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The East African HEALTH RESEARCH JOURNAL

The basis for better health policy and practice

Volume 2 | Issue 1 **ISSN 2520-5277**

ORIGINAL ARTICLES

Photos top to bottom: (1) Biosafety Level 2 Laboratory at RBC, Rwanda, (2) Laboratory for HIV resistance testing at JCRC, Uganda, (3) Laboratory for vaccine development studies at KCRI, Tanzania

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The East African
HEALTH RESEARCH JOURNAL
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The East African Health Research Journal is a no-fee, open-access, peer-reviewed journal published online at www.eahealth.org. It is published three times per year by the East African Health Research Commission, an institution of the East African Community, P.O. Box 1096, Arusha, Tanzania. The journal is made possible by the support of the American People through the United States Agency for International Development (USAID) (www.usaid.gov) through the Knowledge for Health Project (www.k4health.org) and the East African Community (www.eac.int). EAHRJ is editorially independent and does not necessarily represent the views or positions of the East African Community.

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ORIGINAL ARTICLE

Prevalence, Aetiological Agents, and Antimicrobial Sensitivity Pattern of Bacterial Meningitis Among Children Receiving Care at KCMC Referral Hospital in Tanzania

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ABSTRACT

Background: Bacterial meningitis is an inflammation of the meninges that occurs in response to bacteria, causing a significant number of morbidity and mortality worldwide, especially in newborns and people living in low-income countries. Diagnosis of bacterial meningitis combines a high index of clinical suspicion and laboratory confirmation through cerebrospinal fluid (CSF) analysis. Despite antibiotic treatment, mortality remains high and many children end with long-term consequences, which include neurological deficits, hearing loss, and cognitive impairment.

Objective: To determine prevalence, aetiological agents, and antimicrobial sensitivity pattern among children aged less than 13 years with bacterial meningitis at Kilimanjaro Christian Medical Centre (KCMC), Moshi, Tanzania.

Methods: This was a hospital-based cross-sectional study carried out in the KCMC paediatric ward from December 2013 to May 2014 and from June 2015 to April 2016. In total, 161 children aged less than 13 years suspected of having meningitis were consecutively recruited. Each child submitted to a lumber puncture and CSF collected for microscopy, cultures, antimicrobial sensitivity testing, a latex agglutination test, and a polymerase chain reaction (PCR) test. PCR was run on 129 of the selected CSF samples. Data were collected using structured questionnaires and laboratory data sheet. Aetiological agents were identified, and antibiotic sensitivity was tested. Analyses were performed using SPSS version 20.0.

Results: Overall, 24 children had confirmation of having acute bacterial meningitis. Of the 161 participants, Gram stain and culture identified 4 (2.5%) children; whereas, of the 129 samples tested using the PCR, infection was confirmed in 24 (18.6%) children. *Escherichia coli* (n=18) was the most common organism isolated followed by *Listeria monocytogenes* (n=3), *Streptococcus pneumonia* (n=1), *Group B Streptococcus* (n=1), and *Klebsiella* species (spp.) (n=1). With the exception of *Klebsiella* spp., the isolated organisms were sensitive to the following commonly used antibiotics: ampicillin, chloramphenicol, gentamycin, and cephalosporin.

Conclusion: PCR yielded more organisms. *E. coli* was the most common organism and was sensitive to the empirically used antibiotics for treatment of bacterial meningitis tested in our study.

INTRODUCTION

Bacterial meningitis is an inflammation of the meninges that occurs in response to bacteria, causing a significant number of morbidity and mortality throughout the world, especially in newborns and people living in low-income countries. Those with infections caused by antimicrobial-resistant bacteria have increased risk.[1]

Even with the challenge of under-reported cases, the mortality associated with neonatal meningitis in low- and middle-income countries varies between one-third and two-thirds of confirmed cases of meningitis.[2] About 70% of the survivors develop chronic conditions like cerebral palsy, deafness, blindness, seizure disorders, and mental retardation.[3,4]

Clinical features with a high index of suspicions for meningitis vary depending on age; some are non-

specific as some overlap with other conditions or diseases, such as sepsis and severe malaria. In neonates and young children, symptoms include poor feeding, lethargy, irritability, apnea, listlessness, apathy, fever, hypothermia, seizures, jaundice, bulging fontanelle, pallor, shock, hypotonia, a shrill cry, hypoglycemia, and intractable metabolic acidosis; whereas, in infants and children the presentation includes nuchal rigidity, opisthotonos, bulging fontanelle, convulsions, photophobia, headache, alterations of the sensorium, irritability, lethargy, anorexia, nausea, vomiting, coma, fever, or hypothermia.[5]

Diagnosis of bacterial meningitis combines a high index of clinical suspicion and laboratory confirmation through analysis of the cerebral spinal fluid. Identification of the type of bacteria causing the disease is either by Gram stain, cultures, a latex agglutination test, or by a polymerase chain reaction (PCR) test. In the absence of cerebrospinal fluid (CSF) analysis results, empiric antibiotic treatment is recommended to cover the major pathogens due to the high mortality of this disease.

The aetiological agents of meningitis vary from one place to another, as does their antibiotic susceptibility. In Tanzania, the most common aetiology previously reported in children include *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Klebsiella* species (spp.).[6,7] However, *Salmonella* spp., *Escherichia coli,* and *Neisseria meningitidis* have also been reported, but at lower rates.[7] A study in Tanzania showed that among children admitted with fever, meningitis contributed to 0.2% and malaria to 10.5%[8] of the cases, while another study showed a prevalence of suspected meningitis being 9.6%, with bacteriological confirmed meningitis of 5.6%, 61% of those cases were children less than 12 months of age.[7] Despite following the recommended management for treating bacterial meningitis, we still find some children ending up with serious neurological consequences and death, suggesting the possibility that the causative microbes in our setting might have developed resistance to the antimicrobial agents that we are using, specifically, ampicillin, chloramphenicol, and ceftriaxone.[5] A study in Dar es Salaam reported mortality for children, isolated *S. pneumoniae, H. influenzae,* and *Klebsiella* spp. each represented 22.5% of the deaths.[7] In 2009 and 2013, the Expanded Program of Immunization (EPI) introduced the *H. influenzae* (Hib) vaccine and pneumococcal conjugate vaccine (PCV), respectively, to prevent *S. pneumoniae* and *H. influenzae,* which are among the major causes of meningitis in children. In Tanzania, the vaccination status for these vaccines shows good coverage for the last doses of Hib3 (97%), PCV3 (96%), polio (93%), and measles vaccination (90%) as reported in 2016.[9]

At the Kilimanjaro Christian Medical Centre (KCMC) paediatric ward, meningitis is one of the top 10 diseases for which children are admitted; however, no data are available on either its mortality patterns or the microbiological pattern. To date, the aetiology and sensitivity of antimicrobials used to treat meningitis has not been systematically studied. Since bacterial meningitis makes up a significant proportion of disease morbidity and mortality and is among the major health problems in our setting, identification of the causative bacteria and pattern of antibiotic sensitivity is a priority for the referral and teaching hospital. Furthermore, for a serious infection like bacterial meningitis, it is necessary for periodic sensitivity assessment of the causative agents, especially where there is limited rapid investigation to guide clinicians on proper antibiotic treatment selection. Therefore, we aimed to determine the prevalence, aetiological agents, and antimicrobial sensitivity pattern of bacterial meningitis among children receiving care at KCMC who are suspected of having bacterial meningitis. The findings of this study may help to improve rational drug use in managing meningitis and, hence, in reducing morbidity and mortality.

METHODS

Study Design

This was a cross-sectional hospital-based study conducted at KCMC between December 2013 and May 2014, and then extended to June 2015 through April 2016. The study was conducted within the paediatric ward at KCMC hospital, which is both a referral hospital and a research and teaching hospital. It serves the Northern zone of Tanzania, which includes six regions: Kilimanjaro, Arusha, Tanga, Manyara, Singida, and Dodoma, with a catchment of more than 10 million people. In the paediatric ward there are three units, which together have 16 rooms, giving the ward a total capacity of 91 beds.

Study Population

All children aged less than 13 years, clinically suspected to have meningitis, and admitted into the paediatric ward at KCMC during the study period were included in this study. Children who had contraindication to lumber puncture and those whose parent/guardian refused to provide consent were excluded from the study.

Case Definitions

A confirmed case of meningitis was defined as one of the following: bacterial isolation from a CSF culture positive, Gram stain, latex agglutination test, or PCR test.

Sample Size Estimation

The minimum sample size was estimated using a formula by the Survey System (Creative Research Systems, Sebastopol, CA, USA) and the Joint WHO and Directorate-General for International Cooperation (1988) expressed as sample size (SS) = $Z^2 \underline{(P)(1\text{-}P)}/\varepsilon^2$, where, Z = value (1.96 for 95% confidence level [CI]). A prevalence (P) of 9.6% for suspected bacterial meningitis was selected based on a study done in Tanzania by Kalokola et al., 2007[7] and ε = minimal tolerable

error at 95% CI, expressed as a decimal (0.05). The minimum estimated sample size was 133 participants. The study used a convenience sampling technique where all children admitted with a diagnosis of meningitis were voluntarily enrolled after consent was given by parents/caretakers.

Data Collection

After enrolment, interviewers administered a questionnaire to collect data on the age, sex, presenting complaint, relevant signs elicited, antibiotic pretreatment, immunization status, HIV serostatus, and socioeconomic status of each patient. The study team used a data sheet to record information from the laboratory tests, including blood glucose, CSF macroscopic, Gram stain, CSF culture, drug sensitivity, latex agglutination test, and PCR results.

Sample Collection

For older children, raised intracranial pressure was ruled out through fundoscopy. For neonates and infants, especially those with an open fontanel and acute onset illness, lumbar puncture was done independent of fundoscopy using 23-gauge spinal needles. Two to 3 ml of CSF was collected aseptically in a sterile test tube. All specimens were labelled and immediately sent to the Kilimanjaro Clinical Research Institute (KCRI) biotechnology research laboratory situated within KCMC campus.

Upon arrival, each specimen was examined macroscopically to determine whether the sample was clear, slight turbid, cloudy, purulent, or bloody. All CSF samples, except those that appeared turbid, were centrifuged for 15 minutes; 500 µl was collected in cryotubes and stored in a freezer at −80 degrees Celsius for later PCR analysis. All CSF samples were immediately processed using Gram stain and culture analysis.

Laboratory Analysis

Laboratory analysis was done at the KCRI biotechnology research laboratory. The sediment was cultured using standard and Gram stain techniques. The sediment of centrifuged CSF from the sterile bottle was inoculated using a sterile loop onto chocolate, blood, and MacConkey agar plates (Becton Dickinson [BD] International Branch of Becton Dickinson BV, Belgium). All isolates were identified on the basis of their colony, morphology, culture characteristics, and biochemical tests following standard procedures. The susceptibility patterns of the isolates were determined by diffusion technique according to the Clinical Laboratory Standard Institute (CLSI).[10] Susceptibility to antibiotics was defined and categorised by Rodloff et al[11] as: susceptible (S), when an organism is inhibited in vitro by a concentration of the drug that is associated with a high likelihood of therapeutic success; intermediate (I), when an organism is inhibited in vitro by a concentration of the drug that is associated with an uncertain therapeutic effect; and resistant (R), when an organism is inhibited in vitro by a concentration of the drug that is associated with a high likelihood of therapeutic failure.

DNA extraction of the stored CSF samples was done using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. Bacteria were detected by using a real-time multiplex quantitative PCR on a ViiA 7 Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, USA), using the Fast track Diagnostics (FTD) bacterial meningitis and neonatal meningitis kits (Fast Track Diagnostics, Junglinster, Luxembourg). Each of these two assays were developed to detect three different targets: FTD bacterial meningitis detects *N. meningitidis, S. pneumoniae,* and *H. influenzae* and FTD neonatal meningitis detects *S. agalactiae/Group B Streptococcus* (GBS), *L. monocytogenes,* and *E. coli*.

Data Processing and Analysis

Data were entered and analysed using Statistical Package for Social Science (SPSS), version 20 (IBM Corp., Armonk, New York, USA). Data summarization was done using mean and standard deviation for continuous variables and frequency and percentage for the categorical variables. Data presentations were done using bar charts, graphs, and tables.

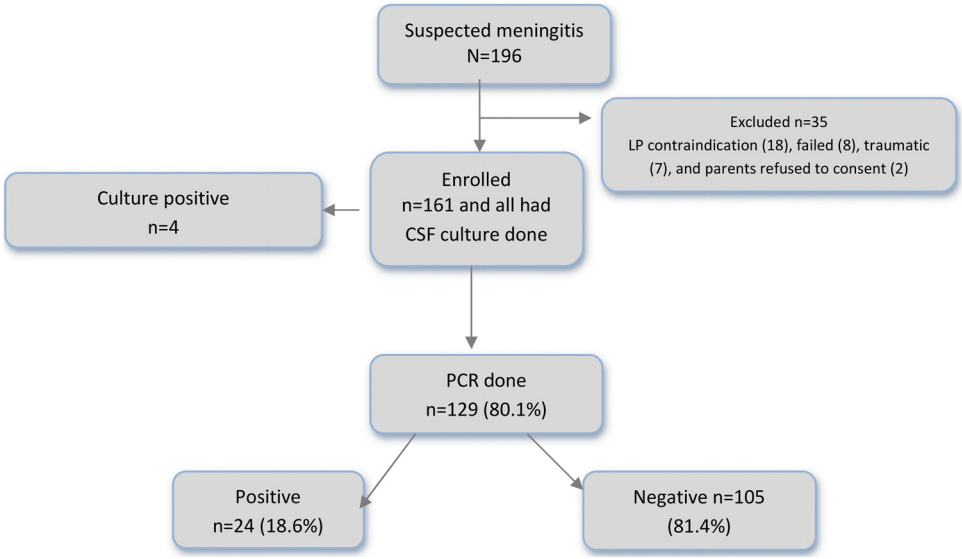
Ethical Consideration

Ethical approval for the study was obtained from the Institutional Ethical Review Board of Kilimanjaro Christian Medical University College (KCMUCo). Parents/caretakers were requested to read and sign a written informed-consent document prior to enrolment. Participation was voluntary, and parents/guardian had the right to withdraw their children from participating in the study at any time. Those parent/guardians who refused to provide consent for their children to participate in the study received equal clinical management.

RESULTS

During the study period, 196 children were hospitalized with a clinical diagnosis of meningitis. After applying exclusion criteria, 35 children were excluded, and the remaining 161 children were enrolled in this study (Figure 1). The median age at enrolment was 8 months, with an age range of birth to 13 years, and most of participants were male (61.5%). Of the enrolled participants, slightly more than half of all study participants (52.2%) reported having used antibiotics prior to admission (Table 1). The majority of antibiotics prescribed at peripheral hospitals prior the present admission included an ampicillin and gentamycin combination (n=26, 34.2%), ceftriaxone (n=22, 29%), an ampicillin and chloramphenicol combination (n=10, 13.2%), or ampicillin alone (n=10, 13.2%) (Figure 2); other antibiotics recorded were metronidazole, cotrimoxazole, and erythromycin.

FIGURE 1. Description of Study Participants Enrolled in Meningitis Study



Abbreviations: CSF, cerebrospinal fluid; LP, lumbar puncture.

Of the 161 study participants, a vast majority 148 (91.9%) had a fever with a body temperature of $>38^0$C at enrolment, 101 (62.7%) presented with fever for less than 3 days and 127 (78.3%) presented with seizures majority being neonates. Slightly less than half (46.0%) of the children presented with a symptom of reduced/poor feeding (Figure 3). Slightly more than one-third (36.6%) of the children demonstrated lethargy/drowsiness. Over a quarter (27.3%) of children suspected to have meningitis had an abnormal cry at presentation. The most presented sign in older children was neck stiffness (Figure 4).

CSF was cultured for all 161 participants. From these cultures, a total of 4 (2.5%) CSF samples had bacteria growth. Gram stains detected 4 bacteria: 1 Gram-negative bacilli, 1 Gram-negative rods, and 2 Gram-positive cocci. A Wellcogen latex agglutination method was conducted with 81 samples. Only 1 CSF sample showed agglutination test positive toward GBS.

PCR testing was done in 129 out of 161 (80.1%) samples collected from all participants, due to shortage in reagents for the first recruitment period. PCR positive test was detected in 24 out of 129 (18.6%) children with meningitis (Table 2). *E. coli* was the most common isolated bacteria (n=18) followed by *Listeria monocytogenes* (n=3), *S. pneumonia* (n=1), GBS (n=1) and *Klebsiella* spp. (n=1) (Figure 5). The most affected age group with *E. coli* isolates (n=17) were neonates aged less than 28 days of life. Based on Gram stain and culture results, *E. coli*, GBS, *Klebsiella* spp., and *S. pneumonia* were each observed in 1 child.

A total of 9 antibiotics were used for determining the antimicrobial sensitivity pattern. All of the isolates were sensitive to ceftriaxone. *E. coli* was not sensitive to cotrimoxazole and cloxacillin. *S. pneumoniae* was not sensitive to cotrimoxazole, however, it was intermediately sensitive to cloxacillin. *Klebsiella* spp. was not sensitive to ampicillin, cloxacillin, gentamicin, cotrimoxazole, amoxicillin/clavulanic acid, and erythromycin, but was intermediately sensitive to chloramphenicol (Table 2).

A majority of children in this study were reported as having received vaccinations according to their age, with almost 95% coverage for measles; only 3 (5%) children above 12 months had not received a measles vaccination (Figure 6). In contrast, only 23 children in the same age group received the PCV and rotavirus vaccines, and none of the neonates had received any vaccination prior to admission.

DISCUSSION

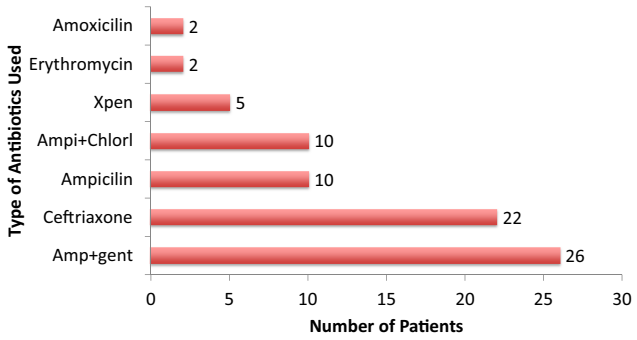
The study of meningitis in children was aimed at identifying the common causative bacteria and their antibiotic sensitivity in children with suspected meningitis. In our study, the rate of identification was 2.5% using conventional methods of CSF analysis, which included Gram stain and culture. The

TABLE 1. Baseline Characteristics of the Participants (N=161)

Baseline characteristics	n	%
Age group, months		
<1	51	31.7
1–12	53	32.9
>12	57	35.4
Median (range); months	8.0 (1–153)	
Sex		
Female	62	38.5
Male	99	61.5
Antimicrobial use		
No	77	47.8
Yes	84	52.2
Comorbidities on admission		
Acute Flaccid Paralysis	1	0.6
Acute Lymphoblastic Leukemia	1	0.6
Coarctation of Aorta	1	0.6
Febrile convulsion	2	1.2
Head Injury	1	0.6
Hydrocephalus	1	0.6
Impetigo	2	1.2
Juvenile Idiopathic Arthritis	1	0.6
Metabolic Alkalosis	1	0.6
Neonatal Sepsis	51	31.7
Otitis Media	4	2.5
Pneumonia	14	8.7
Presumptive HIV	2	1.2
Septicemia	42	26.1
Severe Anemia	1	0.6
Severe Malaria	24	14.9
SOL	1	0.6
URTI	9	5.6
UTI	2	1.2

Abbreviations: SOL, space occupying lesion; URTI, upper respiratory tract infection; UTI, urinary tract infection.

FIGURE 2. Types of Antibiotic Used



Abbreviations: Amp, Ampicillin; Chlorl, Chloramphenicol; Gent, Gentamycine; Xpen, Crystalline penicillin.

observed result was in accordance with prevalence reported in Ilesa, Nigeria (1.6%)[12]; Tehran, Iran (2.9%)[13]; and Ghana (3.3%).[14]

This prevalence is much lower than what was reported in Kenya (17.9%),[15] Nigeria (15.3%),[16] Bangladesh (14.4%),[17] and France (15.0%),[18] which used similar conventional methods. The difference in results found between our study and the others may be due to their exclusion of patients who had received antibiotics before admission, which could have resulted in having a higher yield of live bacteria using both the culture technique and advanced techniques such as the latex agglutination and PCR tests. In our study, slightly more than half (52.2%) of all study participants reported having used antibiotics prior to admission at KCMC. The use of antibiotics was either the result of self-medication, parent/guardian administration, or empiric treatment according to the Integrated Management of Childhood Illness (IMCI) guidelines[19,20] used in the lower health facilities the patients were referred from. The IMCI guidelines recommend giving antibiotics to all patients with severe febrile illness before referral. This could have contributed to low number of isolates by culture.

