits including leaf area, plant height, taproot length and plant biomass were physically measured at flowering from 30 randomly sampled representative plants.[34,28] To determine the total leaf area, the leaf length (L) was measured from the insertion of the petiole up to the apex and the widest width (W) perpendicular to the rib alignment[28]; for the leaves on the plant, then multiplied by a Correction Factor (cf) of 0.7.[27] Taproot length (cm) was determined by measuring the uprooted plant from the soil surface level to the end of the taproot using a ruler. Plant height (cm) was measured from the soil surface up to the end of the main plant stem using a ruler.[28] Plant biomass was determined by first washing off the soil of the uprooted plant, oven dried at 40-65°C and weighed to achieve a constant mass on scale.[14]

Statistical Analysis

Study data was computed and expressed as mean ± standard error. Principal Component Analyses (PCA) for soil and plant ionome characteristics were performed to provide insights on whether the study sites of different agro-ecological zones would be clustered differently in SASS JMP version 11. To study the mean variability of soil physicochemical characteristics, ionome translocation ratio and agro-morphological traits across study sites,one-way variance analysis (ANOVA) for parametric and normal distributed data was later run at 0.05 level. Pair-wise comparisons to show significant difference among the means were conducted using Tukey post-hoc method, in SPSS, version 21. Pearson's product-moment correlation was later run to relate soil physicochemical characteristics with plant ionome and agro-morphological traits at significant levels of 0.01 and 0.05.

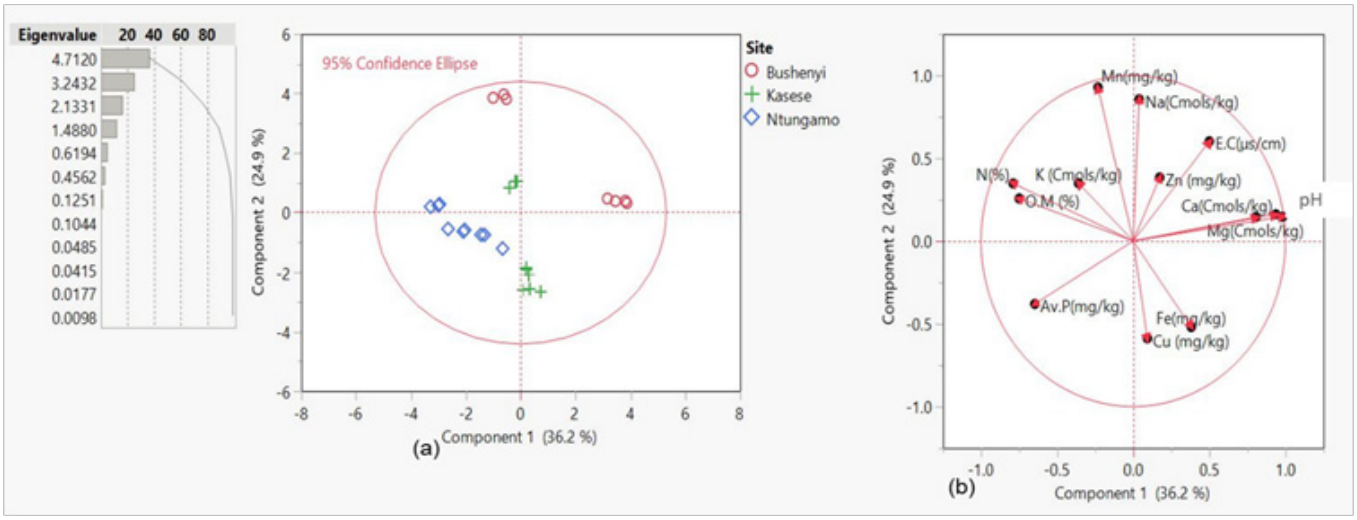
RESULTS AND DISCUSSION

Variability of Soil Physicochemical Characteristics

In the present study, the principal component analysis of soil physicochemical characteristics across the 3 study sites, showed a significant level of variability, explained by 36.2 and 24.9% of the corresponding Principal Components (PC1) and (PC2) respectively (Figure 1). Variability in soil physicochemical characteristics was attributable to the difference in the anthropogenic activities carried out in the neighbourhood of the sites, including house constructions and livestock farming. For instance, livestock residues which are rich in organic matter could have caused variations in soil variability, particularly in soil pH. Our findings are supported by the work done by Gisilanbe *et al.*,(2018) along the 3 slope positions in Ganye, North-Eastern Nigeria where they found that environmental factors such as leaching and erosion processes[35] cause variations in soil characteristics. According to the review done by Rietra *et al.*, (2017), on "Effects of nutrient antagonism and synergism on yield and fertiliser use efficiency" agronomical experiments were identified to be affected by the confounding factors including environmental temperature, rainfall among others. Thus, based on the fact that the design of the current study was agronomical, there is need for further studies that would focus on laboratory-controlled experiments and determine the variability in soil mineral status for comparison purposes.

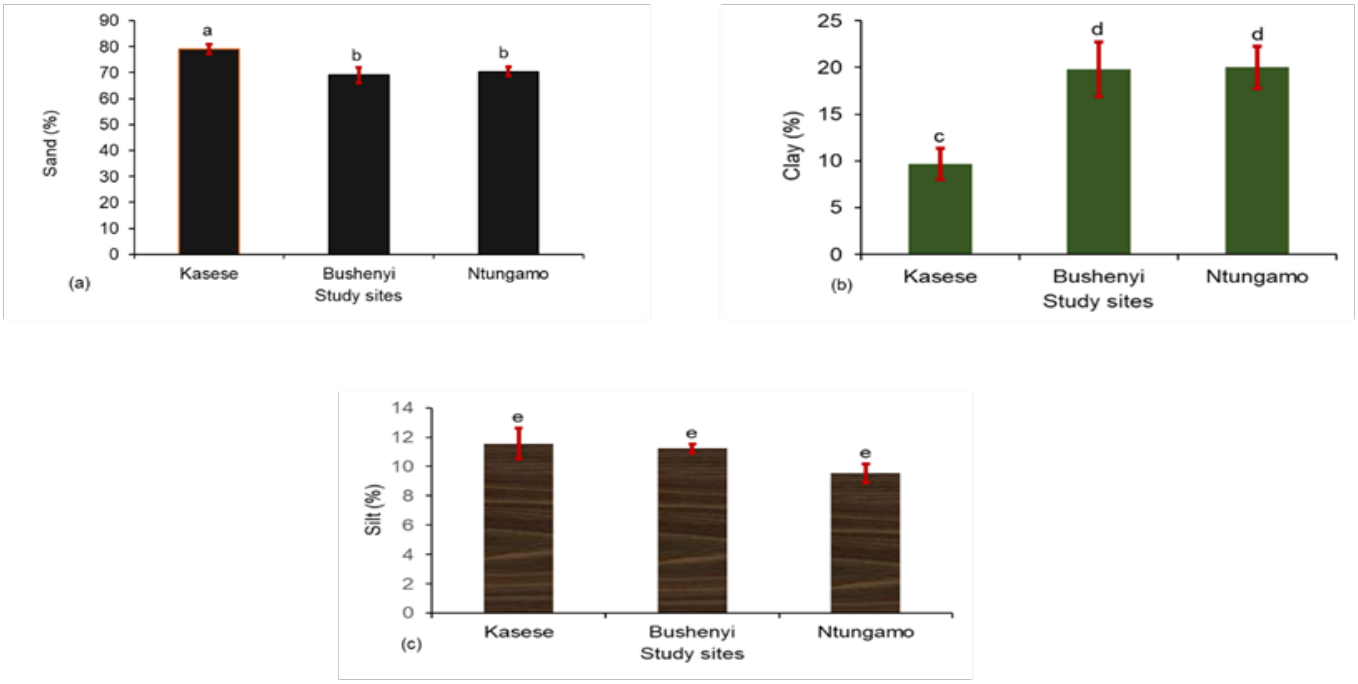
The overview of soil analysis using one-way Anova test revealed that all the soil physicochemical characteristics significantly differed across the study sites ($p \leq .05$), except potassium ($p = .247$), manganese ($p = .053$) (Table 2). According to Rao *et al.*, 2020, soil pH is a measure of acidity, which is a portion of the hydrogen ions that are