In our study, the prevalence increased when CSF tested by PCR. This supports the higher sensitivity of PCR in CSF examination for identification of meningitis. Similar higher yield reported in France (15.0%), where PCR was also used.[18] The higher sensitivity of PCR can be demonstrated by the increase in yield when specimens from our study were run through PCR against common bacteria causing meningitis in neonates and children—specifically, GBS, *E. coli*, *L. monocytogenes*, *H. influenza* type b, *N. meningitidis*, and *S. pneumonia*, which substantially increased the CSF yield to

FIGURE 3. Symptoms Presented by Age Groups

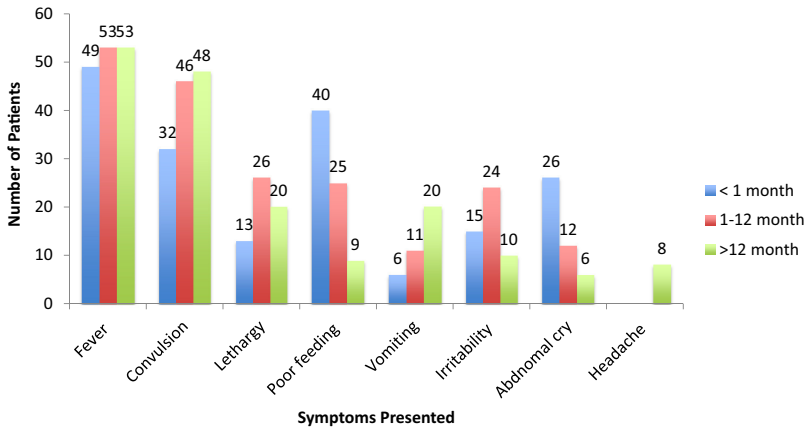
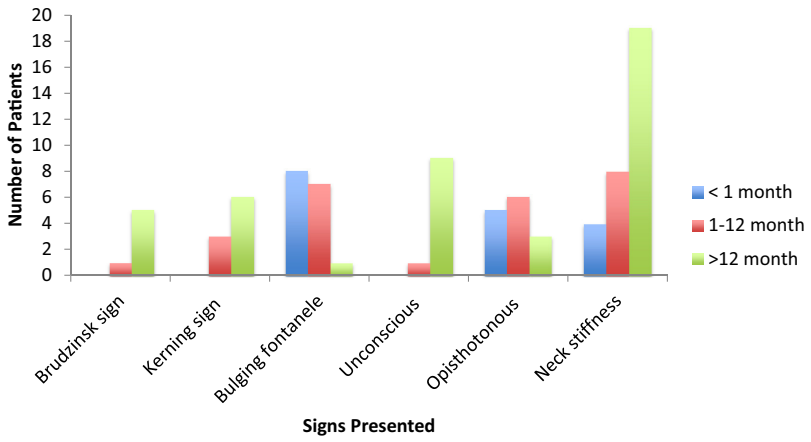


FIGURE 4. Signs of Meningeal Irritation by Age



24 (18.6%) of 129 tested samples; however no *N. meningitidis* was isolated. This was similar to a study of CSF samples by Ceyhan et al in Turkey, whereby PCR analysis was by far the most reliable method of confirming acute bacterial meningitis, accounting for 243 (59.6%) of the 408 CSF samples tested, while CSF cultures only confirmed 41 (10%).[21] In Burkina Faso, researchers had similar findings showing that PCR is both reliable and more sensitive than other conventional methods for detecting the aetiological agents of meningitis.[22] In this study, the most common aetiological agent was *E. coli* (n=18). The predominance of *E. coli* was similar to what was reported in Nigeria[3] and Kenya[15] where both studies reported *E. coli* being the most common bacteria isolated.[3,15] However, in Taiwan[23] and Niger state,[24] *E. coli* was the second most common bacterial isolate; it has also been infrequently reported in Bangladesh,[17] Nigeria,[12,25] Ghana[14] and in Dar es Salaam, Tanzania.[7] The identification of higher predominance of *E. coli* in the current study may also be related to the young age group in which *E. coli* is predominant.

In neonates, *L. monocytogenes,* a food-borne disease, was isolated from three neonate samples. *L. monocytogenes* is counted as one of the infectious causes of sepsis in neonates. The disease is common in individuals with low immunity,

TABLE 2. Distribution of Study Participants According to Methods Used to Identify the Aetiological Agents of Meningitis (N=161)

Method	Results n (%)		Total n
	Positive	Negative	
Gram stain	4 (2.5)	157 (97.5)	**161**
Culture	4 (2.5)	157 (97.5)	**161**
Wellcogen agglutination	1 (1.2)	80 (98.8)	**81**
PCR	24 (18.6)	105 (81.4)	**129**

Abbreviation: PCR, polymerase chain reaction.

including pregnant women, which makes neonates more susceptible. The diagnosis is challenging with convectional culture methods; PCR makes the isolation possible, as shown in previous case studies in neonates.[26,27] If not recognized and treated properly, *L. monocytogenes* can lead to meningitis and hydrocephalus, as complication[27]; therefore, this bacteria should be considered in neonates with sepsis and meningitis.

The second bacteria identified by conventional methods in a child aged above 12 months was *S. pneumonia* (n=1). *S. pneumonia* is one of the most common bacteria that cause meningitis in Africa, as shown in studies in Ghana,[14] Nigeria,[12] and Dar es Salaam, Tanzania.[7] The introduction of the Hib and PCV vaccines in 2009 and 2013, respectively, in the Tanzania EPI programme may have played a role in the low detection of *S. pneumonia* and *H. influenza* observed in this study. In this study, also we observed good vaccination coverage according to age, similar to national coverage levels, with the except of measles, where the coverage was higher (95%) compared to national estimate (90%).[9]

In our study, all children investigated by PCR had received 100.0% immunization as per recommended EPI schedule according to age. It has been demonstrated in other settings that with the introduction of *H. influenza* vaccination, the prevalence of *H. influenza* type b as a cause for meningitis goes down, this was as exemplified in the studies done in Kenya[28] and Uganda.[29]

Ampicillin was effective against *S. pneumonia*, unlike the findings from studies done in Nigeria[16,30] that showed *S. pneumonia* to be resistant to ampicillin. Resistance of *E. coli* to ampicillin was reported in studies done in Bangladesh[17] and Ghana.[14] Although most of the remaining drugs tested were shown to be effective, it is difficult to draw conclusions on the antibiotic sensitivities against the aetiological agents causing meningitis because of the small number of bacterial isolates on CSF cultured.

Limitations

The study was done in a tertiary care hospital and, logistically, it was not possible to conduct investigations for various funguses, viruses, and tuberculosis. It was not possible to conduct PCR tests on all collected CSF; however, cultures were done in all samples. The positive yield for cultures was too small to make proper inference on susceptibility. The team was unable to follow up in the final outcomes of children, which would have shown the effect of management.

CONCLUSIONS AND RECOMMENDATIONS

Bacterial meningitis is an important clinical problem in our hospital setting, with *E. coli being* the most common aetiological bacteria identified in neonates. The effectiveness of

TABLE 3. Antimicrobial Sensitivity Patterns of the Isolated Organism (N=4)

Isolated Organisms	Antibiotics Sensitivity								
	Ampicillin	Cloxacillin	Ceftriaxon	Gentamycin	Chloramphen	Amoxiclav	Cotrimoxazole	Erythromycin	Ciprofloxacin
E. Coli	S	R	S	S	S	S	R	S	S
GBS	S	S	S	S	S	S	I	S	S
S. pneumoniae	S	I	S	S	S	S	R	S	S
K. pneumoniae	R	R	S	R	I	R	R	R	S

Abbreviations: Amoxiclav, amoxicillin/clavulanic acid; *E.*, *Escherichia*; GBS, *Group B Streptococcus*; I, intermediate resistance; *K.*, *Klebsiella*; S, sensitive; *S.*, *Streptococcus*; R, resistant.

FIGURE 5. Isolated Organisms by Age Groups

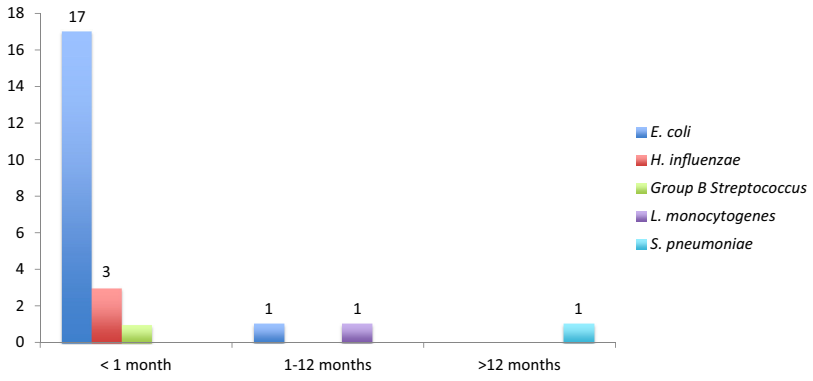
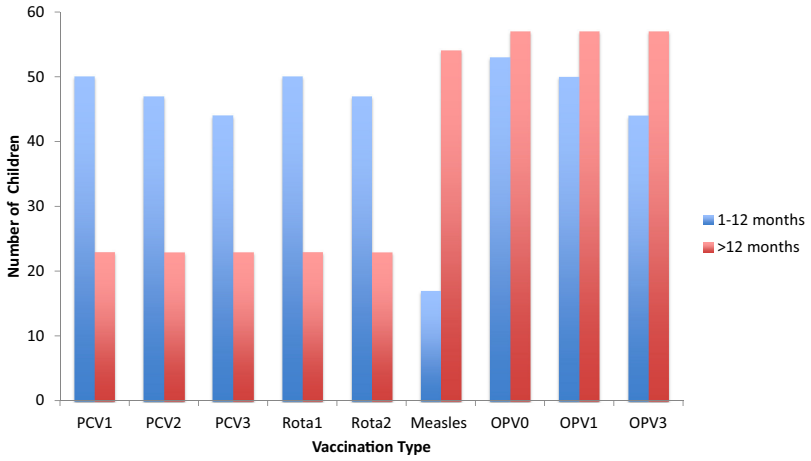


FIGURE 6. Vaccination Status by Age Groups



Abbreviations: PCV, pneumococcal conjugate vaccine; OPV, polio vaccine; 1, 2, 3, first, second, and third doses.

a meningitis diagnosis can be improved with a PCR test. In this study, it was difficult to draw conclusions on the antibiotic sensitivities due to low yield by culture; however, other commonly employed first-line antibiotics were effective against the isolated bacteria, excluding *Klebsiella* spp. We believe that monitoring the aetiological agent causing meningitis and updated information of their antibiotic susceptibility pattern is required.

In light of the discrepancy between the yield of bacteria using PCR and cultures, more sensitive routine testing of patients suspected to have meningitis is needed in order to curb morbidity and reduce complications that can result from untreated meningitis. As PCR testing is still unaffordable for most patients and unattainable by most health facilities, rapid diagnostic tests with higher sensitivity than cultures might be a viable option for low- and middle-income countries, if they can be shown to work in our settings.

Acknowledgements: We acknowledge the support and appreciation to Mentorship Education Program Initiative from KCMC for the funding through the HRSA award T84HA21123. Support provided by the specialists and residents in the Department of Paediatrics and Child Health of KCMC, and KCRI Biotechnology Laboratory team involved includes Happy Kumburu and Athumani Mchana. We acknowledge the

statistical support provided by Mr. Jeremy Damian and Adnan Juma. We finally wish to thank all the parents, guardians, and children for their willingness to participate in this study.

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Peer Reviewed

Competing Interests: None declared.

Received: 28 Oct 2016; **Accepted:** 20 Jan 2018

Cite this article as: Abdallah MS, Philemon R, Kadri A, Al-Hinai A, Saajan AM, Gidabayda JG, et al. Prevalence, Aetiological Agents, and Antimicrobial Sensitivity Pattern of Bacterial Meningitis Among Children Receiving Care at KCMC Referral Hospital in Tanzania. East African Health Res J. 2018;2(1):1-9. https://doi.org/10.24248/EAHRJ-D-16-00358

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Cross-sectional Survey on Antibiotic Prescription Practices Among Health Care Providers in Rombo District, Northern Tanzania

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ABSTRACT

Background: Irrational and inappropriate antibiotic prescription is a worldwide phenomenon – increasing the threat of serious antibiotic resistance. A better understanding of health care providers' knowledge, attitudes, and prescription practices related to antibiotics is essential for formulating effective antibiotics stewardship programmes. The aim of the present study was to assess knowledge, attitudes, and prescription practices toward antibiotics among health care providers.

Methods: A descriptive cross-sectional study was conducted between March and June 2017 to assess knowledge, attitudes, and prescription practices toward antibiotics among health care providers in the Rombo district of northern Tanzania. A total of 217 health care providers were interviewed using a structured questionnaire.

Results: Over half of health care providers (n=111, 51.2%) strongly agreed that the inappropriate prescription of antibiotics puts patients at risk. More than half (n=112, 51.6%) reported that their decision to start antibiotic therapy was influenced by a patient's clinical condition, while 110 (50.7%) reported they were influenced by positive microbiological results in symptomatic patients. Almost two-thirds of the health care providers (n=136, 62.7%) reported that they had access to and used antibiotic therapy guidelines. Less than a quarter (n=52, 24.0%) received regular training and education in antibiotic prescription practice in their work place.

Conclusion: Knowledge and prescription practice of antibiotics among health care providers was generally unsatisfactory. Training and education for health care providers is needed in the area of prescribing antibiotics.

INTRODUCTION

Antibiotics are the most commonly prescribed drugs in clinical practice. In most parts of the world, antibiotic prescription practice and use is either irrational or inappropriate – increasing the threat of serious antibiotic resistance.[1,2] In 2011, the World Health Organization (WHO) estimated that half of all medications are irrationally prescribed or sold worldwide.[3] The irrational prescription of medicines is a phenomenon that prevails across both developing and developed countries.[3,4] In African countries, for example, a systematic review of 43 studies on prescribing indicators at lower-level health care facilities indicated that the median number of medicines prescribed to clients for every encounter with a health provider was 3.1.[5] This recent review of studies published from 1995 to 2015, included 6 studies from Tanzania, and nearly half of all encounters ended with the prescription of an antibiotic.

In Tanzania, studies have indicated that irrational prescription patterns of antibiotics by health care providers is a challenge[6,7] and the magnitude varies widely. A recently published cross-sectional study assessed the rational prescription of medicines in 4 regions of Tanzania by studying 2,067 prescriptions from 67 health care facilities.[6] The study revealed that more than two-thirds (67.7%) of the prescriptions were antibiotics, when compared to the optimal level of 30%, indicating that overprescribing antibiotics is a prevailing behaviour among health care providers. More alarming results were reported by an earlier study in the Northern Zone of Tanzania among 384 children – aged 1 month to 5 years being managed for acute

watery diarrhoea – where 84.9% were given antibiotics.[7] Since most acute watery diarrhoea in that region is caused by viruses, antibiotics should not have been prescribed. The results of these studies, conducted in the same region where the present study was conducted, underscore a need to assess the levels of knowledge and attitudes of the health care providers regarding prescription practices of antibiotics.

Poor knowledge about antibiotics may be a factor influencing prescribers' attitudes toward antibiotic use and prescription practices, which then becomes a problem for patients and the community.[8] Lack of confidence and training about the rational use of antibiotics among prescribers and dispensers is a serious problem, especially when antibiotics are prescribed in the absence of cause of illness, thus increasing the risk of antibiotic resistance and adverse reactions.[9–11] Several factors contribute to the inappropriate prescription of antibiotics, including a health care provider's knowledge and experiences, uncertain diagnosis, patient expectations, pharmaceutical marketing influences, and unregulated antibiotic dispensing.[12] Additional factors, such as inappropriate antibiotic use in agriculture and food-producing animals, also pose a significant threat to human and animal drug resistance. The challenge of the latter is that there is no control of antibiotic use in agriculture and food-producing animals in Tanzania.[13] To that end, there is a risk of humans acquiring resistant pathogens from food-producing animals – such risk must be examined and reduced. The consequences of irrational prescriptions of antibiotics are immense: the practice is associated with immediate economic consequences to the user[4] and the overuse of antibiotics is a cause of bacterial drug resistance, which is increasingly becoming an important global public health problem.[14]

Understanding the prescribing pattern of antibiotics in Tanzania is important, considering that prescriptions in the country are frequently' based on clinical presentation of client's medical conditions. Due to an increasing number of patients and the limitations related to the nationwide availability of reliable laboratory services for all conditions, especially in hard-to-reach rural areas, health care providers tend to base their diagnoses on clinical judgment, while basing their decisions for treatment on the national *Standard Treatment Guidelines and Essential Medicines List* and other related guidelines produced by the Ministry of Health and Social Welfare.[15] Studies at the local level are important to narrow the knowledge gap regarding antibiotic prescription practices among health care providers. A better understanding of physicians' knowledge, attitudes, and practices toward prescribing antibiotics is essential for formulating effective antibiotic stewardship programmes.[16] Therefore, the objective of the present study was to assess knowledge, attitudes, and prescription practices related to antibiotics among health care providers in the Rombo district of northern Tanzania.

METHODS

Setting, Design, and Population Sampling

This descriptive study was conducted from March to June 2017, in Rombo District, Kilimanjaro, Tanzania. Kilimanjaro has 7 districts: Hai, Moshi rural, Moshi urban, Mwanga, Rombo, Same, and Siha. Rombo is bordered to the north and east by Kenya, to the west by the Hai and Siha districts, and to the south by the Moshi rural district. Within the Rombo district is a total of 43 operating health facilities, including 37 dispensaries, 4 heath centres, and 2 hospitals, and 326 health care providers.[17] The Rombo district was randomly selected using the rotary method: each district name was written in a piece of paper, then 1 paper was independently selected. Systematic random sampling was employed to select prospective respondents: a starting point was chosen at random from a list of all health care providers and choices, thereafter, were made at regular intervals. The study included health care providers employed in government and non-government health facilities working in the facility for more than 1 year. We excluded providers in the internship programmes. The study was conducted among 217 health care providers from government and non-government health facilities.

Sample Size Calculation

The minimum sample size was estimated using a formula expressed as $N = [Z^2\ P(1-P)]/(d^2)$, where Z is the value (1.96 for 95% confidence interval [CI]), P represents prevalence (0.846), and d is the minimum tolerable error at 95% CI, expressed as a decimal (0.05). The prevalence of antimicrobial prescription and dispensing was 84.6%.[20] Therefore, N = 200 + 10% (of 200) = 220, with 10% representing the non-response correction.

Questionnaire

Data were collected using a structured, anonymous, and self-administered 4-part questionnaire. The questionnaire content was based on a survey described in an Indian study,[16] but adopted to Tanzania and modified for the purposes of this study. The questionnaire was validated using a small group of medical residents before it was distributed among the target population. Based on the pilot study, the questionnaire was modified and improved on advice of relevant experts in statistics and epidemiology. The final version of the questionnaire had 16 key questions subdivided into 3 categories: knowledge and attitudes, prescription practices, and accessibility and use of antibiotic therapy guidelines.

Briefly, part 1 recorded sociodemographic and health facility characteristics. Part 2 was composed of 8 questions on knowledge and attitudes of antibiotic prescription practices: 'Does inappropriate antibiotic prescribing put patients at risk?', 'Is it always better to overprescribe antibiotics than underprescribe?', 'Should everyone be able to buy antibiotics

without a prescription?', 'Is antibiotic resistance a problem in my daily practice?', 'Is antibiotic resistance a significant problem in my health facility?', 'Antibiotic resistance is a significant worldwide problem?', 'Are infectious diseases services at my hospital easily accessible?', and 'Are infectious diseases services at my health facility very helpful?'. Each question in this part was assessed using a 5-point Likert scale ranging from strongly agree to strongly disagree.

Part 3 had 5 questions about prescription practice: 'Which of these factors may influence your decision to start antibiotic therapy?', 'Do you ever try to make sure that your antibiotic prescribing is cost effective?', 'Which of these do you think are important causes of inappropriate antibiotic use?', 'Which of the following do you think may help control antibiotic resistance?', and 'Have you received regular training and education in antibiotic prescribing in your work place?'.

Part 4 had 3 questions on the accessibility and use of antibiotic therapy guidelines: 'Does your health facility provide guidelines for diagnosis and management of patient with infective problem?', 'How accessible are these guidelines?', and 'Do you follow the recommendations of your health facility antibiotic guidelines?'.

Data Analysis

Data were analysed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Armonk, NY, USA). Data analysis was conducting using descriptive statistics, including frequencies and percentages.

Ethics Approval and Consent to Participate

Permission to conduct the study was gained from the Kilimanjaro Christian Medical University College Research Ethics Review Committee and from the district medical officer. Written informed consent was obtained from all participants who voluntarily agreed to take part in this study.

RESULTS

Demographic Characteristics

The response rate was 98.6%. Out of the 217 participants, 140 (64.5%) were female. A high proportion of participants (n=89, 41.0%) were under 30 years, followed by those between the ages of 31 and 40 years (n=78, 35.9%) and those over 40 years (n=50, 23.1%). Over two-fifths (n=94, 43.3%) of the participants worked at a dispensary. A majority of health care providers (n=149, 68.7%) were assistant nursing officers, followed by clinical officers (n=46, 21.2%), assistant medical officers (n=10, 4.6%), medical doctors (n=6, 2.8%), and nursing officers (n=6, 2.8%). Regarding years of experience, nearly a quarter (n=53, 24.4%) of the health care providers had more than 10 years of work experience and almost half (n=106, 48.8%) had between 3 and 5 years of work experience. Table 1 summarizes the complete sociodemographic characteristics of the study participants.

Knowledge and Attitudes Toward Antibiotics Among Health Care Providers

Over half (n=111, 51.2%) of the participants strongly agreed and almost all of the others (n=97, 44.7%) agreed that inappropriate prescription of antibiotics puts patients at risk. A majority of health care providers either disagreed (n=81, 37.3%) or strongly disagreed (n=65, 30.0%) that overprescribing antibiotics is always better than underprescribing. More than two-fifths (n=90, 41.5%) disagreed that everyone should be able to buy antibiotics without prescription, and

TABLE 1. Demographic Characteristics of the Study Population in Rombo District (N=217)

Variable	n (%)
Gender	
Male	77 (35.5)
Female	140 (64.5)
Age (in years)	
Less than 30	89 (41.0)
31 to 40	78 (35.9)
More than 40	50 (23.1)
Type of health facility	
Hospital	53 (24.4)
Health centre	70 (32.3)
Dispensary	94 (43.3)
Medical role/cadre	
Assistant nursing officer	149 (68.7)
Nursing officer	6 (2.8)
Assistant medical officer	10 (4.5)
Clinical officer	46 (21.2)
Medical doctor	6 (2.8)
Years of experience	
Less than 2 years	16 (7.4)
3 to 5 years	106 (48.8)
6 to 10 years	42 (19.4)
More than 10 years	53 (24.4)

more than half (n=132, 60.8%) agreed that antibiotic resistance was a worldwide problem. A majority of participants agreed that infectious disease services are easily accessible (n=178, 82.0%) and that infectious disease services are helpful (n=172, 79.3%). Table 2 presents participant knowledge and attitudes on antibiotics. Only 82 (37.8%) health care providers were aware of antibiotic resistance rates (Figure 1).

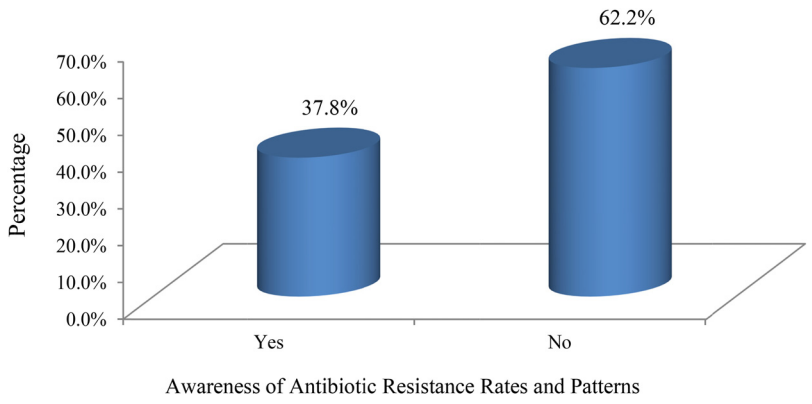
Health Care Provider Practices on Antibiotic Prescriptions

More than half (n=112, 51.6%) of the health care providers reported that their decision to start antibiotic therapy was influenced by patient's clinical condition, while about half (n=110, 50.7%) indicated they were influenced by positive microbiological results in symptomatic patients. Almost two-fifths (n=85, 39.2%) responded that most of the time they try to make sure that the antibiotic prescription is cost effective. Of the important causes of inappropriate antibiotic use, poor skills and knowledge (n=140, 56.5%) were identified as the leading cause, followed by lack of interest in the subject of antibiotic prescribing and infection management (n=63, 29.0%). On important strategies to control antibiotic resistance, 119 (54.8%) were in favour of providing education to physicians on appropriate antibiotic therapy, while 70 (32.3%) suggested including knowledge of pathogens

TABLE 2. Knowledge and Attitude on Antibiotics Among Healthcare Providers in Rombo District (N=217)

Question	Response n (%)				
	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree
Does inappropriate antibiotic prescribing put patients at risk?	111 (51.2)	97 (44.7)	0 (0.0)	7 (3.2)	2 (0.9)
Is it always better to overprescribe antibiotics than to underprescribe?	13 (6.0)	23 (10.6)	35 (16.1)	81 (37.3)	65 (30.0)
Should everyone be able to buy antibiotics without a prescription?	6 (2.8)	24 (11.1)	16 (7.4)	90 (41.5)	81 (37.4)
Is antibiotic resistance a problem in my daily practice?	4 (1.8)	107 (49.3)	1 (0.5)	92 (42.4)	11 (5.1)
Is antibiotic resistance a significant problem in my health facility?	2 (0.9)	100 (46.1)	7 (3.2)	104 (47.9)	4 (1.8)
Is antibiotic resistance is a significant worldwide problem?	26 (12.0)	132 (60.8)	2 (0.9)	57 (26.3)	0 (0.0)
Are infectious disease services at my hospital easily accessible?	19 (8.8)	178 (82.0)	0 (0.0)	19 (8.8)	1 (0.5)
Are infectious disease services at my health facility very helpful?	21 (9.7)	172 (79.3)	9 (4.1)	13 (6.0)	2 (0.9)

FIGURE 1. Awareness of Antibiotic Resistance Rates and Patterns



and antibiotic susceptibility results. Only 52 (24.0%) received regular training and education in antibiotic prescription in their work place (Table 3). Less than one-tenth (n=16, 7.4%) of health care providers were 'very confident' in their knowledge and practices on antibiotic prescription (Figure 2).

Accessibility and Use of Antibiotic Therapy Guidelines

A majority (n=136, 62.7%) of health care providers reported that antibiotic therapy guidelines were accessible and used. About one-third (n=73, 33.6%) reported that the guidelines provided were comprehensive, while half (n=109, 50.2%) indicated that the guidelines were not always accessible when needed. Almost a quarter (n=48, 22.1%) of health care providers reported they never follow the recommendations of the antibiotic guidelines, while more than a quarter (n=63, 29.0%) reported they always follow the antibiotic guidelines (Table 4).

DISCUSSION

This study assessed knowledge, attitudes, and prescription practices related to antibiotics among health care providers

TABLE 3. Responses from Healthcare Providers on Their Antibiotic Prescription Practices (N=217)

Questions	Potential Answers	n (%)
Which of these factors may influence your decision to start antibiotic therapy?	Patient's clinical condition	112 (51.6)
	Positive microbiological results in symptomatic patients	110 (50.7)
	Wanting to satisfy the senior treating physician	0 (0.0)
	Worry of missing patients with possible infections	3 (1.4)
Do you ever try to make sure that your antibiotic prescribing is cost effective?	Always	51 (23.5)
	Most of the time	85 (39.2)
	Never	14 (6.5)
	Rarely	15 (6.9)
	Sometimes	52 (24.0)
Which of these do you think are important causes of inappropriate use of antibiotic?	Poor skills and knowledge	140 (56.5)
	Unrestricted availability of antibiotics	16 (7.4)
	Inadequate supervision	10 (4.6)
	Lack of interest in the subject of antibiotic prescribing and infection management	63 (29.0)
	Lack of effective hospital policies	2 (0.9)
	Overworked health care personnel	9 (4.1)
Which of the following do you think may help control antibiotic resistance?	Treating infection, not contamination or colonization	22 (10.1)
	Physician education on appropriate antibiotic therapy	119 (54.8)
	Consulting with infectious diseases experts	4 (1.8)
	Providing local antibiotic guidelines	8 (3.7)
	Knowledge of pathogens and antibiotic susceptibility test results	70 (32.3)
	Obtaining local antibiotic resistance profile	4 (1.8)
Have you received regular training and education in antibiotic prescribing in your work place?	Yes	52 (24.0)
	No	165 (76.0)

FIGURE 2. Confidence About Knowledge and Practices Related to Antibiotic Prescribing

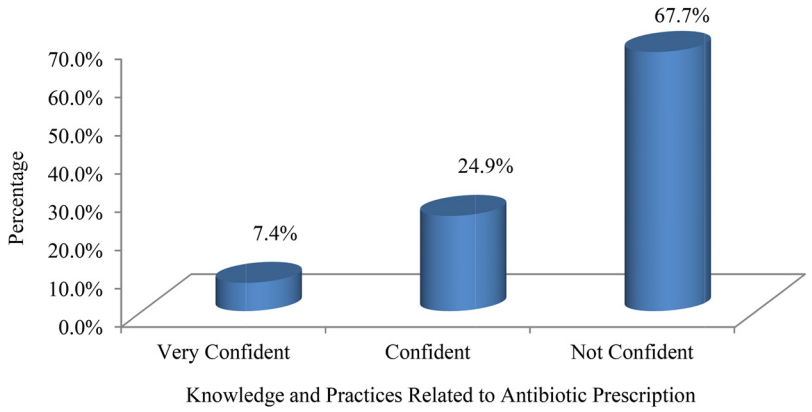


TABLE 4. Accessibility and Use of Antibiotic Therapy Guidelines (N=217)

Question	Potential Answers	n (%)
Does your health facility provide guidelines for diagnosis and management of patient with infective problem?	Yes, but limited	136 (62.7)
	Yes, but not helpful	6 (2.8)
	Yes, comprehensive	73 (33.6)
	I do not know	2 (0.9)
How accessible are these guidelines?	Limited access/access with difficulty	109 (50.2)
	Widely accessible	99 (45.6)
	I do not know	9 (4.1)
Do you follow the recommendations of your health facility antibiotic guidelines?	Never	48 (22.1)
	Rarely	16 (7.4)
	Sometimes	43 (19.8)
	Most of the time	47 (21.7)
	Always	63 (29.0)

in the Rombo district in northern Tanzania. No previous study in this area focused on prescribing practices among health care providers. Health care providers play an important role in antibiotic misuse and, thereafter, the development of antibiotic resistance. This is due to either lacking knowledge about appropriate antibiotic prescription practices or being reluctant to practice caution.

Knowledge and Attitudes of Antibiotic Use Among Health Care Providers

In 2006, a study from the same district documented that antibiotic prescriptions should only be made if health care providers have adequate knowledge of the patient's health and are satisfied that they are serving the patient's needs.[18] In this study, health care providers reported that inappropriate

prescription of antibiotics puts patients at risk. However, in our study, a small proportion of health care providers did not agree with these statements. The same result has also been reported in Saudi Arabia.[16] A study conducted in Ghana reported that 69.1% of health care providers agreed that inappropriate antibiotic use might lead to dangerous allergies, which could cause death.[19] Another study conducted by Tegagn et al reported that inappropriate antibiotic use can lead to resistance, treatment failure, increased adverse effects, and an additional burden of medical cost to the patient.[20] Furthermore, a study by Kumar et al stated that inappropriate antibiotic prescribing results in a 5-fold mortality increase in patients.[21] Our study suggests that there is a lack of knowledge on the effect of inappropriate prescription of antibiotics. Moreover, in this study, a small proportion of health care providers agreed on the statement 'it is better to overprescribe than underprescribe'. Similar observations have been reported elsewhere.[16]

In this study, half of the health care providers disagreed that antibiotic resistance is a significant problem in their respective health facilities, while 38.7% were unaware of antibiotic resistance rates and patterns. This may be due to inadequate surveillance of antibiotic resistance in Tanzania. The same has been reported by Baadani et al.[16] However, several studies have reported antimicrobial resistance in the region.[22,23]

Researchers from Saudi Arabia have suggested that surveillance systems of antimicrobial usage and resistance should include efforts to ensure timely dissemination of information to all health care providers and stakeholders.[16]

Antibiotic Prescription Practices Among Health Care Providers

Several factors, such as clinical conditions and positive microbiological tests in symptomatic patients have been reported to influence provider decisions to start antibiotic therapy.[16] This is encouraging in environments where laboratory facilities and effective hospital policies are available. However, a supportive environment is only 1 of several factors that influence antibiotic prescribing behaviours. Poor skills and knowledge are the leading causes of inappropriate antibiotic prescribing and use. In this study, a lack of interest in the subject of antibiotic prescription and infection management was identified as a cause of inappropriate antibiotic use by 29.0% of participants. This is a higher proportion than that reported by Baadani et al, who reported that only 6.6% of health care providers believed that lack of interest in the subject of antibiotic prescription and infection control was the cause of inappropriate antibiotic use.[16]

On important strategies to control antibiotic resistance, more health care providers were in favour of providing education to physicians on appropriate antibiotic therapy, while others suggested consideration of knowledge of pathogens and antibiotic susceptibility results. Furthermore, 24.0% reported that they did not receive regular training and education in antibiotic prescription. This is nearly the same as the study by Baadani et al, which reported that 34.9% of respondents did not receive regular training.[16] As suggested, there is an urgent need for carefully planned education and training programmes to address the knowledge gaps and support appropriate evidence-based antimicrobial prescribing practices among health care providers.[16]

Accessibility and Use of Antibiotic Therapy Guidelines

This study revealed that access to clinical or treatment guidelines was often limited or nonexistent. This may explain why some health care providers reported they never follow antibiotic guideline recommendations, which could provide them with information on how to safely prescribe antibiotics. Instead, by ignoring recommendations, they continue inappropriate prescription practices and, hence, contribute to increasing antibiotic resistance rates.

As part of a global effort to fight against the development of antibiotic resistance, the Global Antibiotic Resistance Partnership (GARP)–Tanzania aimed to develop policy recommendations to govern the appropriate use of antibiotics. Identified priority areas include, among others, improving hospital practices; rationalising antibiotic use in the community; educating health professionals, policymakers, and the public on sustainable antibiotic use; and ensuring political commitment to meet the threat of antibiotic resistance. However, in Tanzania, there are no current national or local data on antimicrobial surveillance. Moreover, in addition to the recommendations made by GARP, more reports are needed from the local and national levels on surveillance of antibiotic prescribing and resistance.[24] The National Centre for Adverse Drug Reactions monitoring, under the Tanzania Food and Drugs Authority, aims to analyse and disseminate information needed to support drug prescribing and regulation strengthening.

Study Strengths and Limitations

Although this is the first report on antibiotic prescribing among providers in the Rombo district, and probably in the Kilimanjaro region, we feel that the findings are of generalized interest. This study did not interview pharmacists or patients; adding their perspectives could have provided a richer picture of prescription practices and antibiotic use. The study also did not explore the commonly used antibiotics in the study area and did not try to achieve a balance between provider location and level of practice.

CONCLUSION

In this study, health care provider knowledge and prescription practices related to antibiotics were generally unsatisfactory. Our research showed that there is a clear need for training and education for health care providers in the area of antibiotic prescribing, and that antibiotic therapy guidelines should be easily accessible and effectively used.

Acknowledgements: The authors wish to thank the participants for their valuable contribution to this study. This study forms a part of the undergraduate studies of SRL who received a scholarship from the Tanzania Loan Board to pursue his bachelor's degree in laboratory health sciences.

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Peer Reviewed

Competing Interests: None declared.

Received: 7 Aug 2017; **Accepted:** 8 Mar 2018

Cite this article as: Lyimo SR, Sigalla GN, Emidi B, Mgabo MR, Kajeguka DC. Cross-Sectional Survey on Prescription Practices of Antibiotics Among Health Care Providers in Rombo District, Northern Tanzania. East African Health Res J. 2018;2(1):10-17. https://doi.org/10.24248/EAHRJ-D-18-00060

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Potential Value of Qiagen and PrepIT•MAX Kits in Extraction of Mycobacterial DNA From Presumptive Tuberculosis Archived Formalin-Fixed Paraffin-Embedded Tissues

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ABSTRACT

Background: DNA analysis has potential for screening for and diagnosing a variety of conditions as well as the characterization of various pathogens for many purposes including to identify genetic disorders and mutations, study genetic diversity, and establish evolutional trends.

Methods: Our study compared the performance of 2 DNA extraction kits: Qiagen and prepIT•MAX. The study tested 160 formalin-fixed paraffin-embedded (FFPE) human tissue samples that had been collected at Muhimbili National Hospital (MNH) between 2010 and 2016. For each sample, DNA extraction was performed using both the Qiagen and prepIT•MAX kits followed by polymerase chain reaction (PCR) tests to target the RNA polymerase gene and gel electrophoresis.

Results: The findings showed that the Qiagen was 3 times superior to the prepIT•MAX kit in successfully extracting mycobacterial DNA from presumptive tuberculosis (TB) FFPE tissues. Of the 160 previously Ziehl-Neelsen stain-negative *Mycobacterium tuberculosis* suspicious tissue samples, 12 (7.5%) tested positive with the PCR. Of the 12 PCR-detected positive samples, 8 (66.7%) yielded positive results with the Qiagen kit only and 4 (33.3%) yielded positive results with both Qiagen and prepIT•MAX kits. Additionally, 10 (83.3%) came from well-formed granuloma, 1 (8%) from caseous necrosis, and 1 (8.3%) Langhans-type giant cells endorsing their potential for housing infection such as TB adenitis.

Conclusions: A combination of molecular techniques, microscopy, and pathological features increases detection of *M. tuberculosis* from FFPE tissues. Both the Qiagen and the prepIT•MAX DNA extraction kits have shown a remarkable capability for extracting DNA from *M. tuberculosis*, although examination of FFPE tissues is not an intended use for the prepIT•MAX, according to the manufacturer. In resource-limited countries, however, these kits may complement each other. We recommend further studies for validation and optimization, which includes the cost effectiveness of prepIT•MAX extraction kit to advocate for its use in extraction of mycobacterial DNA from FFPE tissues.

INTRODUCTION

With advances in technology, archived biological materials have increasingly become important sources of scientific data. Modern technology can enable, for example, a detailed investigation and analysis of preserved tissue samples at molecular level. As a result, it is possible to retrospectively and prospectively explore large numbers of patients and detect and track genetic changes in infectious agents that occur over time.[1,2] Consequently, this method of analysis provides room for planning appropriate remedies to various disease conditions.

Formalin-fixed paraffin-embedded (FFPE) human tissues have been suitable specimens as DNA sources. DNA analysis is simple, extremely sensitive, and uses small sample amounts (5 to 10 µm sections) for extraction. This method also allows analysis of DNA from a large number of archived tissue samples within short time.[3,4] Nevertheless, for a successful outcome, this approach requires that an appropriate DNA extraction method is used to produce quality DNA data.[5] This is particularly important when analysing various tissue samples from different sources and different storage systems. Archived tissue samples enable analysis of gene expression[6,7] and proteome analysis[8,9] even in preserved

tissues where the collection procedures are largely unknown. FFPE tissues that have been stored for years can provide an opportunity to study macromolecules like DNA, RNA, and proteins for valuable information with no significant difference over storage time.[10]

In extrapulmonary tuberculosis (TB), *Mycobacterium tuberculosis* infects cells in tissues and organs outside the lung. In latent extrapulmonary TB, infected tissues/organs act as reservoirs for reactivation of active TB.[11] This is particularly important in zoonotic TB in multispecies host–pathogen ecosystems where livestock, livestock products, and people co-exist and, thus, are at high risk of infection.[12] In such situations, an innovative, time-efficient, affordable, and applicable-to-field-conditions approach is important for disease surveillance, particularly in low-income countries.

Molecular tools are important for the management, surveillance, and control of diseases. They require appropriate tissue samples that were preserved in ideal conditions and suitable DNA extraction evaluation tools. To obtain DNA for analysis, proper choice of preferential sites where one is most likely to get DNA samples may be necessary to achieve expected outcomes. Detection of *M. tuberculosis* complex in FFPE tissue specimens with necrotizing granulomatous inflammation provides rapid and correct diagnosis on different sources of preserved samples collected prospectively. In so doing, this method offers supplementary opportunity for TB diagnosis in patients where TB was not initially suspected.[13] Polymerase chain reaction (PCR) testing has long been recommended for detection of *M. tuberculosis* DNA in FFPE specimens; it offers increased sensitivity and accuracy crucial for patients with perplexing diagnostic problems associated with a granulomatous tissue response[14] and unusual presentation.[15] Other studies have recommended use of PCR when TB is suspected clinically, especially in cases of chronic inflammation without definite evidence of granulomatous inflammation.[16] Heating the tissue under the influence of variable pH values has been shown to be an effective protocol for DNA extraction from archived paraffin-embedded tissues and may contribute in providing enhanced understanding of changes that occur during formalin-induced modification of nucleic acids.[17] Our study aimed at using FFPE archived human tissue collected retrospectively for diagnosis of *M. tuberculosis* in presumptive TB patients. We compared the performance of 2 commercial kits – Qiagen and prepIT•MAX – to extract DNA from archived FFPE tissue samples, after which each of the samples was tested by PCR amplification of the RNA polymerase (*rpo*B) gene and detection by gel electrophoresis.

METHODOLOGY

Study Design and Population

This retrospective cross-sectional study involved 160 archived FFPE samples that had been collected as part of routine disease management at Muhimbili National Hospital (MNH) central pathology laboratory from 2010 to 2016. The study included tissues that were noted to have histopathological features suggestive of non-specific chronic inflammation and granulomatous lesions. These samples were tested to find out whether they originated from presumptive TB cases. Presumptive TB refers to a patient who presents with symptoms or signs suggestive of TB, previously known as a TB suspect. DNA extraction and PCR analysis from selected samples was done at the Molecular Biology Laboratory, Department of Biochemistry at Muhimbili University of Health and Allied Sciences (MUHAS).

Inclusion and Exclusion Criteria

The inclusion criteria involved all reported cases presumed of non-specific chronic inflammation and granulomatous lesions as detected by haematoxylin and eosin (H/E) staining. All reported cases with benign or malignant features and tissues with normal histological patterns, as detected by H/E staining, were excluded from the study. Archived types of tissues initially submitted for evaluation for other granulomatous inflammatory conditions were tested by the Ziehl-Neelsen (ZN) technique and then DNA extraction by the 2 commercial kits (Qiagen and prepIT•MAX) to establish potential for extrapulmonary TB. Biopsies of individuals submitted for histopathological evaluation were selected using simple random sampling (computer-generated table of random numbers) after obtaining patients' medical records from the archived files and log books. The sample size was then adjusted using the finite population correlation factor to arrive to 160 tissue samples from the calculated 207 sample size. The obtained number of tissue samples was considered enough to provide sufficient information to draw useful conclusions about the compared techniques.