FIGURE 1: Principal Component Analysis of Soil Physicochemical Characteristics across Study Sites



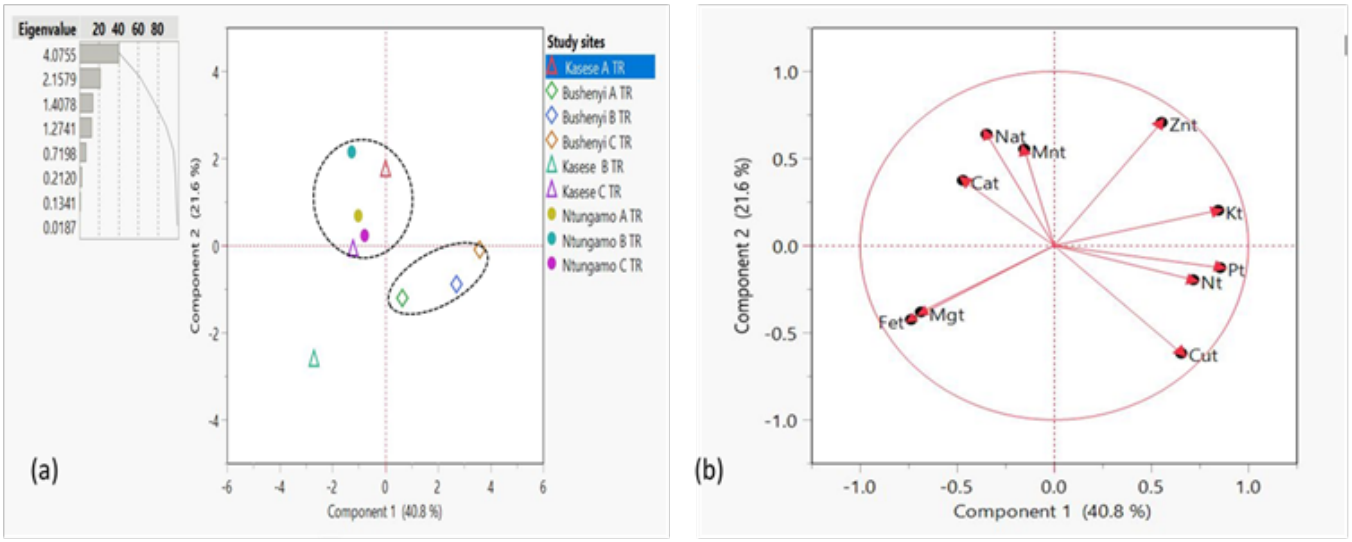
(a) Scores on the two principal components (PC1 and PC2)
(b) Loading plots (PC1 and PC2)

FIGURE 2: Variation of Soil Particle Composition across the Study Sites of Kasese, Bushenyi, and Ntungamo



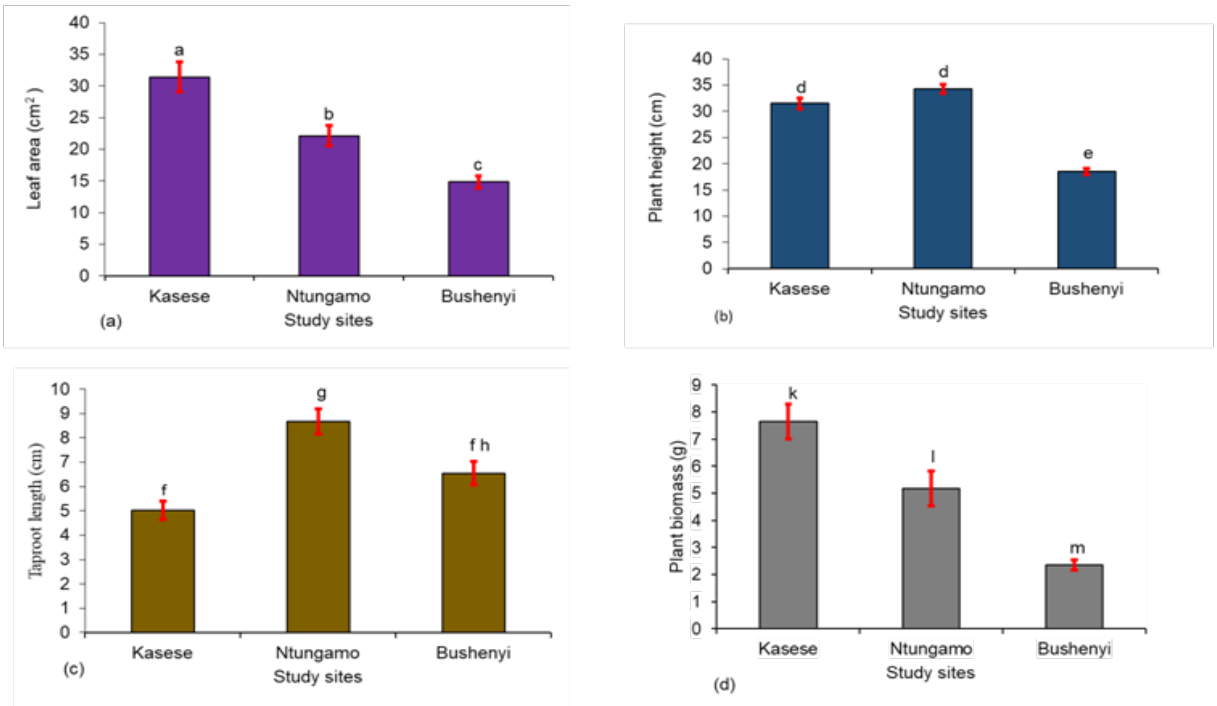
(a) sand, (b) clay, (c) silt.
Mean values superscripted with different letters, which are significant at 0.05 level, those superscripted with the same letters are non-significant

FIGURE 3: Principal Component Analysis of Plant Ionome of S. Pinnata at Flowering Developmental Stage



"t" of elementary symbols represents "tissue". "TR" represents "translocation ratio". Potassium (Kt), nitrogen (Nt), phosphorus (Pt), sodium (Nat), calcium (Cat), magnesium (Mgt), iron (Fet), zinc (Znt), manganese (Mnt), copper (Cut).

FIGURE 4: Variation of Agro-Morphological Traits of Flowering S. Pinnata Grown in Kasese, Ntungamo, and Bushenyi



Mean values subscripted with different letters were considered significant at 0.05 levels (n = 30)

active in the soil solution. In the present study, soil from Kasese had a mean pH value of 6.87 ± 0.05, characterised as slightly acidic and slightly neutral pH. However, this pH value was slightly higher than that of Ntungamo (mean = 6.11±0.09), categorised as acidic and it was lower than that of Bushenyi (mean = 7.78 ± 0.22), which was alkaline. The one-way ANOVA test showed a significant difference in pH mean values among study sites with F (2, 24) = 33.84 and *p*-value of .001. Tukey multiple comparison also showed a significance of p =.003 for Kasese versus Ntungamo and p = .001 for Bushenyi versus either Kasese or Ntungamo. The soil pH across the 3 study sites was highest in Bushenyi, followed by Kasese and lowest in Ntungamo. The soil at Bushenyi was more alkaline compared to other sites. This may be accounted by the observed high electrical conductivity caused by high concentration of salt ions including calcium and magnesium. Also, this is evidenced by the positive correlation between soil pH and calcium (r = 0 .955) , pH and magnesium (r = 0.814; $p \leq .01$) (Table 3). The present finding is in agreement with another study conducted in areas of Perth in Western Australia by Warton and Matthiessen, (2005), where they noted that parent bedrocks are constituted of varying concentrations of calcium salts, including calcium carbonate that causes a liming effect on the soil, which may be responsible for the increase in soil pH.[37] The soil from Ntungamo was slightly acidic, attributed to high nitrogen content, further supported by negative correlation between soil pH and nitrogen (r = -0.699; p = .01). This scenario may be explained by work done by Fageria and Baligar, (2004) in a study under "Nutrient availability", that nitrogen is incorporated in nitrogenous compounds like ammonium compounds which undergo nitrification processes that are accompanied by proton release, thus, reducing soil pH.[13] Furthermore, in a nitrogen fertilisation experiment by Verma *et al.*,(2015), the application of nitrogen containing fertilisers to the soil was also found to cause a decrease in soil pH.[38] The low soil pH in this study was also evidenced by the negative correlation between soil pH and organic matter (r = -0.675; p = .01) and available phosphorus (r = -0.704; p = .01). Interestingly, at Kasese where calcium, magnesium, nitrogen, organic matter and phosphorus were found to be in moderate levels, soil pH was slightly acidic and slightly neutral. Therefore, we conclude that the variability in soil pH could be due to anthropogenic activities and environmental factors such as composition of the parent bedrock, abiotic and biotic conditions of the sites. Also, the information gathered about pH status in this study is for informing the most suitable soil pH for *S. pinnata* growth, to enhance the formulation of an appropriate fertiliser with a target pH value that would reduce toxicity occurrence and optimise nutrient levels to the plant.

In this study, the amount of Organic Matter (OM) to be 3.73± 0.22 % in soil from Ntungamo was higher than that at Kasese (1.80 ± 0.27 %) and Bushenyi (2.28 ± 0.33 %). The statistical analysis indicated a significant difference in mean percentage values of organic matter across the sites with F (2, 24) = 12.70; p =.001 (Table 2). Also, Tukey post-hoc analysis showed significance of p = .001 for Ntungamo versus Kasese and p = .004 for Ntungamo versus Bushenyi. In a study done by Bhatti et al., (2016) at Punjab in India, similar findings were reported. In that study, the organic matter content was reported to be low and ranged between 2.73% to 4.17%[39], which is comparable to our findings in Ntungamo and Bushenyi. The soil in Punjab was considered to be slightly acidic, a similar pH status in Ntungamo and Kasese. Therefore, the anthropogenic drivers including intensive agriculture with the use of agrochemicals seen at Punjab could also have caused the variation in organic matter in our study. The source of organic matter is known to be the death and decomposition of living tissues by microbial activity of extracellular enzymes that releases phosphorus and nitrogen among other nutrients.[40] Thus, it is more likely that increase in organic matter may increase the availability of nitrogen as evidenced by positive correlation between organic matter and nitrogen (r = 0.889; p = .01) (Table 3). Similarly, soil from Ntungamo was the richest in nitrogen (mean = 0.24 ± 0.02%) followed by that of Kasese (0.12 ± 0.15 %) and poorest at Bushenyi (0.14 ± 0.03%). The mean values of soil nitrogen were statistically different across the sites with F (2, 24) = 7.46; p = .003 (Table 2). Tukey post-hoc comparisons showed a significant difference of p = .003 for Ntungamo versus Kasese and p =.004 for Ntungamo versus Bushenyi. According to the work done by Neina, (2019) on the role of soil pH on plant nutrition and soil remediation, plants obtain nitrogen in form of nitrogenous compounds such as nitrates which are often found incorporated in organic matter and are bio available through mineralisation.[41] Thus, the observed difference in nitrogen levels across sites may implicate several environmental factors that enhance biological processes that unlock the nutrients from organic matter. Interestingly, nitrogen and organic matter were also positively correlated (r = 0. 889, p= .01) (Table 3). Thus, based on nutrient-source relationship, organic matter serves as a primary source of organic nitrogen in the soil[13], an implication that increasing availability of organic matter increases nitrogen concentration. According to Achen *et al.*,(2014), the availability and balance of nitrogen in the soil implicates nitrification and de-nitrification processes.[42] Therefore, factors such as microbial reactions that influence such processes might have been different across the sites in this study.

In the present study, the mean values of available phosphorus in soil were statistically different with F (2, 24) = 21.56; p = .001. Soil phosphorus was highest in soil from Kasese (mean = 56.30 ± 0.50 Cmols/kg), followed by that at Ntungamo (mean = 54.98 ± 0.62Cmols/kg) and was the lowest at Bushenyi (12.32 ± 0.74Cmols/kg) (Table 2). Pair-wise comparison showed a significant difference value of p = .001 for Bushenyi versus either Ntungamo or Kasese. The possible explanation for this situation provided by Reitra *et al.*, (2017), that the high concentration of calcium ions in the soil are responsible for the reduced phosphorus availability, as calcium is well known to form a calcium phosphate complexes of very low solubility in soil solution, thus, reducing its availability.[4]

On the other hand, calcium in soil from Bushenyi (mean = 10.26 ± 1.25Cmols/kg) was higher than that of Kasese (mean = 4.90 ± 0.19 Cmols/kg) and Ntungamo (mean = 2.44 ± 0.15Cmols/kg). Statistically, calcium level at Bushenyi was significantly different from that of Kasese and Ntungamo with F (2, 24) = 29.50; p = .001 (Table 2).