Data Collection and Quality Control

For clinical and social-demographic data, a form was developed to capture information including age, gender, area of residence, medical history, HIV status, methods used for their diagnosis, site of the specimen, and final diagnosis. All necessary safety requirements for the investigator were observed during specimen handling. Standard operating procedures and the good clinical laboratory practices were strictly adhered to.

Laboratory Analysis

Tissue sections for ZN staining were prepared according to the site standard protocol. Briefly, the tissue samples were de-paraffinised twice by immersion in xylene-containing vessel, each for 3 minutes, followed by 10 dips immersion in 95%, 80%, and 70% ethyl alcohol. Thereafter, the tissue sections were flooded with carbol fuchsin on the staining rack, warmed until steam rose (not boiled), and left for 10 minutes to cool. Tissue sections were then washed well in distilled water and differentiated in 1% acid in 70% alcohol for

30 seconds. Then, 0.2% methylene blue was applied as counter stain for 1 minute. Stained slides were then blotted dry with Whatman filter paper and, thereafter, transferred to 3 changes of absolute alcohol and cleared in 3 changes of xylene. Finally, stained slides were mounted in distyrene plasticizer xylene (DPX) applied with cover slip.

DNA Extraction Procedures

Extraction of mycobacterial DNA was performed by using 2 different commercial kits, QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and prepIT•MAX (DNA Genotek, Ottawa, Canada). The Qiagen DNA extraction method was performed according to the manufacturer protocol while the prepIT•MAX DNA extraction protocol was modified accordingly to suite extraction of DNA from FFPE tissue specimen. Modifications included, but were not limited to, initial processing from de-paraffinisation to when tissue pellets were obtained before addition of MAX Buffers for DNA extraction. In brief, excess paraffin was trimmed off the sample tissue block and 8 tissue sections of 10 µm thick were cut. One mL xylene was added to the trimmed sections in a 1.5 microcentrifuge tube and then centrifuged at 14,000 rpm for 2 minutes at room temperature. Supernatant was pipetted out. One millilitre (mL) of absolute ethanol was added to the pellets and mixed by vortexing and later centrifugation at 14,000 rpm for 2 minutes and supernatant pipetted out. Thereafter, the 1.5 microcentrifuge tubes containing pellets were opened and incubated at 37°C for 10 minutes.

Polymerase Chain Reaction for *rpo*B Gene

A set of primers – TR8, 5′-TGCACGTCGCGGACCTCCA-3′ and TR9, 5′-TCGCCGCGATCAAGGAGT-3′– was used to amplify the 157 base pairs (bp) segment of *M. tuberculosis* complex *rpo*B gene. In brief, 5 µl of template DNA was added to a final volume of 100 µl containing 10 µl of 10x PCR buffer, 6 µl of 1.5 mM $MgCl_2$ (Promega), 8 µl of 10 mM dNTP's (SIGMA), 2 µl of 0.5 µM of each primer and 0.4 µl(2U) of Taq polymerase (Promega). The PCR conditions included 3 stages: initial denaturation at 95°C for 15 minutes (stage 1), followed by 45 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1 minute (stage 2), and a final extension at 72°C for 10 minutes (stage 3). PCR was done on a programmable PTC-100 thermo cycler (Bio-Rad Laboratories, Foster City, CA, USA). The laboratory *M. tuberculosis* H37Rv standard strain was used as a positive control and DNA-free molecular grade (PCR) water as a negative control.

Gel Electrophoresis

Gel electrophoresis was done at 80V for 2 hours on 1.5% agarose (Promega) in 1x TBE buffer and stained with ethidium bromide (conc 10 mg/mL) (SIGMA). The PCR amplicons were sized using 10 Kb-ladder. PCR amplicons were visualized under UV light and images electronically recorded and stored using a digital mobile phone camera (Samsung Galaxy S5, SM-G900H). The images were later transferred to the computer for interpretation. Expected band size of the target DNA fragment was 157 bp (Figure 1).

Data Management and Statistical Analysis

Data entry, cleaning and analysis was performed using IBM SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA). The data collected included, TB infection as the dependent variable, and gender, site of biopsy, area of residence and microscopy histological features as independent variables. Descriptive statistics (Pearson χ^2-test and Fisher's exact test) were performed to establish frequencies and association of factors where p-values less than .05 were considered statistically significant.

Ethical Consideration

The institutional ethical clearance was obtained from MUHAS Institutional Review Board (IRB), Ref. No. MU/PGS/SAEC/Vol. XVI and permission to conduct research was obtained from MNH. To maintain confidentiality, laboratory and hospital patient identification numbers were assigned and used instead of names.

RESULTS

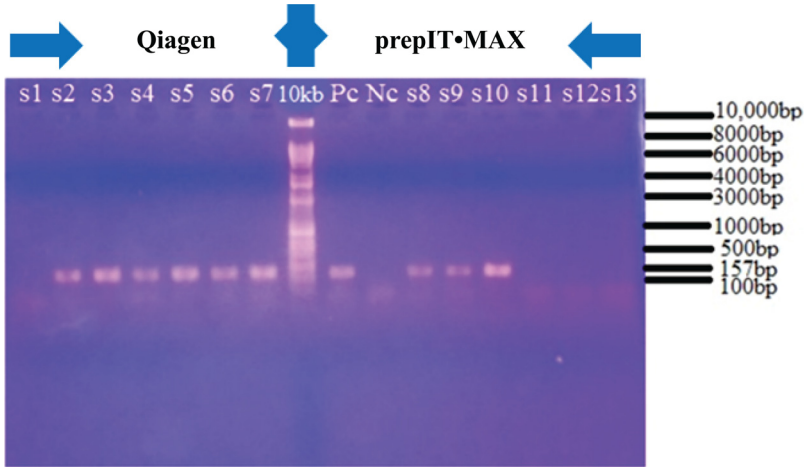
Of the 160 archived tissue samples used in this study, 82 (51.2%) were collected from females and 78 (48.8%) were collected from males. The male-to-female ratio of study subjects was 1 to 1, of whom, 16 (10.0%) subjects were under 15 years, 106 (66.3%) between 15 and 45 years, and 38 (23.7%) over 45 years (Table 1). The mean age range was 33.00 ± 16.7 years. Specific body sites from which most samples were taken include: acetabulum, chest, heel, knee, perianal, peritoneum, pharynx, pleura, scalp, skin, spine, mandible, and lymph nodes.

As stated earlier, DNA extraction was performed from each sample using 2 kits, prepIT•MAX and Qiagen, followed by PCR using extracts from both kits. With PCR results for samples extracted using the Qiagen kit, 12 (7.5%) out of 160 samples were found positive for *M. tuberculosis*, while 4 (33.3%) samples were found PCR positive with the prepIT•MAX kit. Four (33.3%) out of 12 PCR positive samples were positive with both kits and 8 (66.7%) PCR positive samples were detected from the Qiagen kit extraction alone. The 2 kits were significantly different in DNA extraction ($P<.05$) with the Qiagen kit being 3 times superior to the prepIT•MAX DNA extraction kit (Table 2).

Gel Electrophoresis Results

The PCR amplicons obtained were run in 1.5% agarose gel; the results for both Qiagen and prepIT•MAX DNA extraction kits are shown in Figure 1. Comparable gel electrophoresis of PCR amplicons from DNA samples extracted using the

FIGURE 1. PCR Amplicons From Samples Extracted Using Both the Qiagen Kit and prepIT•MAX Kit



Sample numbers s2, s3, s4, s5, s6, and s7 are positive results by the Qiagen kit extracted DNA and sample numbers s8, s9, and s10 are positive results by the prepIT•MAX kit DNA extraction. The 10Kb DNA ladder is located at the center of the figure. Sample number s1 is a negative result by the Qiagen kit, while sample numbers s11, s12, and s13 are negative results by the prepIT•MAX DNA extraction kit.

Abbreviations: Nc, negative control; Pc, positive control.

TABLE 1. Distribution of Sampled Subjects By Gender and Age

Age Group	Male n (%)	Female n (%)	Total n (%)
<15	8 (5.0)	8 (5.0)	16 (10.0)
15 to 45	51 (31.9)	55 (34.4)	106 (66.3)
>45	19 (11.9)	19 (11.9)	38 (23.8)
Total	78 (48.8)	82 (51.3)	160 (100.0)

TABLE 2. Qiagen and prepIT•MAX DNA Extraction Kits: Results By Proportions After Gel Electrophoresis of PCR Products*

	prepIT•MAX		
Qiagen	Negative n (%)	Positive n (%)	Total n (%)
Negative	148 (100.0)	0 (0.0)	148 (100.0)
Positive	8 (66.7)	4 (33.3)	12 (100.0)
Total	156 (97.5)	4 (2.5)	160 (100.0)

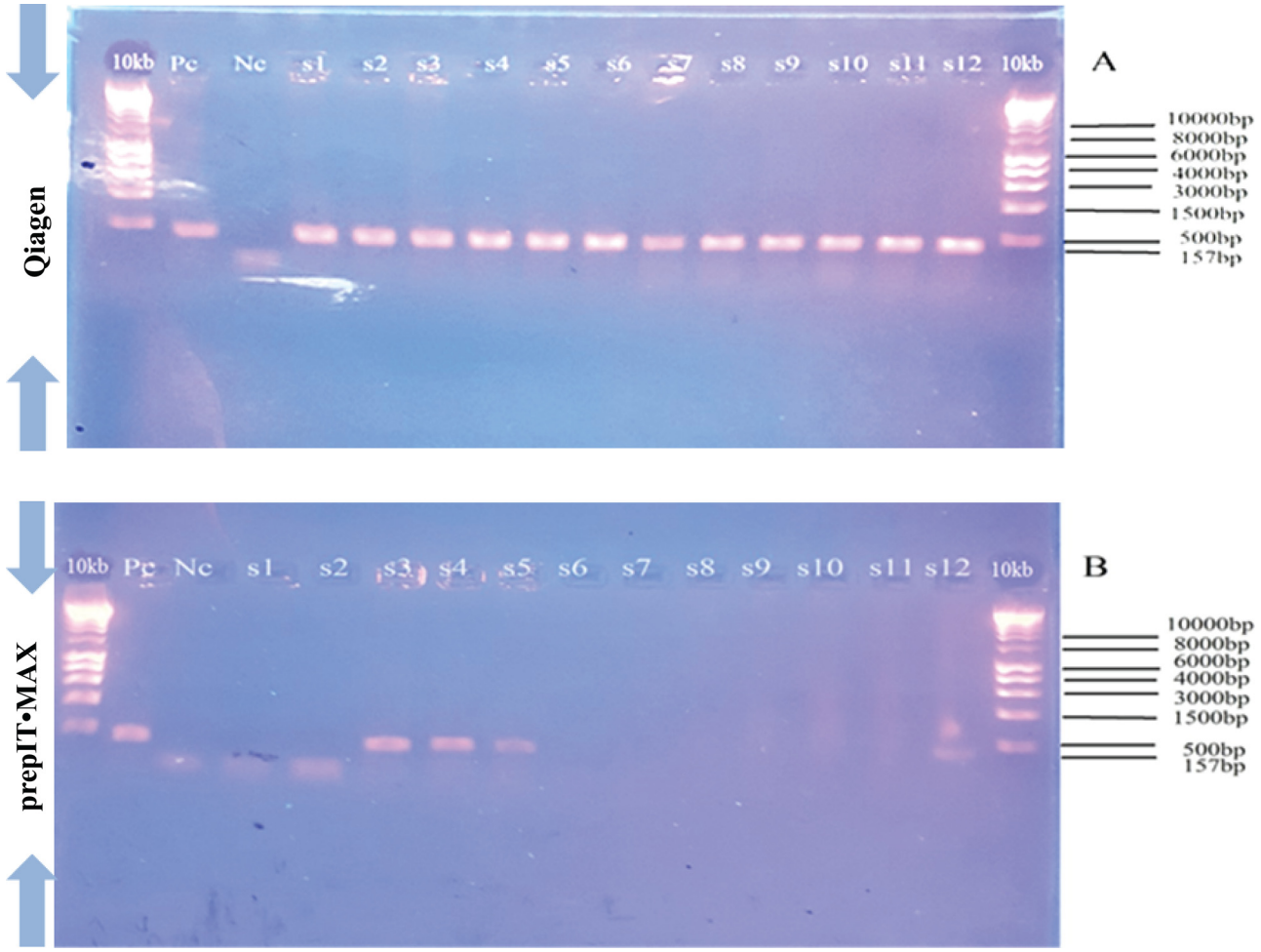
*Primers for *rpo*B gene were used to amplify the DNA target gene.

Qiagen kit (A) and the prepIT•MAX kit (B) are also shown in Figure 2. In these figures, the clear difference between the two test kits is obvious: only 4 samples were extracted using the prepIT•MAX compared to 12 samples extracted using the Qiagen kit.

DISCUSSION

This study focused on the evaluation of conventional versus molecular-based techniques for the identification of *M. tuberculosis* in FFPE tissues. No such study had previously been conducted in Tanzania, hence the proportion of cases detected based on the technique in the tissue samples is unclear. The value of archived FFPE tissue samples is in their ability to enable retrospective and prospective evaluation of large numbers of samples and track genetic changes over time in respect of infectious agents and genetic diseases.[2] FFPE are suitable specimens that can provide sources of DNA from even deteriorated samples and can provide valuable information from small amounts of specimens within a

FIGURE 2. Gel Electrophoresis of PCR Amplicons From DNA Samples



Gel electrophoresis of PCR amplicons from DNA samples extracted using Qiagen kit (A). Pc and Nc are positive and negative controls, respectively. Lanes are marked by samples labeled s1 to s12 showing positive result by using *rpo*B primers that amplify a 157-bp of DNA. DNA 10kb ladder is located at each end of the sample lanes. The PCR amplicons from DNA samples extracted using prepIT•MAX kit (B) are also indicated with only 4 samples being positive. The sample lanes are marked s1, s2, s6, s7, s8, s9, s10, and s11 (negative on gel electrophoresis) and lanes marked s3, s4, s5, and s12 (positive on gel electrophoresis). Amplification used the *rpo*B primers that amplify a 157-bp region of DNA. Faint bands for positive samples were obtained for samples s5 and s12.

Abbreviations: Nc, negative control; Pc, positive control; PCR, polymerase chain reaction; *rpo*B, a portion of the Mycobacterium tuberculosis gene encoding the beta subunit of RNA polymerase.

short time.[3,4] The 160 samples used in this study were collected over a period of 7 years.

Of 160 participants, 106 (66.3%) presumptive TB patients were 15 to 45 years of age suggesting that a larger proportion of relatively young to middle-age and active individuals reported as presumptive TB patients than any other age group. Similar findings were recently reported in the Serengeti ecosystem survey.[18,19] The present study has shown that of 12 PCR positive samples, 8 (66.7%) were collected from patients aged 15 to 45 years. Available evidence reveals TB lymphadenitis to occur relatively early, after *M. tuberculosis* primary infection, frequently affecting young people in endemic countries.[20] The female-to-male ratio of 1 to 1 obtained in this study is indicative of TB

infection to be related to exposure rather than gender (Table 1).

This study obtained samples from different tissue sites. However, lymph node samples were the most common (n=127, 79.4%), constituting 10 (83.3%) out of 12 PCR positive specimens. This was similar to previous reports[21] that used tissue samples from selected sites corresponding to TB infection. Lymph nodes are primary immune response sites prior to infection spread, and the first sites to show features suggestive of extrapulmonary TB. In children, however, the most serious forms are disseminated TB and TB meningitis. TB lymphadenitis is the most common form, accounting for up to 50.0% of extrapulmonary cases in children.[22] Thus, finding a larger proportion of PCR positives in lymph nodes is not surprising.

The present study has shown superiority of PCR to detect TB in tissues previously reported as acid-fast bacillus (AFB) negative by ZN. Of the 160 FFPE tissue specimens tested, none were AFB ZN positive contrary to the previously reported 61.7% AFB positive.[20] Our retrospective study used laboratory microscopic findings that are generally nonspecific with other conditions, such as sarcoidosis, syphilis, leprosy, Crohn's disease, rheumatoid arthritis, systemic lupus erythematous, and pneumoconiosis, having similar features.[23] The failure of ZN to detect AFB in FFPE tissue specimen could be a result of paucibacillary nature of the tissue specimen.[24,25] Presence of a smaller number of bacteria in tissue specimens might necessitate future bacterial culture plans to increase the DNA yield if similar studies are done. This can help to minimize false negative results that could be anticipated following direct extraction of mycobacterial DNA from FFPE tissues.

Twelve (7.5%) out of 160 tissue samples were PCR positive. Results from this molecular study, which used primers for *rpo*B gene segment in mycobacterial DNA, are important and highlight the superiority of PCR over conventional ZN-stain method. Similar results were reported in India, in a study that used insertion sequence IS6110 as a target gene for *M. tuberculosis* in tissues.[25] Our study used FFPE tissues as DNA source. Such samples provide high-quality DNA with reported PCR success rates of 97%.[26] In addition, others have reported that properly preserved and stored paraffin-embedded specimens can yield high-quality DNA given use of appropriate methods of extraction.[27] Thus, we are confident that the PCR-positive samples confirmed TB cases in tissue specimens.

From this study, it is worthwhile to note the potential inability of the ZN stain to detect infection in paucibacillary tissues,[28,29] 12 of which were PCR positive after DNA extraction using the Qiagen kit. The pathological features associated with such samples included well-formed granuloma (WFG), caseous necrosis (CN), and Langhans-type giant cells (LGC) (Table 3), signifying preferential localization of extrapulmonary TB lesions in these specific sites and their potential role of histopathology in TB diagnosis. Four (100%) of prepIT•MAX kit-extracted PCR-positive DNA samples had all of the main features (WFG, CN, and LGC). In general, 4 (33.3%) samples were detected by both Qiagen and prepIT•MAX extracted DNA while 8 (66.7%) were detected by Qiagen Kit extracted DNA alone. This

TABLE 3. Association Between the Polymerase Chain Reaction and Microscopic Features Diagnosed Using Hematoxylin and Eosin Staining Technique

	Microscopic Features Diagnosed in H/E Staining Technique									
PCR Results	**WFG**	**PFG**	**CN**	**LGC**	**WFG+CN**	**PFG+CN**	**WFG+CN+LGC**	**WFG+LGC**	**LGC+CN**	**Total**
Qiagen										
Positive	1	0	0	1	0	0	10	0	0	12
Negative	2	4	1	5	4	1	121	9	1	148
Total	3	4	1	6	4	1	131	9	1	160
prepIT•MAX										
Positive	0	0	0	0	0	0	4	0	0	4
Negative	3	4	1	6	4	1	127	9	1	156
Total	3	4	1	6	4	1	131	9	1	160

Abbreviations: CN, caseous necrosis; H/E, hematoxylin and eosin; LGC, Langhans-type giant cells; PCR, polymerase chain reaction; PFG, poorly formed granuloma; WFG, well-formed granuloma.

reflects a 3 times superiority of Qiagen kit over the prepIT•MAX kit. Our findings provide clues of future complementary use of both Qiagen and prepIT•MAX kits in tissue samples to conventional ZN method. The prepIT•MAX kit has never been used in detection of mycobacterium DNA in FFPE, thus, the different outcomes might be attributed to the fact that the kit was not designated for that purpose.

Two studies previously conducted in India, compared the conventional diagnostic modalities of BACTEC culture and PCR test[30] and IS6110-based PCR[31] for detection of *M. tuberculosis* clinical samples of diversified nature and revealed an association between histological features – WFG, CN, and LGC (Table 3) – and molecular findings. Our study revealed a similar likelihood that *M. tuberculosis* presence in tissue samples might correlate with non-specific histopathological features. In endemic areas, diseases with chronic granulomatous lesions similar to extrapulmonary TB, such as sarcoidosis, syphilis, leprosy, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, and pneumoconiosis, can be differentiated using molecular methods[23] and, thus, indicate precise medical treatment.

Archived tissue samples are valuable source of biological material for epidemiologic research of the disease.[32] DNA concentration may influence the sensitivity of PCR as suggested in previous studies,[21,33] hence the need for an appropriate and optimized method of DNA extraction. Therefore, the extraction procedure should ensure effective lysis of mycobacteria and good recovery of the DNA from a complex mixture of tissue debris with minimal PCR inhibitors. Combined inhibitory factors that might interfere with MTB detection in archived samples are inevitable.[34] This necessitates for dedicated control of inhibitory factors in order to suit molecular tools used for diagnosis. While the kits used in this study might not have the ability and level of quality for extracting of mycobacterial DNA from FFPE, we are sure these findings provide insights for valuable use of the kits in the future. A number of studies have addressed the problem of initial processing of mycobacterial samples and range from simple boiling and centrifugation[35] to trapping of DNA on Chelex resin and bead-beating.[33] It is important to observe these useful procedures for quality DNA.