The multiple comparison showed that the mean values of calcium across the sites significantly differed with p = .001 for Bushenyi versus either Kasese or Ntungamo. Often, the source of soil nutrients is majorly the existing parent bedrock, which undergoes weathering through physicochemical processes. This fact was confirmed by a study conducted by Kabrick and colleagues, (2011) as they identified soil pH as a factor that influenced the distribution of exchangeable cations including calcium in Ozark highland forest soils.[43] Therefore, the same factorcould also explain the variation of calcium across the study sites. Similarly, in our study, variation in calcium concentration was seen to correlate positively to soil pH (r = 0.955; p = .01), which may be accounted by the finding of Warton and Matthiessen, (2005) that the increasing effect of soil pH on the solubilities of calcium ions, increases its availability in soil solution.[37] Also, calcium correlated positively with magnesium concentration (r = 0.925; p = .01) (Table 3). This particular relationship between calcium and magnesium may be linked to their chemical behaviour that relates to the combining powers. In support to our findings, Addis and Ahebaw, (2017) noted that cations of the same combining powers are likely to have the common chemical origin.[44] On the other hand, calcium levels were found to correlate negatively with organic matter (r = -0.554; p = .01), nitrogen (r = - 0.395; p = .01) (Table 3) and available phosphorus (r = -0.645; p = .01) in this study. In this regard, a recent study done by Wangalwa *et al.*, (2021) on occurrence of *Citropsis articulata* (Willd.ex Spreng) Swingle & Kellerm, of family Rutaceae in 3 tropical forests of Uganda, noted a similar scenario between calcium and phosphorus.[45] This observation may indicate that calcium has a potential to cause a decrease in the availability of nitrogen and phosphorus, inducing their nutritional deficiencies in *S. pinnata*.

Magnesium was highest in soil from Bushenyi (mean = 2.25 ± 1.17Cmols/kg), followed by that at Ntungamo (mean = 0.81 ± 0.05 Cmols/kg) and was the least in soil from Kasese (mean = 0.60 ± 0.42 Cmols/kg). The one-way Anova test showed that magnesium concentration in Bushenyi soil was significantly higher than that of Kasese and Ntungamo with F (2, 24) = 15.25; p = .001 (Table 2). The pair-wise tukey's post-hoc test indicated a significant difference of p = .001 for Bushenyi versus either Kasese or Ntungamo. Likewise calcium, magnesium was found to vary across the sites, which may also be due to the nature of the parent bedrock. Variation in magnesium concentration may be responsible for the variation seen in soil pH (r = - 0.814; p = .01) across sites. Also, there are evidences from the correlations that magnesium is likely to reduce the availability of nitrogen (r = - 0.435;p = .05), phosphorus (r = - 0.617; p = .01) and potassium (r = - 0.467; p= .01). Consequently, this may lead to nutritional deficiencies in *S. pinnata*, compromising its medicinal value.

There was a significant difference among the mean values of sodium across the sites with F (2, 24) = 8.74; p = .001. Sodium in the soil samples from Bushenyi was found to be the highest (mean = 1.34 ± 0.29 Cmols/kg), followed by that at Kasese (mean =0.46 ± 0.05 Cmols/kg) and Ntungamo (mean = 0.44 ± 0.04 Cmols/kg). Tukey post-hoc analysis further indicated that sodium in the soil samples from Bushenyi was significantly higher than that from Kasese (p = *.004*) and Ntungamo (p = *.003*) (Table 2).

The electrical conductivity of the soil from Bushenyi (mean = 171.17 ±1.98 µS/cm) was higher than that at Kasese (mean = 129.52 ± 1.81 µS/cm) and Ntungamo (mean= 74.42 ± 0.24 µS/cm). Statistically, there was a significant difference among the electrical conductivity mean values with F (2, 24) = 17.89; p =*.001*. Multiple comparison conducted using Tukey post-hoc test showed significant difference values of p =*.006* for Kasese versus Ntungamo, p = *.043* for Kasese versus Bushenyi and p =*.001* for Ntungamo versus Bushenyi. According to Rao *et al.*, (2020), electrical conductivity (EC) of a solution is a measure of the ability of the solution to conduct electricity.[36] Notably, the EC indicates the presence or absence of salts but does not indicate which salts might be present.[46] Thus, the variation in electrical conductivity across study sites might have been due to environmental conditions including rainfall, moisture, microbial reactions together with the nature of the parent bedrock. Although Pearson's product-moment correlation in this study only indicated a positive relationship between electrical conductivity and exchangeable cations i.e., calcium (r = 0.520) and sodium (r =0.634) at $p \leq .05$), a different study conducted by Salem *et al.*, (2020) noted a positive correlation between electrical conductivity and micronutrients i.e., iron (r = 0.85), zinc (r = 0.81), manganese (r = 0.90) at $p \leq .05$ (Table 3). Therefore, variation in EC across the sites could be due to varied concentrations of both macro and micronutrients of the parent bedrocks of the study sites.

Iron is one of the trace nutrients that is required by plants in small quantities and at the same time expresses plant nutritional deficiencies.[4] In this study, soil from Kasese had the highest amount of iron and presented a mean value of 33.31 ± 1.77 mg/kg, followed by that at Bushenyi (mean = 24.07 ± 1.31 mg/kg) and iron was lowest at Ntungamo (mean = 18.10 ± 8.00 mg/kg). One-way Anova test showed that the concentration of iron significantly differed across sites with F (2, 24) = 4.39; p = *.024* (Table 2). Multiple comparison test did not show significant difference in mean values of iron from Kasese and Bushenyi. However, a significant difference value of p= *.019* for Kasese versus Ntungamo was shown. We attributed the variation of iron to mineralisation processes that release minerals into the soil. Mielki *et al.*, (2016) studied the relationship between iron availability in *Zea mays*' leaf tissue and its nutrient source, the soil organic matter in a semi-hydroponic system. They noted a positive correlation between organic matter and iron accumulation in the leaf tissues.[47] Similarly, the variation in iron concentration across the study sites may be accounted by the difference in organic matter content and influence of both abiotic and biotic factors on mineralisation processes.

Zinc concentration was highest in soil from Kasese (mean = 15.41± 3.32 mg/kg) and lowest in the soil from Ntungamo (mean = 8.16 ± 0.38 mg/kg). These mean values were not statistically different from that of zinc in the soil samples from Bushenyi (mean = 12.51 ± 0.31mg/kg). Thus, the F (2, 24) = 3.54; p=*.046* (**Table 2**). Pair-wise comparison test indicated a significant difference of p=*.037* for Ntungamo versus Kasese. Factors affecting the availability of zinc in soil solution including the pH of the

TABLE 1: Geographical Coordinates and Weather Parameters at the Study Sites

Site	Latitude	Longitude	Elevation (m)	Annual rainfall (mm)	Annual temperature (oC)
Kasese	0o 11′ 30.85″ N,	30o 5′ 24.68″ E	964.8	800	17.7 - 30.2
Bushenyi	00 36′ 59.814″ S	300 39′ 20.442″E	1417.7	1200	14.0 - 26.0
Ntungamo	10 8′ 40.15″ S	300 7′ 38.22″E	1774.0	1780	13.0 - 24.3

TABLE 2: Soil Physicochemical Characteristics of Kasese, Ntungamo and Bushenyi Sites