So far, the study has shown a significant difference in performance between the 2 kits with $p \leq -.05$. The kits comparatively differed in performance with reflection of the Qiagen kit 3 times superior to the prepIT•MAX. Previous studies clearly indicated a greater power of the Qiagen commercial kits in extracting genomic DNA with lysis reagents.[21,35] The performance of prepIT•MAX in FFPE tissues is very promising, given that prepIT•MAX was used for the first time.

CONCLUSION AND RECOMMENDATIONS

Based on the findings from this study, it is recommended that in a setting like the Tanzania national referral hospital, where a majority of tissue specimens are examined and archived, the PCR technique could be used for diagnosis of TB. The use of PCR will increase the detection rate and differentiate extrapulmonary TB from other chronic granulomatous diseases with similar lesions. In this study, a significant number of TB presumptive patients were found to be missed prior to the use of a molecular approach on archived tissues implying that under detection of extrapulmonary TB cases is not uncommon in African TB diagnostic settings. It is also important for clinicians to pinpoint exactly the most preferred specific tissue sites for *M. tuberculosis* in extrapulmonary TB cases. We further conclude that protocol optimization for prepIT•MAX kit is still needed for it to perfectly suit deployment as a tool for DNA extraction from tissues. Having this 'dual purpose' tool will particularly fit well in resource-limited countries by enabling diagnosis and screening of TB cases from both sputa and tissues where TB is clinically suspected. This preliminary information provides an insight and potential for future improvement of the kit for better results. Nevertheless, there is a great need for cost effectiveness studies as the technique might be important, but it is cost driven.

Acknowledgements: The Biochemistry Department at MUHAS is acknowledged for hosting the lab work and MSc Degree study for YA. The management of MNH, particularly, the head of the histopathology section at the central pathology laboratory is acknowledged for their great support on this research. Generally, the data-collection support obtained from staff at MNH as well as in the Institute of Traditional Medicine at MUHAS is highly appreciated. ITECH-Tanzania and MUHAS are acknowledged for their financial support to make this study a success. Ms. Kaitlyn Shaw from the DNA Genotek in Ottawa, Canada, is acknowledged for providing, as a gift, the prepIT•MAX kit that was used parallel with the Qiagen kit. The Ministry of Health, Community Development, Gender, Elderly and Children Tanzania, is acknowledged for granting permission for pursuing this study.

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Competing Interests: None declared.

Received: 25 May 2017; **Accepted:** 28 Nov 2017

Cite this article as: Ayub Y, Mollel JT, Mbugi EV. Potential Value of Qiagen and PrepIT•MAX Kits in Extraction of Mycobacterial DNA From Presumptive Tuberculosis Archived Formalin-Fixed, Paraffin-Embedded Tissues. East African Health Res J. 2018;2(1):18-25. https://doi.org/10.24248/EAHRJ-D-17-00256

EAHRC
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COMMENTARY

Diagnostic Opportunities for Optimizing Management of Multidrug-Resistant Tuberculosis (MDR-TB) in Tanzania

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ABSTRACT

Background: Tanzania is one of the countries confronting a multidrug-resistant tuberculosis (MDR-TB) epidemic.

Research: Research studies on drug susceptibility testing (DST) for second-line TB drugs given to Tanzanian MDR-TB patients has demonstrated mycobacterial resistance to important MDR-TB drugs, such as ethionamide, ofloxacin, amikacin, kanamycin, and pyrazinamide. Likewise, pharmacokinetic studies have shown a high frequency of patients with circulating serum drug levels below the expected ranges, especially for levofloxacin and kanamycin – key drugs in MDR-TB treatment that also affect ex-vivo plasma drug activity.

Recommendations: We suggest using molecular diagnostic assays, such as the GenoType MTBDRplus test, and *inhA* and/or *katG* genotypic results to optimize MDR-TB treatment. Quantitative drug susceptibility can guide the selection of options for second-line anti-TB drugs. The TB drug assay, an alternative biomarker for therapeutic drug monitoring, can identify patients who have extensively drug-resistant TB or are exposed to suboptimal serum drug levels of, specifically, levofloxacin and kanamycin.

BACKGROUND

Multidrug-resistant tuberculosis (MDR-TB) is a public health crisis requiring novel approaches to diagnosis and treatment.[1] In many resource-limited settings, treatment is empirical and not based on known *Mycobacterium tuberculosis* (MTB) susceptibility patterns to the drugs that comprise the multidrug treatment regimen. The empirical MDR-TB regimen recommended by the World Health Organization (WHO) consists of pyrazinamide – which belongs to the first-line TB regimen, or group 1 TB drugs – and at least 4 other second-line TB drugs.[2] The second-line drugs are categorized in different groups, and the proposed regimen includes at least 1 drug from each group: 1 from the fluoroquinolone class, also called group 2 (ofloxacin, levofloxacin, or moxifloxacin); 1 from the injectable agents or group 3 (amikacin, kanamycin, or capreomycin); and 2 from group 4 (ethionamide/prothionamide, cycloserine/terizidone, or para-aminosalicylic acid). Of the group 4 drugs, the order of preference is ethionamide, followed by cycloserine and para-aminosalicylic acid. These drugs are added until 4 effective drugs are established.[2] If 4 drugs are not established or the efficacy of the combination is doubtful, 2 group 5 drugs (clofazimine, amoxicillin clavulanate, linezolid, imipenem, clarithromycin, high-dose isoniazid, or thiacetazone) are selected – 2 of these group 5 drugs are counted as a single effective drug to strengthen the regimen.[3] The recommended treatment duration is at least 20 months, with a minimum duration of 8 months for the injectable agent.[2]

MULTIDRUG-RESISTANT TUBERCULOSIS CLINICAL RESEARCH STUDIES IN TANZANIA

We have conducted several MDR-TB studies in Tanzania, aimed at improving treatment through an individualized approach.[4–8] One of the studies sought to describe the application of second-line drug susceptibility testing (DST) using minimum inhibitory concentration (MIC) results and compare those results with the empirical regimen used during a patient's treatment with a second-line regimen.[5] MIC allows categorization of isolates near the resistance breakpoint, 'borderline susceptible', that may be labelled as 'susceptible' by conventional testing but are subject to clinical resistance with poor drug absorption, altered metabolism, or inhibited protein binding. In this study, we found the majority of patients had at least 1 medication that could have been modified with the application of MIC guidance.

The most common medication modification in the MDR-TB regimen was changing ethionamide to para-aminosalicylic acid, which could have happened for more than 50% of patients.[5] Although ethionamide is considered tuberculocidal at higher concentrations, a different study showed that only 22% of subjects had pharmacodynamic indices (serum concentration 2 hours post medication [C_{2hr}]/MIC) ratios more than 2.[6] While ethionamide may have a less predictable time to peak concentration than the other oral agents tested–which may result in an underestimation of the measured C_{2hr} in some subjects–alternative second-line agents, such as para-aminosalicylic acid, may provide a more reliable benefit in the subset of patients with reduced drug exposure and borderline MICs. Additionally, research has shown that 22% of MTB isolates on kanamycin had borderline or resistant MICs, while amikacin retained full susceptibility and, therefore, required substitution within the aminoglycoside class.[5] WHO recommends to substitute aminoglycosides in case of resistance into polypeptides, such as capreomycin, in group 3.[3]

Fortunately, in all of the studies we have conducted, resistance to the fluoroquinolone class was low, ranging from 5% to 15%; however, 45% of patients had isolates of borderline susceptibility.[4,5,8] Moreover, 52% of our MDR-TB patients had circulating levofloxacin serum drug levels below the expected range when the levofloxacin was given as a 750 mg daily dose.[6] Recent studies of levofloxacin demonstrate that the best pharmacokinetic properties may be achieved at a dose of 1000 mg daily.[9] Such optimization may be applicable in our setting, given that no subject in this study was on doses of levofloxacin as high as 1000 mg. The C_{2hr} of levofloxacin for all subjects was below the median maximum serum concentration (C_{max}) of 15.5 μg/mL.[6] Correlating with what we observed, that a high proportion of MDR-TB isolates had borderline susceptibility on ofloxacin in MIC plates, these findings suggest a value to conducting clinical trials to evaluate levofloxacin at a dose of 1000 mg.

We also looked further into the resistance patterns of MDR-TB isolates using genotypic methods in the following mutations: rifampicin (*rpoB*), isoniazid (*inhA or katG*), ethambutol (*embB*), pyrazinamide (*pncA*), ofloxacin (*gyrA*), amikacin (*rrs or eis*), and ethionamide (*inhA*).[5] The results were compared with MICs, which showed a good correlation to the drugs tested. The few discrepancies showed resistance with MICs but were genotypically wild on the known regions conferring resistance. Although pyrazinamide MIC assays were not conducted, the *pncA* mutation was common, meaning that the suspected pyrazinamide resistance could have impact on treatment outcome.[10]

Our results suggest that in Tanzania, *inhA* and/or *katG* genotypic results could be used to optimize MDR-TB treatment. This can be done by screening MDR-TB with a GenoType MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany), which identifies *inhA* and/or *katG*. The research suggests that a mutation in the *inhA* region alone can exclude ethionamide from the MDR-TB empirical regimen, while adding high-dose isoniazid and para-aminosalicylic acid to the regimen. While an exclusive mutation on *katG* includes ethionamide, a mutation of both *inhA* and *katG* excludes ethionamide and adds para-aminosalicylic acid only. Further research is required to determine the empiric choice of ethionamide or para-aminosalicylic acid while processing for MIC testing.

Given the low proportion of isolates with resistance to injectable agents, the lack of *rrs* or *eis* mutations noted in the isolates, and the borderline susceptibility to ofloxacin, the new GenoType MTBDRsl assay (Hain Lifescience GmbH, Nehren, Germany) for these targets may be of less value. Instead, MIC testing would allow for selection within the class of aminoglycosides and support the use of high-dose levofloxacin.

We, therefore, believe that quantitative susceptibility methods would prove as useful and cost-effective as MDR-TB programmes that individualize management based on second-line drug susceptibility. This approach can be made even more cost effective by developing in-house laboratory platforms with the capacity to perform TB cultures.

Our findings on quantitative MICs in MDR-TB patients led to research work on MDR-TB drug concentrations relative to MICs, particularly when compared to the TB Drug Activity (TDA) assay.[6,7] The TDA assay uses a patient's plasma or serum collected during TB treatment and the patient's own MTB isolate and measures time to detection in liquid culture. Following extensive in-vitro studies, the TDA assay revealed that it predominantly measures the concentration-dependent activity of the aminoglycoside and fluoroquinolone components of the standard MDR-TB regimen.[6] The study further demonstrated the inactivity of pyrazinamide at the pH of the media used and the comparatively low concentration/MIC achievable for ethionamide and cycloserine. As such, in a patient on a standard MDR-TB regimen in Tanzania, a TDA value approaching 1.0 may be considered to have little plasma-killing potential. If this phenomenon occurs in the aminoglycoside or fluoroquinolone, it is similar to having XDR-TB, which indicates higher mortality and overall treatment failure. While the TDA assay cannot assess drug activity at the site of infection, subjects from Tanzania who had a faster time-to-sputum culture conversion were more likely to have high TDA values.[6,7] However, it is important to note that plasma drug activity was not exclusively predictive of sputum culture conversion and the TDA assay could not discriminate the relative contribution of individual drugs in the MDR-TB regimen, particularly at the highest TDA values. This may be a consequence of the range of C_{2hr} observed in subjects on the MDR-TB regimen and the unknown target concentration/MIC for a drug such as cycloserine that is not entirely concentration dependent or tuberculocidal in action.

Several limitations were recognized in these studies: critical concentrations for second-line medication still remain a subject of debate and not all regions of mutations or MTB

mechanisms for conferring drug resistance have been identified. Despite these limitations, we believe we have established options and evidence for optimization of MDR-TB management in Tanzania. With the scale up of rapid molecular diagnostics – specifically, GeneXpert and GenoType MTBDRplus – in the country, we expect more MDR-TB cases will be diagnosed and effectively treated than have been in the past. Those who need special attention, for instance those harbouring extensively drug-resistant TB (as diagnosed by second-line MIC, or the functional equivalent by TDA with TDA $\leq$1.0), drug reactions, or delayed culture conversion will continue to be treated at the National Centre of Excellence for MDR-TB management. Previously, we compared the overnight-pooled method to the current standard spot technique for quality, quantity, and time to MTB detection by culture using the BACTEC mycobacterial growth indicator tubes (MGIT) system (BD Diagnostics, Sparks, MD, USA) for pulmonary TB-suspected patients.[11] The study found that modifications of the overnight-pooled sputum collection method improved the time to detection in the MGIT system among culture-positive samples. This is important not only for identifying susceptibility in phenotypic methods, but also for microbiological monitoring of patients during the course of MDR-TB treatment. We, thus, recommend improving sputum collection for efficient monitoring of microbiological responses by use of the overnight-pooled method.

CONCLUSION AND RECOMMENDATIONS

Examination of second-line DST conducted with Tanzanian MDR-TB patients showed resistance to the important MDR-TB drugs, such as ethionamide, ofloxacin, amikacin, kanamycin, and pyrazinamide. Likewise, pharmacokinetic studies on existing MDR-TB regimens showed a high frequency of patients with circulating drug levels below the expected ranges, especially for levofloxacin and kanamycin – 2 key drugs in MDR-TB treatment that also affect ex-vivo plasma drug activity.

To optimize MDR-TB management in Tanzania and similar settings at all levels, we propose the following: patients diagnosed with MDR-TB by either the GenoType MTB/RIF or GenoType MTBDRplus rapid molecular diagnostic test shall submit pretreatment overnight-pooled sputum for culture to establish baseline mycobacteriology for culture-based DST. Next, the empirical second-line (MDR-TB) regimen will be started, but the regimen will be based on *inhA* or *katG* results to determine if ethionamide, para-aminosalicylic acid, or high-dose isoniazid should be added to the empirical regimen. Then, quantitative MIC results for second-line DST should be determined within 2 months of initiation of treatment and used to alter the regimen, if needed. Monthly overnight-pooled sputum should be collected to monitor the time-to-culture conversion. Blood should be taken at weeks 2 to 4 for the TDA assay or alternate therapeutic drug-monitoring (TDM) test. In combination with other standard clinical factors, quantitative second-line drug MIC and TDA/TDM results should be used to tailor the appropriate regimen.

Acknowledgements: The author would like to acknowledge The European & Developing Countries Clinical Trials Partnership (EDCTP), through the Pan-African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA) and the National Institutes of Health/Fogarty Training Grant (D43 TW008270), awarded to professors Eric R Houpt and Gibson S Kibiki. The studies discussed in this article were part of the PhD training of the author.

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Peer Reviewed

Competing Interests: None declared.

Received: 29 Oct 2016; **Accepted:** 8 Mar 2018

Cite this article as: Mpagama SG. Diagnostic Opportunities for Optimizing Management of Multidrug-Resistant Tuberculosis (MDR-TB) in Tanzania. East African Health Res J. 2018;2(1):26-28. https://doi.org/10.24248/EAHRJ-D-16-00357

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ORIGINAL ARTICLE

Tuberculosis Contact Screening and Isoniazid Preventive Therapy Among Children Under 5 in the Mbeya and Songwe Regions, Tanzania

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ABSTRACT

Introduction: The World Health Organization (WHO) recommends contact screening and initiation of isoniazid preventive therapy (IPT) for children under 5 years of age exposed to a sputum smear-positive (SS+) tuberculosis (TB) source case. We conducted this study in order to assess implementation of these recommendations in southwestern Tanzania.

Methods: We conducted this cross-sectional study from June to August 2015 in 12 selected health facilities in the Mbeya and Songwe regions of Tanzania. Adult SS+ pulmonary TB patients living in the same household as children under 5 were enrolled. Structured questionnaires were used to obtain sociodemographic information and details about screening and intervention activity related to contact children under 5. Data were analysed using Stata version 11.0.

Results: We enrolled 257 index cases, who collectively had 433 contact children under 5. The median age of the index cases was 34 years (interquartile range 28 to 41) and 52.9% were male. Out of 433 contacts, 31 (7.2%) were screened for TB, of whom 7 (22.6%) were treated for presumptive TB. Among those screened, 24 were not diagnosed with TB, of whom only 8 (33.3%) received IPT.

Conclusion: Low uptake of TB contact screening and IPT administration among eligible children under 5 was observed in this study. Health-care workers should be sensitized to screening of household contacts of adults with SS+ TB and initiate IPT in those who are eligible.

INTRODUCTION

Childhood tuberculosis (TB) contributes a significant proportion of the global TB burden. In 2015, 10% of the 10.4 million incident TB cases worldwide occurred in children under 15 years of age.[1] Children are usually infected via household contact with an adult who has sputum smear-positive (SS+) pulmonary TB (PTB).[2]

In line with recommendations set by the World Health Organization (WHO) and governed by national TB programmes, strategies for preventing childhood TB include contact tracing and isoniazid preventive therapy (IPT) for children under 5 in contact with TB source cases.[3,4] IPT has been shown to reduce the risk of progression from latent TB infection to active TB disease by as much as 59% among children aged 15 years or younger.[5–7] Despite the known benefits of contact screening and IPT, these strategies remain widely underutilized, even in countries severely affected by TB.[8–12] For example, a study in Malawi found that only 9% of contacts were screened for TB and only 6% were initiated on IPT.[13]

Tanzania is among the world's 22 high TB-burden countries and childhood TB is estimated to contribute about 10% of the national TB burden.[1] In keeping with WHO recommendations, Tanzania's National Tuberculosis and Leprosy Control Programme (NTLP) recommends contact investigation and IPT provision for all TB contacts under 5, after excluding active TB disease.[4] TB treatment services, including IPT initiation and follow-up, are provided for free by the NTLP and supervised by district TB and leprosy coordinators

(DTLCs), under the guidance of regional TB and leprosy coordinators (RTLCs).[4] Despite these recommendations and deployments, uptake of contact tracing and IPT initiation has not been well documented in most facilities, which has made it difficult to evaluate performance and adherence to guidelines. We conducted this study in 2 regions of Tanzania to assess uptake of contact screening and IPT administration, in 2 regions of Tanzania, for children under 5 who are contacts of SS+ TB patients.

METHODS

Study Design and Setting

Data for this cross-sectional study were collected from June to August 2015 in the Mbeya and Songwe regions located in the Southern Highlands Zone in southwestern Tanzania. These regions have TB notification rates ranging from 3,700 to 4,000 cases per year, with childhood TB contributing about 10% of the total TB burden. At the time of data collection, the 2 regions had 448 health facilities, which included 18 hospitals, 36 health centres, and 394 dispensaries. We randomly selected 10 hospitals and 2 health centres among sites with TB diagnostic and treatment capabilities.

Participants

We enrolled consenting adult SS+ PTB patients who lived in the same household with children under 5. Parents or caregivers who had other forms of TB were excluded. A household contact was defined as a child who shared the same house with an index TB case for at least 3 months prior to diagnosis.

Sample Size and Data Collection

The minimum sample size was estimated using the following formula: $n=Z^2P(1-P)/d^2$, where, Z is the level of confidence that the chosen sample was not representative of the population (using the value 1.96 for 95% confidence interval [CI]), P represents the proportion of the sample that is assumed to practice contact screening (50% or 0.5), and d is margin of error that the probability that the desired sample size was not representative of the study population at 95% CI, expressed as a decimal (0.05). We estimated the minimum sample size to be 384 contact children under 5, but included 433 children in the study to minimize errors. Data were obtained through interviews with adult index patients whose clinic TB treatment cards revealed positive TB results from sputum. Participants confirmed the presence of contact children under 5 before consenting to and proceeding with questionnaire-guided face-to-face interviews during routine TB clinic visits. Interviews captured sociodemographic information and details of contact TB screening, prevention with IPT, and treatment.

Data Analysis

Completed questionnaires were entered into Microsoft Excel and then exported into Stata version 11.0 (Stata Corp, College Station, TX, USA) for analysis. Categorical variables were summarized as proportions and continuous variables as medians with interquartile range (IQR).

Ethical Consideration

Ethical approval was obtained from the joint Catholic University of Health and Allied Sciences and Bugando Medical Centre (CUHAS/BMC) Ethics and Review Committee. The respective district administrative authorities granted permission to carry out the study. All patients provided written informed consent before participating.

RESULTS

Characteristics of Tuberculosis Index Cases

We enrolled 257 index TB cases, who accounted for 433 contact children under 5. The median age of the index cases was 34 years (IQR 28 to 41) and 136 (52.9%) were male. All index cases had at least 1 contact living in the same household and 150 (58%) were biological parents of the contacts (Table 1).

Household Contacts and Isoniazid Preventive Therapy

Out of 433 contacts, only 31 (7.2%) were reported to have been screened for TB. Among the 31 who were screened, 7 (22.6%) were found to have presumptive TB disease and were treated with anti-TB therapy. Eight of the 24 remaining contacts (33.3%) started IPT, while the other 16 eligible contacts did not receive any intervention (Figure 1).

DISCUSSION

This study investigated the extent of TB contact investigation as an entry point to IPT initiation among children exposed to adult SS+ PTB patients in southwestern Tanzania. The main purposes of contact investigation were to identify contacts with presumptive TB disease who require anti-TB therapy and to provide a gateway to IPT for those without presumptive TB but who are susceptible to developing disease following recent infection.[7]

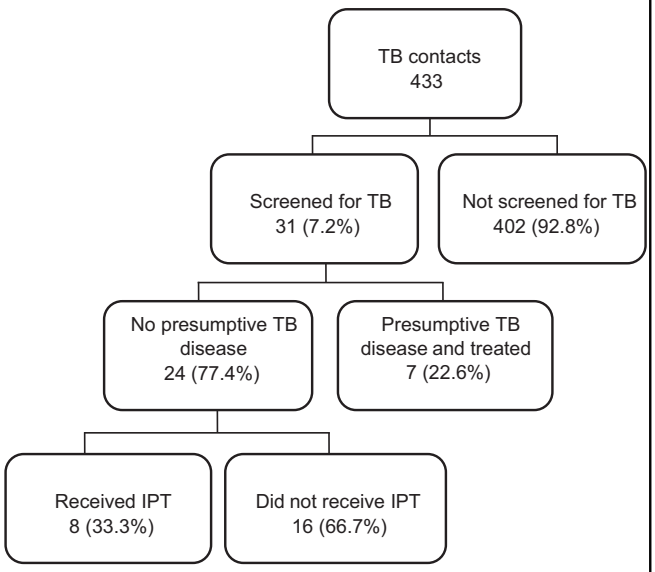
Despite the availability of both WHO and national guidelines, which clearly recommend clinical evaluation of household contacts of SS+ PTB adult index cases, only 31 (7.2%) contact children under 5 of SS+ TB source cases were screened for TB. Our findings are similar to screening rates of 9.0% and 5.5% among TB contact children under 5 in Malawi and Vietnam, respectively.[13,14] The low TB screening rate in our study may have left many children without appropriate interventions, putting them at risk of developing active TB disease.

TABLE 1. Characteristics of Tuberculosis Index Cases in the Mbeya and Songwe Regions, Tanzania

Characteristic	Frequency (n) or median	% or IQR
Age, median	34	28–41
Sex		
Male	136	52.9
Occupation		
Peasant/subsistence farmer	106	41.3
Pet trader	84	32.7
Housewife	18	7.0
Others	49	19.1
Education		
No formal education	23	9.0
Primary education	190	73.9
Secondary education	36	14.0
College education	8	3.1
Walking time to health facility on foot (minutes)		
0 to 30	60	23.4
31 to 60	66	25.7
61 to 90	52	20.2
>90	79	30.7
Number of contacts per household		
1	125	48.7
2	98	38.1
3	25	9.7
4	9	3.4
Relation to contact children		
Mother or father	150	58.4
Guardian/caregiver	107	41.6

Abbreviations: IQR, interquartile range.

FIGURE 1. Contact Screening and Isoniazid Preventive Therapy Initiation among Tuberculosis Contact Children Under 5 in the Mbeya and Songwe Regions, Tanzania



Abbreviations: IPT, isoniazid preventive therapy; TB, tuberculosis.

One-third of the IPT-eligible children – those who were screened and found not to have presumptive TB – were started on therapy. This is lower than proportions reported from studies conducted in Ethiopia (64.3%)[15] and South Africa (68.0%),[16] but higher than what has been observed in Malawi (6.0%).[13] Possible reasons for low IPT uptake in the study area included the limited availability of documentation tools during the study period, which negatively affected the national TB programme's ability to monitor IPT implementation. Contact screening and IPT provision have substantially improved in countries that have introduced IPT registers. For example, in South Africa, the introduction of official documentation forms increased the number of contacts screened per TB patient as well as the proportion of children initiated on IPT.[11] In India, the introduction of an IPT register increased IPT provision from 19.0% to 61.0%.[17] As with the low uptake of contact screening, failure to provide IPT to high proportions – two-thirds in our study – of eligible children may contribute to TB-related morbidity and mortality. In South Africa's Western Cape, for example, a study found that 81.4% of missed opportunities for IPT in at-risk children under 3 years of age who later presented with confirmed TB – 25.0% had disseminated TB and 5.1% died.[18]

Limitations

This study relied solely on patient recall regarding information about contact tracing, TB screening, and IPT administration. Review of IPT provision data tools may have provided a cross-checking mechanism, but unfortunately, these tools were not available in the study sites during the study period.

However, this study has provided important evidence to the Tanzanian NTLP on the low uptake of contact investigation and low IPT provision to eligible TB contact children under 5.

CONCLUSION

The uptake of contact screening and IPT administration among children under 5 observed in this study was low. We advise the NTLP to fully implement the IPT programme and sensitize health-care workers on the importance of contact screening and IPT for those who are eligible. Further research is needed to inform the national TB programme on factors associated with low uptake of contact investigation by health-care workers and the feasibility of implementation of IPT recording tools under the programme setting.

Acknowledgements: The authors thank the patients who volunteered to take part in the study. We acknowledge the support received from the RTLC and DTLC offices and the health-care workers in the TB treatment clinics in the Mbeya and Songwe regions. This research was supported by the Tanzanian Ministry of Health and Social Welfare through an unconditional grant to YKM.

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Peer Reviewed

Competing Interests: None declared.

Received: 11 Mar 2017; **Accepted:** 15 Mar 2018

Cite this article as: Mwasubila YK, Sabi I, Kabyemera R, Ntinginya N, Sauve R, Kidenya B. Tuberculosis Contact Screening and Isoniazid Preventive Therapy Among Children Under 5 in the Mbeya and Songwe Regions, Tanzania. East African Health Res J. 2018;2(1):29-32. https://doi.org/10.24248/EAHRJ-D-17-00186

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Adherence to Exclusive Breastfeeding and Associated Factors in Mothers of HIV-Exposed Infants Receiving Care at Kilimanjaro Christian Medical Centre, Tanzania

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ABSTRACT

Background: More than 90% of HIV in children occurs through mother-to-child transmission. Breastfeeding is one of several factors associated with transmission of HIV, and, because of this, infant feeding practice is one of the cornerstones in the effort to reduce HIV transmission in children. World Health Organization guidelines from 2012 recommend exclusive breastfeeding to all infants for first 6 months of life. However, many factors contribute to the adherence of mothers to exclusive breastfeeding (EBF) recommendations. The aim of this study was to determine what factors likely influence adherence to exclusive breastfeeding in mothers of HIV-exposed infants receiving care at Kilimanjaro Christian Medical Centre.

Methods: A cross-sectional hospital-based study was conducted from September 2012 to March 2013 at Kilimanjaro Christian Medical Centre in Moshi. All mothers of HIV-exposed infants aged 6 to 12 months receiving care at child-centred family care clinic who consented to participate in the study were included. Data were collected using a pretested structured questionnaire and analysed using SPSS version 16.0 statistical package.

Results: Of the 128 mothers of HIV-exposed infants enrolled in the study, 71 (55.5%) adhered to EBF for 6 months. No statistical significance was seen between adherence status and maternal characteristics in bivariate analysis ($P>.05$). The mean age and standard deviation of initiating other foods by mothers who did not adhere was 3.32 months ± 1.24. Of 57 (44.5%) non-adherent mothers, one-tenth of them practised mixed breastfeeding and the rest stopped breastfeeding completely. Fear of postnatal transmission of HIV through breastfeeding and inadequate breast milk production were the most common reasons for non-adherence to EBF.

Conclusion: Adherence to the recommended duration for EBF by mothers to their HIV-exposed infants is still a challenge. Ongoing intensive feeding counselling and education on EBF may increase the number of mother who can adhere to EBF in our society.

BACKGROUND

In 2011, about 330,000 children, approximately 1,000 each day, were newly infected with HIV, more than 90% of whom were from sub-Saharan Africa.[1] Since 2010, significant efforts have been made by prevention of mother-to-child transmission (PMTCT) programmes to reduce the transmission of HIV in children in low- and middle-income countries (LMICs).[2] A recent study in Moshi, Tanzania, using birth registry data from mothers with known HIV status, showed the percentage of infants who tested HIV positive declined from 17.4% in 2000 to 4.0% in 2014,[3] and the proportion of mothers who knew their HIV status at the time of delivery increased from 5.4% in 2000 to 98.8% in 2014.[3]

Globally, over 90% of children acquire HIV through mother-to-child transmission (MTCT) as reported in 2010.[4] Mothers living with HIV, who are not virally supressed, can transmit the virus during pregnancy, labour, delivery, and breastfeeding. Without any intervention, the risk of MTCT is 15% to 45%, while transmission through breastfeeding alone ranges from 5% to

20%. However, with PMTCT interventions the risk of MTCT of HIV reduces to 1% to 15%, while MTCT through breastfeeding reduces risk to less than 5%.[5] The critical component of an effective PMTCT program is infant-feeding counselling and practice. Therefore, a primary focus for reducing MTCT of HIV is through infant feeding practices; this is especially true in LMICs, especially in the breastfeeding communities.[6]

In 2012, the World Health Organization (WHO) recommended exclusive breastfeeding (EBF) of infants for the first 6 months to all mothers, regardless of the HIV status, and emphasized this recommendation for mothers living with HIV.[7] In contrast, mixed breastfeeding (MBF) has a fourfold higher risk of postnatal transmission of HIV compared to EBF.[8] Additionally, replacement feeding is recommended only when it is acceptable, feasible, accessible, sustainable, and safe (AFASS), which is rarely the case in a resource-limited setting.[7] Despite WHO recommendations, with Tanzanian mothers living with HIV, the mean age for cessation of EBF was 3 months.[9,10] A majority of mothers living with HIV who initially choose to practice EBF did not adhere to it for the entire first 6 months, usually reverting to MBF until weaning. This increases the risk of HIV transmission to the infant.[11] For PMTCT programmes, EBF for 6 months before weaning is crucial for reducing risk of HIV infection in infants. Many factors contribute to the adherence of EBF by mothers living with HIV. The 2016 WHO breastfeeding guidelines updates the 2010 guidelines to state that breastfeeding should not to be restricted in settings where health services provide and support lifelong ART and adherence counselling. While emphasizing EBF for 6 months, breastfeeding should be promoted and supported for at least 12 months, and women may continue breastfeeding for up to 24 months or longer, similar to the general population.[12]

The aim of this study was to determine the proportion of mothers living with HIV adhering to EBF for 6 months and the factors that influence adherence to EBF. This information could be used by concerned bodies to improve feeding counselling strategies in order to reduce MTCT of HIV.

METHODS

Study Design

This was a cross-sectional hospital-based analytical study carried out at Moshi municipality in Kilimanjaro region at child-centred family care clinic of Kilimanjaro Christian Medical Centre (KCMC) in the northern zone of Tanzania. Moshi municipality has an estimated population of about 200,000 people. The study was conducted for 6 months – from September 2012 to March 2013. KCMC was selected because of its long-standing PMTCT clinic and experience in feeding counselling.

Study Population

All mothers with HIV-exposed infants aged 6 to 12 months who opted for EBF, were receiving care at the HIV-exposed baby follow-up clinic at KCMC, and gave informed consent to participate in the study were eligible for enrolment. Mothers who opted for alternative feeding (formula feeding or cow's milk) or mixed-feeding options or breastfeeding mothers who did not sign consent for participation were excluded from the study.

Sample Size and Procedure

The minimum sample size was estimated using a formula by the Survey System (Creative Research Systems, Sebastopol, CA, USA) and Joint WHO and DGIS (1988) expressed as sample size (SS) = $Z^2P(100\text{-}P)/\epsilon^2$, 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). A prevalence of 13.3% for EBF was selected based on a study done by Young et al.[10] The minimum estimated sample size was 178 mothers. Of the 157 mothers living with HIV with infants born between October 2011 and September 2012 and received care at KCMC, only 128 opted for EBF and consented to be recruited for this study.

Operational Definitions

At the time of this study, the following WHO definitions were used:[13]

- *Exclusive breastfeeding* is defined as giving ONLY breast milk, except for prescribed medicines, vitamins, and mineral supplements for the first 6 months of life.
- *Mixed breastfeeding* is defined as giving breast milk and ALSO other liquids such as water, tea, formula, cow's milk, or foods such as porridge or rice for the first 6 months of life.
- *Replacement feeding* indicates that the infant NEVER receives breast milk but instead is given a breast-milk substitute, such as commercial infant formula or modified animal milk for the first 6 months of life.

Data Collection Procedure

Data were collected using a structured questionnaire by the principal investigator who also identified the mothers who opted for EBF during the clinic visit. The questionnaire included questions about sociodemographic characteristics, factors that likely influence adherence to EBF, and barriers for non-adherence to EBF, and was pretested for comprehension and accuracy. Respondents were interviewed in Kiswahili language.

Data Processing and Analysis

Data were cleaned, manually entered, and analysed using Statistical Package for the Social Sciences for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). Univariate and bivariate tables and multiple logistic regressions were used to investigate associations between the outcome and different determinants. Data were summarized into frequency tables, bar charts, pie charts, and percentages of different variables related to the outcome. Factors found to be significant in the univariate analysis were included in a full model with all potentially important social covariates and were adjusted for confounders. Finally, the odds ratio (OR) was calculated with 95% CI. A *P* value of <.05 was considered to yield statistical significance.

Ethical Considerations

Ethical clearance was obtained from KCMC Research Ethical Committee. Informed consent was obtained from each mother prior to study participation. All mothers recruited were voluntarily participated in the study and signed an informed consent form. Participation in the study did not change the services rendered to PMTCT mothers in the clinic. Hospital file numbers were used instead of mothers' names to keep the confidentiality of any information provided.

RESULTS

Characteristics of the Study Participants

A total of 128 mothers living with HIV were recruited in this study. The age range of the mothers was 19 to 49 years with a mean ± standard deviation (SD) of 31 ± 6 (Table 1) and the mean age ± SD of their infants during interview was 8.2 ± 2.5 months. All mothers had formal education, 87 (68%) of whom had received up to primary education. About three quarters (n=93, 72.7%) were Christians and the rest were Muslims. Of the study population, over half (n=72, 56.3%) were from urban areas, and the rest from the rural areas. A majority (n=107, 83.6%) lived in nuclear family – defined as a mother, father and their own children – and the rest lived in a joint family – defined as an extended family consist of more than 1 family or generation. Over half (n=66, 51.6%) were self-employed, about two-fifths (n=51, 39.8%) were housewives, and the remaining were employed. Almost three-fifths (n=74, 57.8%) of the participants reported their monthly income to be less or equal to 150,000 Tanzanian shillings (<US$100) and the remaining reported a monthly income of more than 150,000 Tanzanian shillings. About 46 (35.9%) of the respondents indicated that they were the sole income generators for their families, and 44 (34.4%) said they generated income with their partners. A majority (n=89, 69.5%) of the mothers interviewed were either married or cohabiting, almost a quarter (n=23, 18.0%) were single, and the remaining were either divorced or separated. More than three quarters (78.1%) were multiparous – having had multiple births – with a mean number of 3 infants.

Other HIV-Related Concerns

Among the 100 multiparous mothers, 26.0% had another child infected with HIV. Of the 128 total respondents, the majority (89.1%) had had at least 4 antenatal care (ANC) visits. Almost three-quarters (n=94, 73.4%) of the mothers said they learned about their HIV status during the current child's pregnancy; only 16 (17.0%) of whom knew their status before they became pregnant, while others found out during previous pregnancies. All mothers delivered at a health facility. Of 128 infants, 113 (88.3%) were born with normal birth weight, while the remaining 15 (11.7%) were born low birth weight (Table 1).

HIV-Status Disclosure

Of the 128 respondents, 118 (92.2%) had disclosed their HIV status: 95 (80.5%) to their spouses, 75 (63.6%) to their close relatives, and only 2 (1.7%) to their friends (Figure 1). Fifty-four (45.7%) of 118 mothers indicated that they had disclosed to more than 1 group of people. A majority (89.0%) of the mothers stated they disclosed before this birth and only 13 (11.0%) disclosed after birth of the last child.

Feeding Counselling During Antenatal and Postnatal Care Visits

Among 128 mothers, all but 3 had received feeding counselling at any stage during pregnancy and after delivery. About three-quarters (n=94, 75.2%) received counselling at all 3 stages – antenatal, perinatal, and postnatal periods, while 19 (14.8%) received counselling during 2 of the stages and the remaining 12 (9.4%) received counselling at only 1 stage (Figure 2). Over a quarter (27%) of those married or cohabiting reported that their partners attended the counselling sessions. More than 85% reported that they had discussed EBF with their partner and that the partner agreed to support EBF. More than half (n=69, 53.9%) reported that the decision to do EBF was solely theirs (Figure 3), while others were influenced by their spouse (n=42, 32.8%), health-care provider (n=17, 13.3%), family members (n=5, 3.9%), or mother-in-law (n=1, 0.8%). Of the 59 mothers influenced by other people to choose EBF, 5 were influenced by more than 1 person.

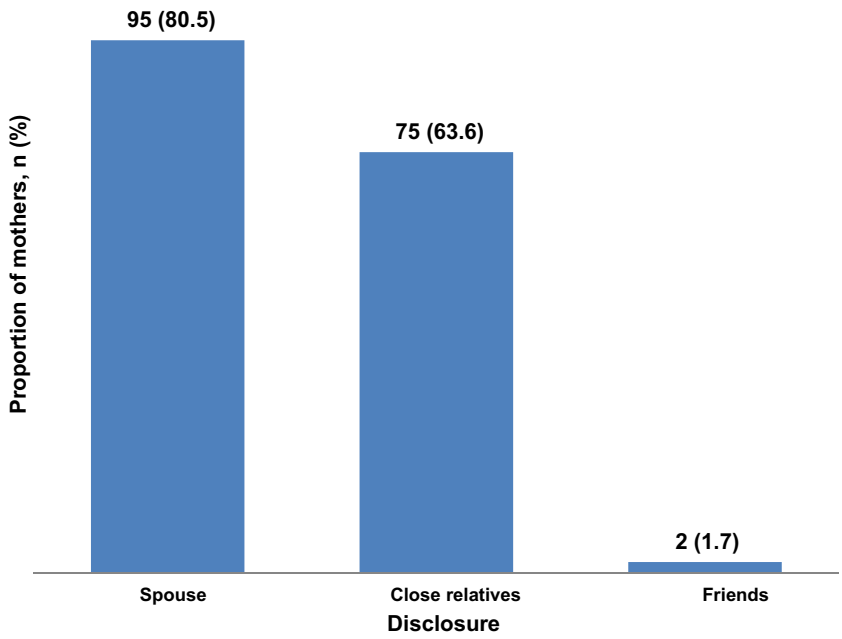
Adherence to Exclusive Breastfeeding

Among the total 128 respondents, 71 (55.5%) reported they exclusively breastfed their children for the first 6 months of their child's life. No maternal characteristics showed a significant association with adherence status to EBF. Mothers who were the sole source of family income were more likely to adhere compared with those mothers who were dependent on spouses and relatives for their daily needs. This was the

TABLE 1. Characteristics of Study Participants (N=128)

Variable	Attribute	n (%)
Mother's age (years)	Mean (±SD, range)	31 (±6, 19–49)
	18–25	22 (17.2)
	26–35	79 (61.7)
	36–49	27 (21.1)
Child's age (months)	Mean (±SD, range)	8.2 (±2.5, 6–12)
	6–9	86 (67.2)
	10–12	42 (32.8)
Mother's parity	Mean (±SD, range)	3 (1, 1–7)
	Primipara	28 (21.9)
	Multipara	100 (78.1)
Mother's education	Up to primary	87 (68.0)
	Secondary	36 (28.1)
	Above secondary	5 (3.9)
Mother's marital status	Single	23 (18.0)
	Married/co-habiting	89 (69.5)
	Divorced/separated	16 (12.5)
Mother's occupation	Formal employment	11 (8.6)
	Self-employment	66 (51.6)
	Not employed/housewife	51 (39.8)
Self-reported monthly income (T.Shs)	Up to 150,000	74 (57.8)
	More than 150,000	54 (42.2)
Type of family	Nuclear	107 (83.6)
	Joint	21 (16.4)
Main income provider	Self	26 (20.3)
	Husband	46 (35.9)
	Husband + self	44 (34.4)
	Other family member	12 (9.4)
Religion	Christian	93 (72.7)
	Muslim	35 (27.3)
Residence	Rural	56 (43.7)
	Urban	72 (56.3)

FIGURE 1. Disclosure of HIV Status to Any Person (N=118)



Note: Almost half (n=54; 45.7%) disclosed to more than one group (eg, spouse and relative), therefore, the numbers exceed 100% in total.

only factor showed statistical significance (*P*=.014) in univariate but not in bivariate analysis (Table 2).

Almost half (n=57, 44.5%) of infants were switched to other forms of feeding before the age of 6 months. The mean age ± SD was 3.32 months ± 1.24. Of the 57 infants, 52 (91.2%) stopped breastfeeding completely, while the remaining received MBF. Of the 52 infants who were stopped breastfeeding completely, 41 (78.8%) were given cow's milk and maize/millet porridge and 11 (21.2%) were given formula milk alone or with porridge.