Soil physicochemical parameter	Study Site			F-value	P-value
	Kasese	Ntungamo	Bushenyi		
pH	6.87 ± 0.05a	6.11±0.09b	7.78 ± 0.22c	33.84	.001
O.M (%)	1.80 ± 0.27d	3.73± 0.22e	2.28 ± 0.33d	12.70	.001
N (%)	0.12 ± 0.15f	0.24 ± 0.02g	0.14 ± 0.03f	7.46	.003
Av. P(mg/kg)	56.30 ± 0.50h	54.98 ± 0.62h	12.32 ± 0.74i	21.56	.001
K (Cmols/kg)	0.83 ± 0.3j	0.72 ± 0.19j	0.65 ± 0.05j	1.49	.247
Ca (Cmols/kg)	4.90 ± 0.19k	2.44 ± 0.15k	10.26 ± 1.25l	29.5	.001
Mg (Cmols/kg)	0.60 ± 0.42m	0.81 ± 0.05m	2 .25 ± 1.17n	15.25	.001
Na (Cmols/kg)	129.52± 1.81q	74.42 ± 0.24r	171.17 ±1.98s	17.89	.001
Fe (mg/kg)	33.31 ± 1.77t	18.10 ± 8.00u	24.07 ± 1.31tu	4.39	.024
Mn (mg/kg)	21.32 ± 2.98v	26.09 ±1.74vw	36.56 ±6.54w	3.34	.053
Zn (mg/kg)	15.41± 3.32x	8.16 ± 0.38y	12.51 ± 0.31xy	3.54	.045
Cu (mg/kg)	22.28 ± 1.63z	0.00 ±00a	1.26 ± 0.04a	14.84	.001

TABLE 4: Translocation Ratio of *S. Pinnata* ionome across the Study Sites

Nutrient	Kasese	Ntungamo	Bushenyi	F-value	p-value
Nitrogen	30.38 ± 7.01b	6.69 ± 1.56a	31. 40 ± 9.24b	3.355	.041
Phosphorus	0.43 ± 0.01c	0.07 ± 0.06d	0.19 ± 0.02e	15.128	.005
Potassium	3.62 ± 0.64f	4.54 ± 0.61f	5.69 ± 1.11f	2.696	.146
Calcium	0.14 ± 0.02g	0.22 ± 0.09g	0.11 ± 0.04g	0.812	.487
Magnesium	0.52 ± 0.25h	0.25 ± 0.06h	0.25 ± 0.12h	2.922	.130
Iron	8.33 ± 2.25i	10.52 ± 2.73i	4.77 ± 1.49i	1.710	.258
Sodium	30.13 ± 7.91j	28.86 ± 5.68j	17.25 ± 1.95j	1.530j	.290
Manganese	12. 54 ± 4.23k	7.15 ± 0.56k	4.75 ± 1.20k	2.437	.168
Zinc	13.78 ± 4.67l	16.32 ± 1.08l	17.56 ± 1.65l	0.432	.668
Copper	0.93 ± 0.37m	0.000 ± 0.00n	2.57 ± 0.18o	28.770	.001

Data were computed as mean ± standard error. Mean values superscripted with different letters in a given row are significant at 0.05 level (n = 9).

Turkey post-hoc comparison: Nitrogen, p =.384 for Kasese Vs Ntungamo, p =.521 for Kasese Vs Bushenyi, p = .046 for Ntungamo Vs Bushenyi. Phosphorus, p =.533 for Kasese Vs Ntungamo, p = 0.005 for Kasese Vs Bushenyi, p =.015 for Ntungamo Vs Bushenyi.

Potassium, p=.588 for Kasese Vs Ntungamo, p = 0.125 for Kasese Vs Bushenyi, p = 0.450 for Ntungamo Vs Bushenyi. Calcium, p= .660 for Kasese Vs Ntungamo, p=.942 for Kasese Vs Bushenyi, p=.479 for Ntungamo Vs Bushenyi.

Magnesium, =.175 for Kasese Vs Ntungamo, p = 0.168 for Kasese Vs Bushenyi, p =.999 for Ntungamo Vs Bushenyi. Iron, p=.114 for Kasese Vs Ntungamo, p=.529 for Kasese Vs Bushenyi, p=.238 for Ntungamo Vs Bushenyi.

Sodium, p=.986 for Kasese Vs Ntungamo, p = 0.321 for Kasese Vs Bushenyi, p = 0.385 for Ntungamo Vs Bushenyi. Manganese, p=.359 for Kasese Vs Ntungamo, p=.158 for Kasese Vs Bushenyi, p =.179 for Ntungamo Vs Bushenyi. Zinc, p=.819 for Kasese Vs Ntungamo, p =.654 for Kasese Vs Bushenyi, p=.952 for Ntungamo Vs Bushenyi.

Copper, p =.022 for Kasese Vs Ntungamo, p =.022 for Kasese Vs Bushenyi, p =.001 for Ntungamo Vs Bushenyi.

TABLE 3: Correlations of soil Soil Physicochemical Characteristics of Kasese, Ntungamo and Bushenyi Sites

Soil parameter	pH	O.M (%)	N (%)	P (mg/ kg)	K (Cmols/ kg)	Ca (Cmols/ kg)	Mg (Cmols/kg)	Na (Cmols/ kg)	E.C (µs/ cm-)	Fe (mg/ kg-)	Mn (mg/kg-)	Zn (mg/ kg)	Cu (mg/k g)
O.M	-0.675**	1											
N	-0.699**	0.889**	1										
Av. P	-0.704**	0.301	0.317	1									
K	-0.293	0.131	0.240	0.269	1								
Ca	0.955**	-0.554**	-0.395**	-0.643**	-0.396	1							
Mg	0.814**	-0.383	-0.435*	-0.617**	-0.467**	0.896**	1						
Na	0.160	0.264	0.365	-0.431*	-0.467*	0.144	0.039	1					
E.C	0.576**	-0.240	-0.204	-0.287	0.204	0.520**	0.316	0.634**	1				
Fe	0.339	-0.276	-0.394	-0.121	-0.204	0.209	0.029	-0.180	0.154	1			
Mn	-0.094	0.393	0.513*	-0.270	0.417	-0.100	-0.138	0.886**	0.460	-0.472*	1		
Zn	0.229	-0.319	-0.237	0.167	0.648**	0.141	-0.026	0.135	0.646**	-0.102	0.240	1	
Cu	0.026	-0.189	-0.249	.0271	-0.065	-0.057	-0.296	-0.219	0.058	0.860**	-0.438*	-0.049	1

TABLE 5: Correlation between Soil Physiochemical Characteristics and *S. Pinnata* Ionome

		Soil physicochemical characteristics												
		pH	O.M (%)	N (%)	Av. P (Cmols/kg)	K (Cmols/ kg)	Ca (Cmols/ kg)	Mg (Cmols/kg)	Na (mg/kg-)	E.C (µs/cm)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Plant ionome	N	0.788*	-0.401	-0.378	-0.812**	-0.420	0.758*	-0.645	0.602	0.576	0.192	0.319	-.069	-.042
	P	-0.663*	0.729*	0.669*	0.582	0.391	-0.573	-0.362	-0.294	-0.370	-0.292	-0.024	.053	-.213
	K	0.203	0.502	0.456	-0.228	0.190	0.312	0.334	0.591	0.553	-0.135	0.561	.244	-.257
	Ca	0.692*	-0.432	-0.414	-0.684*	-0.002	0.514	0.386	0.509	0.723*	0.312	0.297	.334	-.038
	Mg	0.740*	-0.638	-0.589	-0.646	0.139	0.607	0.373	0.446	0.711*	0.131	0.283	.484	-.072
	Fe	-0.125	-0.463	-0.406	0.485	0.397	-0.280	-0.522	0.594	0.014	0.449	-0.372	.417	0.690*
	Na	0.334	0.064	-0.011	-0.739*	-0.366	0.308	0.487	0.215	-0.010	-.097	0.138	-.355	-.548
	Mn	-0.402	-0.094	-0.074	0.402	-0.248	-0.382	-0.460	-0.427	-0.611	0.233	-0.446	-.491	0.559
	Zn	0.731*	-0.125	-0.162	-0.578	-0.255	0.740*	0.627	0.594	0.743*	0.369	0.305	.112	0.154
	Cu	0.070	-0.540	-0.507	0.225	0.004	-0.034	-0.323	-0.262	-0.014	0.589	-0.406	0.059	0.826**