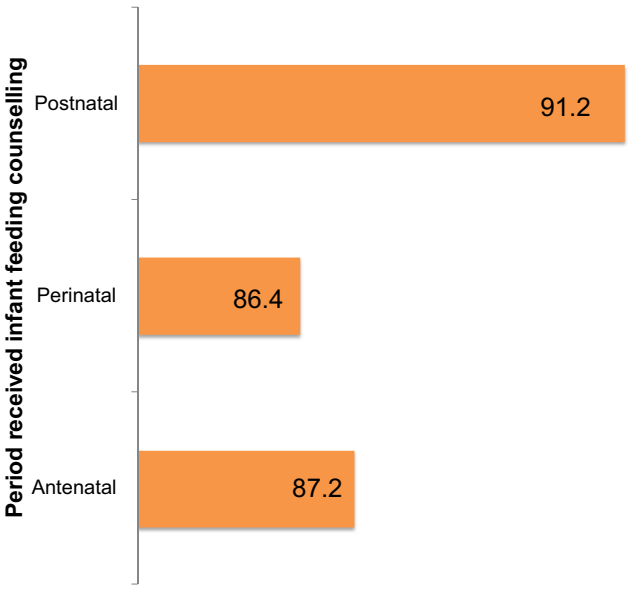
Barriers for Non-Adherence to Exclusive Breastfeeding

Barriers to adherence to EBF were divided into mother-, infant-, and community-related factors. Mother-related barriers for non-adherence of EBF were the most common (91.2%). Five of the most common responses by the mothers were: fear of HIV transmission to the infant through breastfeeding (67.3%), inadequate breast milk production (48.1%), mother had planned to breastfeed for 3 months only (23.1%), mother had to resume/go back to work (19.2%), and mother wished to stop after the first polymerase chain reaction (PCR) result was negative (15.4%) in order to avoid further exposure to breast milk. The most common infant-related responses given by study participants were increased demand of milk by the infant (46.1%) and 38.5% complete refusal of breast milk. Only 5 (8.8%) of the study participants gave community-related reasons, the most common of which was the tradition of early weaning (n=3, 60.0%) (Figure 4).

DISCUSSION

In this study, 55.5% of mothers living with HIV adhered to EBF for the first 6 months. This timeframe was recommended by both WHO and the Tanzania Ministry of Health and Social Welfare (MOHSW) for feeding HIV-exposed infants.[7,14] This finding was lower compared to those reported in Ethiopia (83.7%) and Nigeria (68.3%)[15,16]; and higher than those in India (44.0%), Guinea (15.5%), Uganda (24.0%), and Dar es Salaam, Tanzania (13.3%).[10,17–20] The difference between this finding and that of the Dar es Salaam study can be explained by the involvement of several health facilities in the latter study. Information provided during feeding counselling may have differed at each health facility, depending on the counsellors' experiences, which may have contributed to the lower prevalence of EBF.[10] The higher rate of

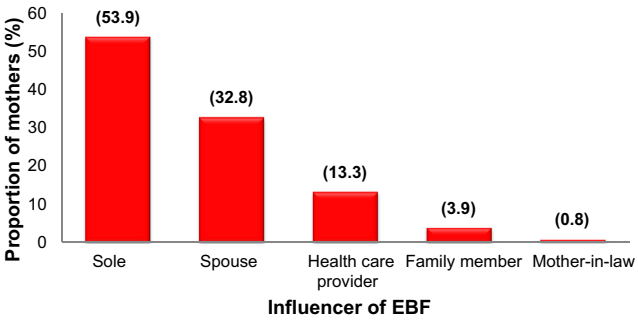
FIGURE 2. Infant-Feeding Counselling to Mother at Different Periods of Care (N=125)



Note: The percentages/proportion exceed 100% in total as some mothers received counselling in more than one period.

EBF in our site may also be explained by the support provided during the frequent follow-up visits by ongoing counselling to mothers on infant-feeding practice done by counsellors at our centre. The lower proportion in India could be due to higher non-disclosure to partners and relatives and insufficient breast milk production, forcing mothers to wean early. In Uganda, the difference may be due to the tradition of prelacteal feeding – before breastfeeding is established – and the early introduction of staple foods, which was not the case in our study. The higher proportion in Ethiopia and Nigeria could be due to the study design comparing EBF with replacement feeding. The differences in adherence could also be explained by different social and cultural factors related to infant feeding.

FIGURE 3. Person With Influence on Decision to Practice Exclusive Breastfeeding (N=128)



Abbreviation: EBF, exclusive breastfeeding.

In our study, the mean age for stopping EBF for mothers who did not adhere was 3.32 months. This was similar to that reported by Leshabari et al. and Young et al. in Tanzania.[9,10] However, it was higher than the 1.8 months reported in a 2004 nationally general population representative sample[21] and the 2.4 months in 2010 general population representative sample.[22] This addresses that mothers still need more support and more adequate feeding counselling in order to follow the national recommendations of EBF for 6 months. The drop off by study participants after 3 months could be due to mothers resuming work after the national maternal leave of 84 days or that the mothers initially only planned to breastfeed for 3 months.

One-tenth of the mothers in our study who did not adhere to EBF practiced MBF and the remaining had stopped EBF completely. MBF is an unsafe mode of feeding and has shown to increase the risk of HIV infection fourfold compared to EBF.[8] The proportion of MBF was lower than that reported in India (29.0%) and Zimbabwe (68.6%), and in a qualitative study conducted in the Kilimanjaro region with a small sample size involving only 13 mothers (100.0%).[8,9,17] Our proportion was similar to that reported in Ethiopia and South Africa.[16,23] The higher proportion in Zimbabwe could possibly be due to cultural norm to introduce liquids and solid foods very early life. In India, those mothers who practiced MBF may have done so because of insufficient breast milk, non-disclosure to their partners and relatives due to social repercussions, or their decision to change the mode of feeding. The lower proportion in this study may indicate that mothers might be provided with adequate knowledge on risks of MBF during feeding counselling in our centre.

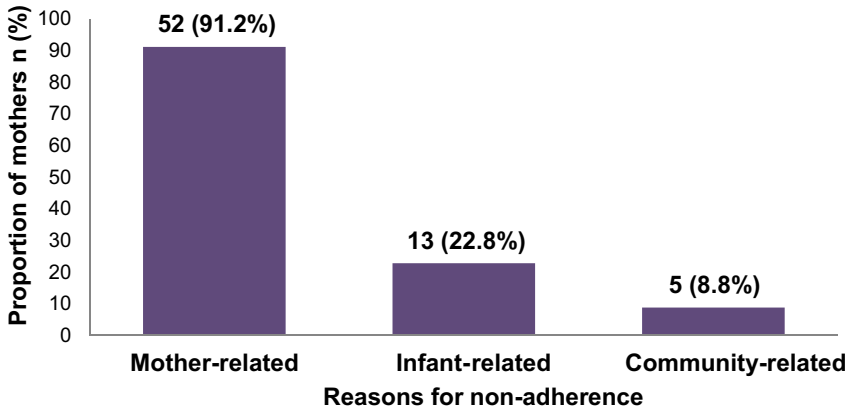
While we acknowledge the low rate of MBF, some mothers stop breastfeeding completely before their infants are 6 months of age. Early cessation of breastfeeding can lead to serious gastroenteritis, which usually peaks at 3 to 4 months of life and is more severe if stopped during this period than when stopped later in life.[19] In Tanzania, predictors of breastfeeding among mothers living with HIV in Dar es Salaam had shown that introduction of cow's milk at 4 months of age and becoming pregnant again contributed significantly to early cessation of breastfeeding.[20] The tight clustering of stopping breastfeeding completely likely also reflects the effects of infant-feeding counselling. Material and social support can also contribute to reducing the number of mothers who stop breastfeeding earlier.

TABLE 2. Selected Maternal Characteristics and Their Adherence Status With Exclusive Breastfeeding (N=128)

Variable	Total	Adherence Status		*P* Value	OR (95% CI)
		Adherent n (%)	Non-adherent n (%)		
Age (years)					
25 or younger	22	12 (54.5)	10 (45.5)		
Older than 25	106	59 (55.7)	47 (44.3)	.924	1.0 (0.4–2.4)
Education level					
Up to primary	87	47 (54.0)	40 (46.0)		
Post-primary	41	24 (58.5)	17 (41.5)	.632	0.8 (0.4–1.8)
Marital status					
Single/Divorced	39	25(64.1)	14 (35.9)		
Married/Cohabiting	89	46 (51.7)	43 (48.3)	.193	1.7 (0.8–3.6)
Occupation					
Formally employed	11	7 (63.6)	4 (36.4)		
Not formally employed	117	64 (54.7)	53 (45.3)	.754	1.4 (0.4–5.2)
Place residence					
Rural	56	31 (55.4)	25 (44.6)		
Urban	72	40 (55.6)	32 (44.4)	.982	1.0 (0.5–2.0)
Parity					
Primipara	28	14 (50.0)	14 (50.0)		
Multipara	100	57 (57.0)	43 (43.0)	.510	0.8 (0.3–1.7)
Family income generation					
Self	26	20 (76.9)	6 (23.1)		
Others	102	51 (50.0)	51 (50.0)	.014*	3.3 (1.2–9.0)
Income per month (T-Shs)					
Up to 150,000	74	46 (62.2)	28 (37.8)		
More than 150,000	54	25 (46.3)	29 (53.7)	.074	1.9 (0.9–3.9)
Type of Family					
Nuclear	107	62 (57.9)	45 (42.1)		
Joint	21	9 (42.9)	12 (57.1)	.203	1.8 (0.7–4.7)
Number of antenatal visits					
Less than 4	13	7 (53.8)	6 (46.2)		
At least 4	115	64 (55.7)	51 (44.3)	.902	0.9 (0.3–2.9)
HIV status of siblings (n=100)					
Infected	26	11 (42.3)	15 (57.7)		
Not infected	74	46 (62.2)	28 (37.8)	.079	0.4 (0.2–1.1)
Counseling status					
Inadequate	19	13 (68.4)	6 (31.6)		
Adequate	109	58 (53.2)	51 (46.8)	.218	1.9 (0.7–5.4)
Disclosure status					
Not disclosed	10	6 (60.0)	4 (40.0)		
Disclosed	118	65 (56.1)	53 (44.9)	1.000	1.2 (0.3–4.6)
Influence to choice of EBF					
Own	69	41 (59.1)	28 (40.6)		
Other person's	59	30 (50.8)	29 (49.2)	.331	1.4 (0.7–2.9)

Abbreviations: CI, confidence interval; EBF, exclusive breastfeeding; OR, odds ratio.

FIGURE 4. Barriers for Non-Adherence to Exclusive Breastfeeding (N=57)



Note: Total exceeds 100% as some of the mothers reported more than one barrier.

Surprisingly, maternal demographic characteristics were not statistically significant with adherence to EBF. Olandokun et al. in Nigeria had also seen no association between maternal characteristics and infant-feeding choices and practices.[18] The association between mothers characteristics and infant-feeding patterns could have been neutralized by infant-feeding counselling at our centre or they may have been significant when mothers opted for replacement feeding, as reported in Ethiopia, because breastfeeding is norm in Africa.[16] Mothers who were the sole income generators for the family adhered to EBF compared to mothers who were dependent on spouse and relatives. In Ethiopia, mothers who were daily labourers and generated income on their own were more likely to choose and adhere to feeding option in a recommended way.[16] This higher level of EBF by sole earners may be due to the power these mothers have on make decisions on whether to adhere to EBF or not. In Nigeria, mothers who were more than 31 years of age adhered to the feeding option chosen and was proven significant.[15] However, they only compared 2 modes infant feeding, and EBF was not an option.

Maternal characteristics were not significant but those who had post-primary education; were single or divorced, formally employed, multiparous, or living in a nuclear family; and whose monthly income was up to 150,000 Tanzanian shillings, were more adherent to EBF. This could be because mothers with a post-primary education or higher may have more easily understood the concept of EBF. The single or divorced group had good adherence, which may be explained by the fact that they make decisions on their own and not dependent on spouses. Multiparous mothers were more adherent; this could be due to their maturity and past experience. Those living in nuclear family were more adherent than those living in a joint family. This difference may be due to non-nuclear family pressures to add other foods in early life and to families deeply involved in culture and tradition.

Disclosure of HIV status had also shown no significance with adherence to EBF. While those who disclosed in Ethiopia and Nigeria were more likely to practice the recommended feeding option,[15,16] this may be because in both studies some mothers had opted for replacement feeding. In Burkina Faso, partners of mothers who opted for replacement feeding and had disclosed were seen to support the mothers by making false statements, like having lesions on the breast to the community, if questioned why they were not breastfeeding.[24] Disclosure status may, however, have no association with breastfeeding because breastfeeding is a norm in Africa.

Although the study did not set out to test the effectiveness of infant-feeding counselling status, counselling on feeding and adherence status had no significance to EBF; however, the proportion of mothers who adhered to EBF in our study was good, with low proportion of MBF. In Nigeria, mothers who had more than 1 counselling session during ANC adhered more to the feeding option, however, that study only compared 2 modes of feeding.[15] This indicates that the more ANC visits the mother makes the more likely she is to adhere to the mode of feeding chosen. This is

because they get enough time to discuss their concerns with the counsellors. The impact of EBF counselling programs for African mothers living with HIV should consider individual maternal, social, and health contexts. Therefore, counsellors at ANC clinics play a major role in infant-feeding counselling, particularly EBF. The Tanzania MOHSW and implementing partners have done much to strengthen the infant-feeding counselling aspect of PMTCT services.[6] However, a large gap still remains in achieving adequate number and trained counsellors especially at ANC facilities.[25] This gap is a concern for Tanzania as well as sub-Saharan Africa.

The 5 most common mother-related reasons given by the study participants were fear of transmitting HIV through breastfeeding, poor or inadequate milk production, mother had planned during ANC visit to feed for 3 months only, mother had to resume/go back to work after maternal leave, and mother chose to stop breastfeeding once she found that the infant's first PCR was negative. In Ethiopia, insufficient breast milk was found to be significant in practising feeding in a recommended way.[15] Similar reasons were reported in Zimbabwe, where the primary barrier to mothers living with HIV continuing breastfeeding for the entire 6-month period was to protect their child from HIV infection. They worried less about infant health and survival associated with early cessation of EBF. Other reasons for stopping EBF were negative PCR results at 6 months of age and poor nutrition of the mother, which was indirectly related to inadequate milk production.[26] However, there was no significance to HIV-free survival of infants of mothers who stopped EBF completely at 4 months compared to those who continued EBF and then complementary feeding.[27] WHO recommends EBF to all mothers, regardless of the HIV status, and with continued breastfeeding for 2 years.[7] These recommendations were also emphasized in the 2016 WHO breastfeeding guidelines, especially where counselling on adherence and provision of ARVs is provided.[12] When counselling mothers living with HIV, it is important to identify barriers that could mitigate adherence to EBF.

Other common reasons reported by different countries in Africa for not adhering to EBF are: mother's level of education, mother always involved in household activities, stigma of HIV, local culture and/or traditions, and family pressure to add other foods, especially traditional gruels.[9,15,24,26] In the current study, education was the least among the reasons followed by culture and/or tradition. This may be because a higher percentage of mothers had at least a primary education, which is completed at ages 13 to 15 years, and the mothers in this study were ages 19 years or older. Community-related barriers were mentioned by few mothers, this may be because most of the mothers in this study were living in a nuclear family, which usually means decisions are made by a woman and her husband, without pressure from mothers-in-law and other relatives.

Limitations

There were few important limitations to this study. This was a hospital-based study in a tertiary-care hospital where counselling is expected to be of good quality, which might limit the generalization. Also, recall bias may be a possibility because infant-feeding information depended on mother self-reporting. However, we suspect majority of mothers could recall duration of exclusive breastfeeding and barriers associated accurately since the study enrolment was done while they were still practicing breastfeeding. The study did not perform assessment of the AFASS criteria to determine the appropriateness of replacement feeding in the infants who were never breastfed.

CONCLUSION

Many of the mothers living with HIV who participated in our study did not adhere to recommendations to exclusively breastfeed their uninfected yet HIV-exposed infants for 6 months after birth. About 45.0% of the participants indicated that they did not adhere to recommended duration of EBF. Of this group, one-tenth practised MBF and the rest stopped breastfeeding completely. Of the reasons for non-adherence to EBF extrapolated from study participants, the most common reasons were mother related: fear of postnatal transmission of HIV through breastfeeding and poor milk production were the most common responses given. To support the 2016 breastfeeding guidelines, more emphasis should be put on mothers living with HIV to exclusively breastfeed their infants for the first 6 months of life and to continue breastfeeding while introducing complementary feeding.

As a result of our research, we suggest that more effective and intensive feeding counselling and additional psychological counselling and education on the risks of early cessation of EBF should be provided to mothers and their partners throughout pregnancy and after delivery. Further longitudinal research is needed to see the outcomes of HIV naive or positive mothers who do or do not adhere to EBF.

Acknowledgements: We acknowledge the Tanzania MOHSW for funding the research. We thank Professor Ben Hamel, Dr. Kweka, Dr. Amos Nsheha, Dr. Mohammed Salehe, and all other paediatric specialists and residents of KCMC for their support and contributions relevant to drafting and reviewing the manuscript. We would also like to thank Mr. Gibson Kapanda for making the database and assisting in data analysis. We thank the health institution, KCMC, for allowing research data collection and, finally, with a grand salute to all mothers who participated in this study and their children.

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Peer Reviewed

Competing Interests: None declared.

Received: 30 Oct 2016; **Accepted:** 26 Oct 2017

Cite this article as: Husain Rasheed M, Philemon R, Damas Kinabo G, Maxym M, Mamuu Shayo A, Theophil Mmbaga B. Adherence to Exclusive Breastfeeding and Associated Factors in Mothers of HIV-Exposed Infants Receiving Care at Kilimanjaro Christian Medical Centre, Tanzania. East African Health Res J. 2018;2(1):33-42. https://doi.org/10.24248/EAHRJ-D-16-00365

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

The Novelty of Inhalable Medications: Interest, Use, and Impact of Inhalant Medications in Low-Resource Settings

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ABSTRACT

Introduction: Postpartum haemorrhage (PPH) claims more than 100,000 maternal lives annually worldwide, most of them in low-resource settings. To address morbidity and mortality from PPH, the global health community is exploring novel drug formulations, such as inhalable medicine, to improve treatment availability and use, especially in community settings with limited access to skilled birth attendants. A major limitation in the ability to prevent or treat PPH in resource-limited settings is that the most effective medications for prevention and treatment are injectables, which require administration by skilled birth attendants.

Methodology: We conducted formative research, including online surveys and in-person interviews, with a range of providers across a variety of health-care settings in Guatemala, Indonesia, Kenya, and Nigeria, to better understand the standard of care for mothers and newborns in low-resource settings, including care practices related to PPH.

Results: It is estimated that up to 40% of PPH deaths could be averted if an inhalable prevention and treatment were available. However, survey and interview respondents noted a desire for more intravenous and oral medicinal formulations over inhalable formulations.

Discussion/Conclusion: Lack of knowledge and use of inhalable medicines among these health workers illuminates key challenges to introducing novel formulations in low-resource settings.

INTRODUCTION

Postpartum Haemorrhage in Context in Low- and Middle-Income Countries

Each year, postpartum haemorrhage (PPH) claims the lives of more than 100,000 mothers worldwide.[1] Most of these maternal deaths occur in low-resource settings at the community level, where it is challenging to prevent and treat PPH due to the lack of adequate health-care infrastructure, long distances from care, and technologies not available in or not appropriate for low-resource settings.[2] In a World Health Organization (WHO) systematic analysis on maternal mortality, regional PPH-related mortality was estimated at 13.3% (n=9,200) in Latin and Central America, 15.2% (n=200,000) in sub-Saharan Africa, and 23.1% (n=34,000) in Southeastern Asia.[3]

Most low- and middle-income countries (LMICs) follow WHO recommendations for PPH prevention and treatment guidelines. Intravenous or intramuscular oxytocin for prevention and intravenous oxytocin for treatment of PPH is the WHO-recommended uterotonic.[4] In cases where oxytocin is not available, misoprostol or injectable uterotonics are recommended as alternative treatments.[4]

Initial Challenges: Unmet Need and Barriers

In its current formulation, oxytocin is included in treatment guidelines and is considered the standard of care for PPH; however, it is often not available in resource-limited settings where injection is not possible.[5] A study by Smith et al surveyed 37 countries about the inclusion of oxytocin on their Essential Medicines List (EML) and distribution of oxytocin.[5] Of the countries surveyed, 4 reported not having oxytocin regularly available in facilities and only Equatorial Guinea did not include oxytocin on its EML for PPH prevention and treatment.[5] Unfortunately, despite the availability of oxytocin to health-care providers within a country, it is often not available or cannot be used appropriately in community settings where many births occur. This unmet need

persists throughout sub-Saharan Africa, where only 50% of births are facility-based.[6]

In low-resource settings, providing care for women suffering from PPH can be challenging for myriad reasons. including numerous barriers to care-seeking and limited skills of providers. For women in LMICs, obstacles to care-seeking and the timely effective treatment of PPH include the decision to deliver at home instead of a health-care facility.[7]

Formulations of Oxytocin Under Development

Because oxytocin requires a refrigerated environment and administration by trained providers, women who give birth in community settings are unlikely to receive the medication when they need it.[8] This unmet need for PPH treatment has spawned innovative approaches to bring oxytocin to communities in a form that can be administered by the local health workforce, many of whom lack formal training. The United Nations Commission on Life-Saving Commodities for Women and Children reports several examples of potential product innovations, including temperature monitoring devices for oxytocin packaging; heat-stable formulations; pre-loaded, single-use injection devices for use by lower-skilled cadres of health-care providers; and non-parenteral inhalation/intranasal spray-dried (dry powder) formulations.[9]

The inhalant form of oxytocin that is under development is described as an innovative, heat-stable, and low-cost form of oxytocin that could help manage PPH treatment in resource-poor settings.[10,11] Such a novel approach is needed for women who do not deliver in facilities that have appropriate interventions for PPH.