**r denotes correlation coefficient at 0. 01 level, *r denotes correlation coefficient at 0.05 level.

TABLE 6: Correlations between Agro- Morphological Traits and Soil Physicochemical Characteristics

Agro-morpho-logical trait	Soil physicochemical characteristics												
	pH	O.M (%)	N (%)	Av. P (Cmols/kg)	K (Cmols/ kg)	Ca (Cmols/ kg)	Mg (Cmols/kg)	Na (mg/kg-)	E.C (µs/cm)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Leaf area (cm2)	-0.299	0.020	0.183	0.504	0.684*	-0.428	-0.602	-0.328	0.095	0.358	-0.135	0.477	0.602
Plant height (cm)	-0.838*	0.310	0.101	0.869**	0.317	-0.841*	-0.774	-0.617	-0.687	-0.171	-0.356	-0.143	0.234
Taproot length (cm)	-0.429	0.833**	0.131	0.313	0.458	-0.360	-0.084	0.058	-0.021	-0.366	0.306	-0.118	-0.353
Plant Biomass (g)	-0.266	0.094	-0.195	0.500	0.659*	-0.372	-0.494	-0.352	0.110	0.418	-0.183	0.414	0.610*

**r denotes correlation coefficient at 0. 01 level, *r donates correlation coefficient at 0.05 level.

soil solution[48], soil texture, and water availability in the substrates[49] have been identified. Our present study is not in any way different from other studies conducted elsewhere. Therefore, similar factors could account for the varied concentrations of zinc across the study sites.
Copper in the soil from Kasese (mean = 22.28 ± 1.63mg/kg) was the highest, but undetectable from soil at Ntungamo (0.00 ±00 mg/kg), while that at Bushenyi was found to have a mean value of 1.26 ± 0.04 mg/kg. There was a significant difference in mean values of copper across sites with F (2, 24) = 14.84; $p = .001$. Pair-wise comparison test indicated that copper at Kasese significantly differed from that at Ntungamo and Bushenyi ($p = .001$). The immense concentration of copper in soil from Kasese may be linked to mining and industrialisation activities carried out in that particular district. According to Kasese District Integrated Disaster Risk Reduction and Management Plan Report, (2017-2020), Kasese experienced floods from 2013 to 2020.[50] The floods may have washed down the copper residues from Kilembe copper mines to the study site. Our findings agree with what Oves *et al.*, (2016) highlighted that heavy metals are added to the soil through anthropogenic activities including fertiliser application, waste disposal, use of toxic pesticides, coal combustion and smelting.[9] However, there are other drivers for heavy metal contamination. These include volcanicity and weathering of heavy metal enriched parent bedrocks by geochemical processes.[9]

Important to note, concentrations for all soil micronutrients in the present study were found to be below the recommended maximum safe limit levels of Cu-100 mg/ kg, Mn- 2000 mg/ kg, Zn- 300 mg/ kg and Fe- 50,000 mg/ kg by the Food and Agricultural Organisation / World Health Organisation (FAO/WHO).[51] This implied that the soil currently studied was safe for growth of the plant with no suspicion for any possible toxicity occurrence.

An evaluation of soil texture was performed using one-way Anova test and the proportions of sand varied significantly ($p = .008$). Sand percentage composition was highest in the soil from Kasese (mean = 79.00 ± 1.86 %), followed by that from Ntungamo (mean = 70.44 ± 1.66 %) and was lowest at Bushenyi with mean percentage value of 69.00 ± 2.93 % (Figure 2a). This study's results are comparable to previous findings by Bhatti *et al.*, (2016) who studied soil surrounding Sutlet and Beas rivers neighbouring Punjab, an agricultural area in India. Their study reported a sandy soil texture with sand percentage composition ranging between 78.0 and 93.68%.[39] This is similar to our findings in soil from Kasese. Their study also found similar amounts of soil organic matter as what we found in this study. Thus, the variation in sandy nature of the soil in this study can be explained by the organic matter available at the sites.

Also, the percentage composition of clay particles was significantly different ($p = .006$) across the study sites. However, in this particular case, a reverse trend was observed where the soil from Bushenyi presented the highest percentage composition mean value of (mean = 19.78 ± 2.92%), followed by soil from Ntungamo (mean = 20.00 ± 2.24 %), and was lowest at Kasese (9.67± 1.68 %) (Figure 2b).

The silt composition did not show any significant variation across the sites ($p \geq .05$) (Figure 2c). The variability in soil particle sizes may lie on the nature of the parent rock and anthropogenic activities neighbouring the sites. Also, in this study, soil textural classes were determined and we found soil from Kasese to be sandy and loamy, that from Ntungamo to be sandy loam, and finally soil from Bushenyi was sandy clay loam. According to the investigation done by Jimoh *et al.*, (2019) on the effect of soil texture on the phytochemical accumulation and biological activity of *Amaranthus caudatus* L[52], soil characterised as clay loam was found to have the highest accumulation of flavonoids in ethanolic extracts. This implied that the soil textural classes determined in this study could also influence the phytochemicals and antimalarial activity in *S.pinnata*. Therefore, our study may provide a scientific foundation for determination of optimal phytochemical and biological activities, antimalarial in particular in varied soil textures. Together, the soil from Kasese was characterised by slightly neutral and slightly acidic soil pH, moderate levels of phosphorus, calcium, salinity, moderate levels of nitrogen and organic matter, but sand loamy soils. In Ntungamo, soils were characterised by slightly acidic pH, high levels of nitrogen and organic matter, phosphorus, low levels of calcium and salinity and soil texture was sandy loam. On the other hand, soil from Bushenyi was characterised by alkaline pH, high levels of salinity, calcium, moderate levels of nitrogen and organic matter low levels of phosphorus, whereas the soil texture was sandy clay loamy.

S. pinnata Ionome at Flowering

The plant ionome reported in the present study comprised of mobile ions i.e., nitrogen, magnesium, phosphorus, potassium, as well as immobile ions i.e., calcium, copper, zinc, iron, manganese in plant tissues.[13] A principal component analysis showed that translocation efficiency of plant ionome significantly varied across the sites andwas explained; 40.8 and 21.6% of PC1 and PC2 respectively (Figure 3a-b). However, Sauerbeck and Helal, (1990) indicated that translocation of plant nutrients implicates several factors, which may include; root morphological and physiological characteristics, root-soil interaction, rhizosphere conditions and shoot-root relations.[53] According to work done by White, (2011) under "Ion uptake mechanisms of individual cells and roots: Short-distance transport", phenolics and carboxylates are produced by plant roots within the rhizosphere which increase nutrients uptake.[54] Furthermore, Rietra *et al.*, (2017) noted that nutrients are transported across the root membranes by multiple plasma membrane transporters, which may be attributed to the increased nutrient accumulation in the tissue.

One-way Anova test for normally distributed data indicated that translocation ratios for plant ionome did not significantly vary ($p \geq .05$) except for nitrogen, phosphorus and copper ($p \leq .05$) (Table 4). In regard to this, nitrogen was the most effectively translocated element with a ratio (mean = 31.40 ± 9.24) in Bushenyi, followed by nitrogen in Kasese (mean = 20.28 ± 7.01) and the least translocated in Ntungamo (mean = 6.69 ± 1.59). The variation was significantly different with F (2, 6) =3.36; $p = .04$. Pair-wise comparison indicated that nitrogen only significantly varied between Bushenyi and

Ntungamo (p =.046). Although nitrogen concentration was highest in Ntungamo soils, its translocation efficiency in plant tissues was the poorest, which confirms that the presence of a nutrient in the growth medium does not necessarily guarantee its equivalent absorption and translocation in a plant.[6] This implies that nutrient translocation implicates several other factors in addition to nutrient availability in the soil. According to Rietra *et al.*,(2017), soil parameters tend to interact with each other,which may result into either antagonism or synergism of nutrients in plants.[4] In our study, this was evidenced by positive correlation between plant nitrogen and soil calcium (r = 0.758); plant nitrogen and soil zinc (r = 0.740); $p \leq .05$). In addition, soil pH was observed to positively correlate with the nutrient efficiency of calcium (r = 0.692), magnesium (r = 0.740) and zinc (r = 0.731; $p \leq .05$) (Table 5) in *S. pinnata*. Thus, suggesting that increased soil pH may have raised the solubility coefficients of the particular nutrients in the soil solution, thus, a booster for absorption of plant ionome. On the other hand, Sauerbeck and Helal, (1990) identified root morphology, root physiological processes, shoot-root relations entailing how nutrients are absorbed at root level, transported and redistributed into plant organs, root products and exudates, microbial nutrient turnover and action of root enzymes on the available nutrients in the rhizosphere, as factors that affect nutrient translocation efficiency in plants.[53] Therefore, such factors could also have affected the nutrient translocation in the present study.

Phosphorus translocated better in *S. pinnata* grown in Kasese (mean = 0.43 ± 0.00) than Bushenyi (mean = 0.19 ± 0.02) and Ntungamo (mean = 0.07 ± 0.06; p =.005). One-way Anova test showed significant difference with F (2, 6) and p =.005. According to Tukey's post-hoc comparison, there was significant difference of p =.005 for Kasese versus Bushenyi, p =.015 for Ntungamo versus Bushenyi. The difference may probably be due to variations in soil parameters including soil pH, nitrogen and organic matter.

Although copper was significantly abundant in Kasese soils than Bushenyi, it was poorly translocated in plants grown in Kasese (mean = 0.93 ± 0.37), but instead translocated better in plants of Bushenyi (mean = 2.57 ± 0.18). One-way Anova test showed a significant difference with F (2, 6) = 28.77; p =.001. Tukey's post-hoc test indicated p =.022 for Kasese versus either Ntungamo or Bushenyi, p =.001 for Ntungamo versus Bushenyi. The possible explanation for the observed variation could be environmental factors i.e., limited moisture content, soil aeration and biological processes i.e., microbial activity among others. Notably, soils from Ntungamo were found to have undetectable amounts of copper in both soil and plant tissue. Hence, the translocation efficiency for copper could have followed a similar pattern as the abundance levels in the soil. Remarkably, copper availability in soil positively correlated with copper in the tissue (r = 0.826, $p \leq .01$) (Table 5), confirming that copper availability in soil solution granted its uptake by *S. pinnata*, hence, an indicator for the absence of antagonistic interactions of other nutrients with copper.

Variability of Agro-Morphological Traits across Study Sites

In this study, we presented quantitative agro-morphological traits of *S. pinnata*, grown and monitored under different study sites. The variability in agro-morphological traits of a particular plant mostly implicates environmental factors that encompass soil physiochemical characteristics among others. This is supported by the fact that soil nutrients regulate vital processes for the overall growth and development of plants including photosynthesis[55], protein synthesis[56], cell metabolism and cell division[57], and respiration[58], to mention but a few. Thus, the observed variations in agro-morphological traits of *S. pinnata* in this study were determined using one-way Anova test for parametric and normally distributed data and later discussed in relation to variability in soil physicochemical characteristics across the sites. The total leaf area across the sites significantly varied(p =.001). Plants grown in Kasese had the largest total leaf area (mean = 31.43 ± 2.41cm^2), followed by those at Ntungamo (24.07 ± 1.34 cm^2), while those at Bushenyi had the smallest (mean = 14.82 ± 1.01 cm^2) (Figure 4). Pair-wise comparison using Tukey post-hoc test indicated that a significant difference of p =.001 for Bushenyi versus Kasese and p=.004 for Bushenyi versus Ntungamo. However, the comparison did not show any significant difference in total leaf area of plant from Kasese and Ntungamo (p=.065). Although our results only showed positive correlation between leaf area and potassium, other studies including that conducted by Bagale *et al.*, (2018) in a physiological study for strawberries under hydroponic system noted a positive relationship between electrical conductivity and leaf number up to optimal EC level. However, beyond the optimal levels, vegetative growth such as leaf area reduced due to salt stress[6] thus the same situation may explain the present results. Another soil mineral that could have influenced the leaf area in this study is nitrogen. Nitrogen is a component of chlorophyll pigment, that increases photosynthetic rates and leaf area.[42] Also, according to Mikkelsen and Hartz, (2005), lack of nitrogen in plants presents deficient symptoms such as stunted growth, and chlorosis, particularly in older leaves that finally fall off.[59] Therefore, such physiological processes affect leaf area and consequently variations in leaves from one environment to another. Considering soil pH as a 'master of soil variables'[35], it could have influenced the agro-morphological traits in this study including leaf area. A comparison between suitable soil pH range for the cultivation of *Artemisia annua* (Lam.), a medicinal plant of family Asteraceae showed a pH between 4.5 and 8.5[60], whereas that of *S. pinnata* in this study was found to be between pH of 6.5 and 7.5. This indicated that *S. pinnata*'s soil pH range for its growth is likely to be limited as compared to other medicinal plants. This has an implication on its colonisation range, abundance, distribution as well as usage.

The plants grown in Ntungamo were found to be the tallest and had height mean value of 34.27± 0.85cm, while those grown in Bushenyi were the shortest (mean = 18.54 ± 0.61cm). The plants grown in Kasese were intermediates in height (mean = 31.19 ± 1.16 cm) (Figure 4). Analysis using one-way Anova showed that the plant height was significantly different (p =.001). Turkey' multiple comparison test indicated a significant difference of p =.001 for Bushenyi versus either Kasese or Ntungamo

However, the same test did not show any significant difference in plant height of plants from Kasese and Ntungamo ($p = .866$). Lack of soil nitrogen has been identified with stunted growth in most plants.[59] Similarly, the dwarfness observed in plants from Bushenyi could have been due to inadequate amounts of nitrogen in the soil as compared to the rest of the sites. Furthermore, our findings agree with Giel and Bojarczuk, (2010) who found out that high calcium levels and alkaline pH significantly reduces the assimilation of other nutrients such as phosphorus and manganese, limiting proper growth of plants that grow better in non-alkaline soils.[61] Remarkably, this situation was further demonstrated by the negative correlation between the plant height and either soil pH (r = - 0.838) or soil calcium (r = - 0.841) at $p \leq .05$ (Table 6) in the present study. The fact that potassium promotes photosynthetic processes and protein synthesis and its insufficient levels lead to stunted growth[56], then, the variation in plant height could also be linked to soil potassium. According to Hasanuzzaman *et al.*, (2018), potassium reduces the accumulation of reactive oxygen species by activating antioxidant defence machinery in plants, thus, increasing the stress tolerance. However, our Pearson-moment product correlation did not reflect any significant relationship between these 2 variables. Therefore, this discrepancy may be explained by confounding factors including leaching and erosional drivers which are often associated with agronomical studies. Nevertheless, we noted a significant positive correlation between plant height and available phosphorus (r = 0.869, $p \leq .05$) (Table 6). A study conducted by Taliman et al., (2019) on Soybean-Low phytate Line supports our observed relationship with the fact that phosphorus increases the overall plant growth, photosynthesis and dinitrogen fixation of nodules.[62] Therefore, it is more likely that the plant height was influenced by soil pH, soil calcium and phosphorus levels among other soil parameters.

Another agro-morphological trait that demonstrates the health status of a plant is the length of the taproot. In this study, the plants grown in Ntungamo were found to have the longest taproots (mean = 8.67 ± 0.53 cm) followed by those planted at Bushenyi (mean = 6. 35 ± 0.21 cm). Plants grown from Kasese had the shortest taproots (mean = 5.16 ± 0.33 cm). The mean values of the root length were significantly different across the sites ($p = .001$). Comparatively, turkey's post-hoc test indicated the significant difference of $p = .001$ for Kasese versus Ntungamo, $p = .03$ for Kasese versus Bushenyi and $p = .001$ for Ntungamo versus Bushenyi. We suggested that the variation in plant root length could have been influenced by the nature of soil texture which in turn determine the availability of water in the soil. This may further be explained by findings of previous works done by Figueiredo *et al.*, (2016) about the influence of soil texture on nutrient allocation in *Eremanthus erythropappus* (DC) Macleish, where they noted that soil texture increased allocation of potassium, magnesium, phosphorus, sodium and copper into the roots. Similarly, such scenario could have happened in this study, causing the observed variation in root length across the study sites. Also, Pearson-moment product correlation provided evidence for positive relationship between plant root length and soil organic matter (r = 0.833; $p \leq .01$) (Table 6). In support to our observed relationship, a recent study done by King *et al.*, (2020) on soil organic matter as a catalyst of crop resource capture, noted that organic matter reduces the compaction of the soil, improves soil aeration and maintains soil moisture which permit the uptake of soil nutrients required for proper root growth and development.[40] Here, the variation in environmental factors and the key roles of organic matter in the soil could explain the difference in the length of taproots across study sites.

Often, plant biomass is considered among the agro-morphological traits used for selection of cultivars with superior performance potential.[34] In our study, we found that plants from Kasese had the highest mean values of plant biomass (mean = 7.65± 0.64 g), while those from Bushenyi were found to have the lowest plant biomass (mean = 2.03 ±0.18 g). The mean value of biomass of plants from Ntungamo was 5.05 ± 0.45 g (Figure 4).

One-way Anova test showed a significant difference ($p = .001$). When tukey post-hoc test was conducted, a significant difference of $p = .001$ was found to exist between mean value of plant biomass from Kasese and that of either Ntungamo or Bushenyi. On the other hand, the mean values of plant biomass of Ntungamo and Bushenyi were statistically the same ($p = .114$). In this study, we expected significant positive correlations between plant biomass and most of the soil nutrients that regulate the photosynthetic related processes played by; nitrogen in protein synthesis[63], phosphorus in phytic acid uptake[62], potassium in enzyme activation for metabolic reactions[64] magnesium as a constituent of chlorophyll pigment in the plant chloroplasts[65,66], calcium in control of cell division[13] and plant protection through cell wall defence[67], to mention but a few. However, from this study, this was not the case with most of them, except potassium (r = 0.659) and copper (r = 0.610; $p \leq .05$) (Table 6).

The insignificant correlations may be attributable to environmental factors including soil erosion drivers. In line with our argument, Rietra *et al.*, (2017) noted that agronomical experiments are influenced by external factors such as limited and excess water, uncontrolled temperature and fluctuating soil pH. However, they support such agronomical trials to be conducted owing to the facts that results from controlled experiments do not always apply under field conditions. On this, we therefore, conclude that agronomical experiments are as important as those conducted under controlled environments. Also, a controlled laboratory experiment should be considered in further studies.

Generally, plants grown in Kasese were characterised with the largest total leaf area and highest plant biomass while those from Ntungamo were characterised with tallest plants with longer taproots. Exceptionally, plants grown in Bushenyi were the shortest, with the smallest total leaf area. Therefore, the pharmaceutical investors who are always interested in aerial parts (increased total leaf area and highest biomass), should consider growing *S. pinnata* in Kasese where soil pH was slightly acidic and slightly neutral, with phosphorus, calcium, magnesium, electrical conductivity, nitrogen, organic matter in moderate levels.

From this study, therefore, we recommend soil pH between 6.50 and 7.25, low to moderate levels of: nitrogen (0.05-0.20%), organic matter (1.0-3.25%), calcium (4.0-5.0 Cmols/kg), available phosphorus (40.0-70.0 mg/kg), iron (20.0-60.0 mg/kg), copper (9.0-48.0 mg/kg), manganese (10.0-40.0 mg/kg), low to moderate levels of zinc (6.0-15.0mg/kg) to be available for maximum total leaf areas and highest plant biomass. In this study, therefore, phosphorus of about 20.0 to 85.0 mg/kg was associated with highest plant height, whereas for longer taproots, organic matter (2.79-4.74%) should be provided in the growth media. Calcium salts that alter soil salinity must also be maintained within a range of 4.00 to 6.14 Cmols/kg. Thus, the above quantitative measures of soil characteristics should be put into consideration when formulating fertilisers for nutrient-deficient soils for the growth of *S. pinnata*.

CONCLUSION

The physicochemical characteristics of the soils from where *S. pinnata* was grown was associated with the plant ionome and agro-morphological traits. Alkaline soil pH, caused by high concentration of calcium and magnesium available in the soil solution adversely affected the *S. pinnata* growth performance. Soil organic matter, nitrogen as well as availability of phosphorus were found to reduce the soil pH, which resulted to better plant performance. Thus, the present investigation offers a scientific proof for *S. pinnata*'s potential to perform best in soils characterised by slightly acidic and slightly neutral soil pH, sandy loam texture and non-saline at Kasese in Western Medium-High Farmland. For the purposes of designing a suitable fertiliser formula for improved growth of *S. pinnata* and where it does not thrive well, soil pH between 6.5 and 7.5, the synergistic interactions that occurred between calcium and magnesium, soil nitrogen and phosphorus during the nutrient translocation process should be considered.

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