METHODS

We conducted a small-scale, mixed-methods study of maternal and neonatal health care in 4 LMICs—Guatemala, Indonesia, Kenya, and Nigeria. The mixed-methods approach included a comprehensive literature review, an online quantitative survey and qualitative interviews with health-care providers from the target countries, and mathematical modelling to assess the impact of maternal and neonatal health interventions. For the purposes of this paper, we will focus on the availability, practices, and preferences regarding PPH prevention and treatment that we explored in the study.

Data sources, including national demographic and health surveys, grey literature, and peer-reviewed journals, were derived from a literature review in PubMed using a publication date range of 2005 to 2016, with search terms: 'postpartum haemorrhage', 'inhalant medication', 'inhalable medication', and 'oxytocin' combined with 'low-resource' and the countries included in the study. We used a snowball methodology to review additional data sources cited by articles in our initial review.

The original survey was developed in English and then translated into Spanish and Bahasa Indonesia for the Guatemala and Indonesia sites, respectively. The survey was piloted with 1 or 2 practitioners in each country to ensure the appropriate translation of each question and the functionality of the survey platform. The feedback provided from these pilot surveys was incorporated into the final version of each country survey. Across the 4 sites, health-care providers were identified for the online survey through convenience sampling; respondents represented the variety of providers – doctors, nurses, midwives, and traditional birth attendants – who care for pregnant women. By country, the final number of respondents was: Guatemala (n=11), Indonesia (n=11), Kenya (n=10), and Nigeria (n=10). The number of respondents for this small-scale study was determined as a number both sufficient to inform a product development strategy and practical within study constraints. For geographic context, the respondents from Guatemala represent semi-urban health facilities near Guatemala City, while respondents from Kenya are from rural western Kenya. The Nigerian practitioners are from the Kano area, representing a semi-urban region, and the Indonesian respondents are also mainly from an urban area. We sought a broad range of feedback from across the health provider spectrum, as noted in the characteristics of the survey respondents provided in Table 1.

Prior to launching the qualitative interviews, the interview questionnaire, which included scripted questions, was sent to all sites for translation, feedback, and clarification. Each qualitative interview was conducted in person with individual health-care providers from each country. Many of the qualitative interview participants also contributed as survey respondents, including 5 providers from Guatemala, 1 provider from Kenya, and 4 providers from Nigeria. The final number of interview respondents by country was: Guatemala (n=5), Indonesia (n=4), Kenya (n=4), and Nigeria (n=5). Table 2 provides the characteristics of the interview respondents. The data collected during the interviews were analysed through content analysis.

The PPH portion of the survey focused on questions related to available treatments for the third stage of labour, preferred formulations, refrigeration availability in facilities, and the use of inhaled delivery of medications for any cause (see Appendix A for the full set of PPH-related survey questions). Further, through qualitative interviews, we explored the barriers associated with inhalable medicine across a variety of health-care providers. The PPH-related interview question is also included in Appendix A.

As part of the PPH treatment analysis we modelled the potential impact of inhalant oxytocin using the Maternal and Neonatal Directed Assessments of Technology (MANDATE) model, an interactive web-based decision-tree mathematical model. MANDATE, developed by RTI with support from the Bill and Melinda Gates Foundation, informs the development of appropriate and effective

TABLE 1. Characteristics of Survey Respondents

Country	Profession	Average Years of Experience	Type of Facility
Guatemala (n=11)	Doctor (5) Nurse (4) Traditional birth attendant (2)	16–20 years	Public hospital (4) Health clinic (2) Health area (2) Patient's home/village setting (2)
Indonesia (n=11)	Doctor (5) Nurse (3) Midwife (3)	16–20 years	Public hospital (1) Health clinic (10)
Kenya (n=10)	Doctor (4) Nurse (5) Midwife (1)	6–10 years	Public hospital (9) Health clinic (1)
Nigeria (n=10)	Doctor (1) Nurse (3) Midwife (1) Community health worker (5)	10 years	Public hospital (7) Health clinic (2) Tertiary institution (1)

TABLE 2. Characteristics of Interview Participants

Country	Profession	Average Years of Experience	Type of Facility
Guatemala (n=5)	Doctor (3) Obstetrician-gynaecologist (1) Nurse (1)	10 years	Hospital (4) Health centre (1)
Indonesia (n=4)	Doctor (2) Midwife (2)	15 years	Health centre (4)
Kenya (n=4)	Doctor (2) Nurse/midwife (1) Clinical officer (1)	9 years	Hospital (4)
Nigeria (n=5)	Doctor (1) Nurse/midwife (2) Nurse (1) Community health extension worker (1)	14 years	Hospital (2) Public health facility (2)

technologies to improve maternal and neonatal health care in low-resource settings (www.mnhtech.org). Using this baseline model, researchers can evaluate theoretical scenarios that improve baseline data across prevention, diagnostic, transfer, and/or treatment data, resulting in lives saved across care settings. Baseline assumptions for sub-Saharan Africa are outlined in Table 3. The inputs of the MANDATE model and the current estimates of penetration (availability of an intervention), utilization (appropriate use of an intervention), and efficacy (ability of an intervention to successfully prevent, treat, or diagnose a condition) can be used to run scenarios to examine the impact of the improvement of an intervention or a package of interventions in 1 or more care settings.[12]

RESULTS

Modelling Potential Impact

We ran a series of MANDATE scenarios for sub-Saharan Africa to assess the number of maternal lives saved through the scale up of an inhalable form of oxytocin for PPH prevention and treatment. The results are summarized in Table 4. The first scenario shows that people – across all settings – who do not currently have access to (penetration) or use

TABLE 3. Baseline Assumptions for Model

Sub-Saharan Africa 2020 Baseline Assumptions				
Intervention	**Metric**	**Home**	**Clinic**	**Hospital**
Prevention Interventions				
Uterine massage to prevent PPH	Penetration	50%	95%	100%
	Utilization	35%	40%	45%
	Efficacy	40%	40%	40%
Oxytocin to prevent PPH	Penetration	0%	60%	80%
	Utilization	0%	65%	65%
	Efficacy	50%	50%	50%
Misoprostol to prevent PPH	Penetration	5%	15%	20%
	Utilization	55%	65%	65%
	Efficacy	43%	43%	43%
Drape (to determine blood loss)	Penetration	0%	0%	1%
	Utilization	0%	0%	10%
	Efficacy	92%	92%	92%
Recognize PPH	Penetration	50%	0%	0%
	Utilization	60%	0%	0%
	Efficacy	60%	60%	60%
Clinical diagnosis of PPH	Penetration	0%	85%	90%
	Utilization	0%	80%	90%
	Efficacy	0%	70%	70%
Recognize retained placenta	Penetration	50%	0%	0%
	Utilization	15%	0%	0%
	Efficacy	70%	70%	70%
Clinical diagnosis of a etiology of PPH (lacerations, atonic uterus, retained placenta)	Penetration	0%	85%	90%
	Utilization	0%	65%	80%
	Efficacy	0%	85%	85%
Ultrasound for retained placenta	Penetration	0%	0%	25%
	Utilization	0%	0%	10%
	Efficacy	85%	85%	85%
Manual removal of retained placenta	Penetration	30%	75%	99%
	Utilization	30%	40%	45%
	Efficacy	70%	70%	70%

Continued

TABLE 3. Continued

Sub-Saharan Africa 2020 Baseline Assumptions				
Intervention	**Metric**	**Home**	**Clinic**	**Hospital**
D&C, manual removal	Penetration	0%	30%	70%
	Utilization	0%	30%	40%
	Efficacy	90%	90%	90%
Surgery for retained placenta	Penetration	0%	0%	75%
	Utilization	0%	0%	50%
	Efficacy	92%	92%	92%
Recognize lacerations	Penetration	50%	0%	0%
	Utilization	15%	0%	0%
	Efficacy	70%	70%	70%
Treatment Interventions				
Clinical diagnosis of a etiology of PPH (lacerations, atonic uterus, retained placenta)	Penetration	0%	85%	90%
	Utilization	0%	65%	80%
	Efficacy	0%	85%	85%
Suturing	Penetration	30%	80%	95%
	Utilization	50%	70%	95%
	Efficacy	90%	90%	90%
Surgery – lacerations	Penetration	0%	0%	75%
	Utilization	0%	0%	50%
	Efficacy	92%	92%	92%
Recognize atonic uterus	Penetration	50%	0%	0%
	Utilization	15%	0%	0%
	Efficacy	15%	15%	15%
Clinical diagnosis of a etiology of PPH (lacerations, atonic uterus, retained placenta)	Penetration	0%	85%	90%
	Utilization	0%	65%	80%
	Efficacy	0%	85%	85%
Ultrasound – atonic uterus	Penetration	0%	0%	25%
	Utilization	0%	0%	10%
	Efficacy	85%	85%	85%
Uterine massage to treat PPH	Penetration	50%	95%	100%
	Utilization	35%	60%	80%
	Efficacy	40%	40%	40%

Continued

TABLE 3. Continued

Sub-Saharan Africa 2020 Baseline Assumptions				
Intervention	**Metric**	**Home**	**Clinic**	**Hospital**
Oxytocin to Treat PPH	Penetration	0%	60%	80%
	Utilization	0%	65%	65%
	Efficacy	50%	50%	50%
Misoprostol to Treat PPH	Penetration	5%	15%	20%
	Utilization	55%	65%	65%
	Efficacy	43%	43%	43%
Balloon tamponade	Penetration	0%	0%	0%
	Utilization	0%	0%	0%
	Efficacy	0%	0%	0%
Surgery – atonic uterus	Penetration	0%	0%	75%
	Utilization	0%	0%	50%
	Efficacy	92%	92%	92%
Recognize severe haemorrhage	Penetration	50%	0%	0%
	Utilization	70%	0%	0%
	Efficacy	80%	0%	0%
Clinical diagnosis of severe haemorrhage	Penetration	0%	85%	90%
	Utilization	0%	90%	99%
	Efficacy	0%	95%	95%
Fluid resuscitation	Penetration	0%	50%	99%
	Utilization	0%	70%	80%
	Efficacy	5%	5%	5%
Blood replacement and transfusion	Penetration	0%	5%	45%
	Utilization	0%	50%	80%
	Efficacy	95%	95%	95%
Impact of Transfer				
Transfer with diagnosis of PPH	Home to clinic		30%	
	Home to hospital		15%	
	Clinic to hospital		35%	
Transfer with diagnosis of retained placenta	Home to clinic		30%	
	Home to hospital		15%	
	Clinic to hospital		35%	

Continued

TABLE 3. Continued

Sub-Saharan Africa 2020 Baseline Assumptions				
Intervention	**Metric**	**Home**	**Clinic**	**Hospital**
Transfer with diagnosis of lacerations	Home to clinic		30%	
	Home to hospital		15%	
	Clinic to hospital		35%	
Transfer with diagnosis of atonic uterus	Home to clinic		30%	
	Home to hospital		15%	
	Clinic to hospital		35%	
Transfer with diagnosis of severe haemorrhage	Home to clinic		30%	
	Home to hospital		20%	
	Clinic to hospital		40%	

Abbreviations: D&C, dilation and curettage; PPH, postpartum haemorrhage.

TABLE 4. MANDATE Model: Estimated Number of Maternal Deaths and Lives Saved With Improvement of Postpartum Haemorrhage Prevention and Treatment Through an Inhalable Oxytocin, Sub-Saharan Africa, 2020

Scenario Number	Scenario	Maternal Deaths (n)	Maternal Lives Saved From Current Level (n)	Maternal Deaths Prevented From Current Level (%)
1.	Current levels of prevention and treatment	54,290	n/a	n/a
2.	Moderate coverage (50%) inhalable oxytocin prevention and treatment: all settings	45,600	8,690	16
3.	Near perfect coverage (95%) of inhalable oxytocin prevention and treatment: all settings	33,790	20,500	38

(utilization) oxytocin will now have increased access to and use of oxytocin. In this first scenario, both penetration and utilization are increased to ensure that 50% of people who do not have access to oxytocin get access to the medication and 50% of people who do not use oxytocin will now be able to access and use the medication. The second scenario shows near perfect access and use of oxytocin across all settings, which means that penetration (availability) and utilization (use) both raised to 99%. MANDATE estimates approximately 40% of all maternal deaths from PPH could be prevented through the universal use of an inhalable form of oxytocin.

Refrigeration Capabilities

In LMIC health-care settings, drugs that are not heat stable – including current formulations of oxytocin – degrade and, thus, require refrigeration.[8,13]

It is important to note, across all the regions that we surveyed, only a third or less of all clinics and at least half of the health centres in all regions had the ability to store medicine in refrigerated conditions. The majority of hospitals across all regions had the ability to refrigerate medicine; however, in Indonesia, health-care providers stated that hospitals were less likely to be able to refrigerate medicine than health centres. The responses are summarized in Table 5.

The lack of proper storage facilities and ability to maintain the cold chain, especially at the community level, underscores the necessity for innovative formulations that can work within current health systems and contexts.[14]

Formulation Preferences

Despite the potential impact of a universally heat-stable inhalable version of oxytocin that may allow greater access and ease of use for less-skilled health workers in low-

TABLE 5. Refrigeration Capabilities of Health Care Settings

	Clinic	Health Centre	Hospital
Guatemala (n=11)	18%	55%	91%
Indonesia (n=11)	18%	82%	64%
Kenya (n=10)	20%	70%	100%
Nigeria (n=10)	20%	50%	80%

Note: In this study, clinic refers to village/community-based health care; health centre and hospital refer to urban, regionally based care.

resource settings, health-care providers surveyed in the 4 LMICs reported little to no use of any inhalable medicine. Across all sites and all health-care providers, no respondent reported a preference for using an inhalable form of PPH medicine. In all country sites examined, the majority of health-care providers noted that either inhaled delivery medicines were not used or that they were unaware of these formulations being used.

Across all sites, intravenous formulations were a preferred formulation for PPH (see Table 6 for a summary of preferred formulations). Health-care providers also preferred oral formulations in Guatemala and Indonesia; intramuscular formulations in Guatemala, Kenya, and Nigeria; and suppositories in Nigeria. The responses are summarized in Table 6.

Of note, innovative formulations, including inhaled formulations, were not selected by the survey participants. Instead, the formulations they regularly used seem to be the formulations they prefer. Across all settings, efficacy and availability were cited as the primary reasons for their preferred formulation. Further, when asked about their desired PPH formulation, providers expressed the desire for more intravenous and oral medicines over inhalable formulations. These responses are summarized in Table 7.

Notably, 40% of Kenyan respondents, including 2 doctors, 1 nurse, and 1 midwife, were the only respondents across the entire study to indicate that they would desire an inhalant formulation for PPH medication.

Qualitative Insights From Inhalable Questions

Qualitative interviews revealed that respondents had little experience and/or understanding of inhalable medicines in general. Across the countries surveyed, all health-care providers stated that the primary barriers associated with using inhaled medicine were that they have never been used

TABLE 6. Preferred Formulation for Postpartum Haemorrhage Medications

	Inhaled	Intravenous	Oral	Transdermal	Sublingual	Suppositories	Intramuscular	Other	Don't Know
Guatemala (n=11)	0%	64%	55%	0%	9%	18%	40%	0%	9%
Indonesia (n=11)	0%	64%	64%	0%	9%	27%	27%	0%	0%
Kenya (n=10)	0%	100%	0%	0%	10%	10%	60%	0%	0%
Nigeria (n=9)	0%	44%	11%	0%	33%	33%	33%	0%	0%

Note: Answers are not mutually exclusive.

TABLE 7. Desired Formulation of Postpartum Haemorrhage Medicine

	Inhaled	Intravenous	Oral	Transdermal	Sublingual	Suppositories	Other	Don't Know
Guatemala (n=11)	0%	27%	27%	0%	0%	9%	0%	36%
Indonesia (n=11)	0%	9%	27%	0%	0%	9%	0%	27%
Kenya (n=10)	40%	20%	20%	10%	30%	20%	0%	40%
Nigeria (n=9)	0%	33%	33%	0%	44%	22%	11%	0%

Note: Answers are not mutually exclusive.

before and/or were not available. For example, a respondent in Indonesia explained in response to the question 'In your region, what barriers are associated with inhaled medicine?' that 'I do not know anything about inhaled medications, so far all I know is the other methods.' The lack of knowledge about inhalable formulations and their availability are considerable barriers to the uptake of inhalable medicines. It is evident that the respondent health-care providers trust their current formulations. One respondent from Nigeria noted other barriers for inhalable medications, stating the 'people surrounding the patient, they all have effect (of the medication) also, so that one is also a barrier too; and then I think the rule doing an inhalational form is not a fast route.' If a new drug or formulation is introduced to the market, health-care providers will need to trust the supply chain and the impact of the medicine in order to adopt its use and be trained on the new delivery system of inhalables.

DISCUSSION

Our research fits into the global research context outlined in the United Nations Commission on Life-Saving Commodities for Women and Children report, in which oxytocin is identified as one of 13 life-saving commodities.[9] The report provides recommendations for actions needed to increase uptake and use of these crucial commodities.[9]

Our small study begins to address some of the challenges associated with introducing a new formulation for PPH prevention and treatment. Through our research, we identified which countries include oxytocin in their treatment guidelines and measured levels of health-care provider knowledge and use of various formulations of oxytocin. We further explored the availability of oxytocin and challenges to its use, including preferred and desired formulations.

Although paediatric tuberculosis (TB) is a different field, the challenges in introducing new formulations of medication are similar to those posed in the maternal and neonatal health field. In their review of barriers to uptake of paediatric TB treatment, Craig et al[15] highlight the need for more qualitative data about patient, caregiver, and provider knowledge, behavior, beliefs, and attitudes regarding medicine in general and biomedical pharmaceuticals, in particular to inform the introduction of new formulations.[15] Our study begins to fill this gap in qualitative field data to inform formulation uptake that Craig et al note by surveying a variety of health-care providers as well as the mixed-methods approach that was used for data collection. Respondents were chosen from a variety of urban and rural health-care settings, representing each level of care – from the community to the hospital level. Insights from a variety of providers, including a range of community health workers with basic training and physicians with specialty training in obstetrics and gynaecology, further strengthened the results. Moreover, respondents had various years of experience in the health-care field, providing both novice and expert practitioner perspectives. Finally, the combination of literature review, modelling, and quantitative and qualitative data collected through the surveys and interviews provides a comprehensive set of results.

Limitations

It should be noted that the intent of this study was to provide initial insights into maternal and neonatal health care in the selected countries. The sample size was small, which may give results that do not adequately represent the larger population and may not reflect regional variations within the countries. The survey respondents may not have considered the inhalant form of oxytocin because they are unfamiliar with inhalables, in general, or they did not know an inhalable formulation of oxytocin could be available in the future. While valuable information was gleaned from this study, additional studies could examine variations among respondents and/or gather additional insights into the context of the responses from those interviewed and surveyed.

CONCLUSION

Oxytocin is currently formulated as an injectable medicine that is not heat stable – requiring refrigeration and maintenance of the cold chain – and must be administered by trained health-care providers. A heat stable, inhalable version of oxytocin may allow greater access and ease of use, making it possible for it to be administered by less skilled health-care providers in low-resource settings. However, the health-care providers surveyed reported little to no use of any inhalable medicine. Further, providers expressed the desire for more intravenous and oral medicinal formulations over inhalable formulations. Interviews revealed that respondents had little experience and/or understanding of inhalable medicine, and therefore no interest in this formulation.

Given the low use and the lack of knowledge about inhalable medicine among the survey respondents, education around the use of an inhalable formulation would be needed to increase health-care providers' interest and self-efficacy in administering this novel treatment method once it is fully developed.

Additional research opportunities related to the selection of preferred or desired formulations could help put a new form of the gold standard PPH treatment in the hands of frontline health-care providers. Of note, 40% of Kenyan provider respondents stated that their desired formulation would be an inhalable formulation. In Kenya, the Ministry of Health is currently attempting to increase access to oxytocin.[15] Further exploration of Kenyan provider interest in inhalant formulations is needed to understand if there is a link between the Ministry of Health's campaign and the expressed interest in inhalant treatment. Research into why an easier mechanism to deliver PPH treatment did not rise to

the top of preferred or desired formulations in other locations could inform the need to create awareness and demand before an inhalant form of oxytocin is market ready.

Acknowledgements: This study was conducted by RTI International with funding from GlaxoSmithKline.

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Peer Reviewed

Competing Interests: None declared.

Received: 6 Mar 2017; **Accepted:** 27 Nov 2017

Cite this article as: MacGuire ER, Moran K, Hepler BJ, Rouse DJ. The Novelty of Inhalable Medications: Interest, Use, and Impact of Inhalant Medications in Low-Resource Settings. East African Health Res J. 2018;2(1):43-52. https://doi.org/10.24248/EAHRJ-D-17-00080

EAHRC
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ORIGINAL ARTICLE

Prevalence and Risk Factors Associated With Chronic Kidney Disease Among Patients Presenting at a Haemodialysis Unit in Dodoma, Tanzania

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ABSTRACT

Background: Chronic kidney disease (CKD) is a major public health problem worldwide, due to its epidemic proportions and the associated cardiovascular morbidity and mortality. However, data on the burden of CKD among patients attending hospitals in Tanzania are still limited. The aim of this study was to determine the prevalence and risk factors associated with CKD among patients presenting at the University of Dodoma (UDOM) haemodialysis unit in Tanzania.

Methods: In this retrospective study, we reviewed data of 1,395 patients who presented at the UDOM haemodialysis unit from January 2013 to June 2015. Data were descriptively and inferentially analysed using Stata version 11.0.

Results: From January 2013 to June 2015, a total of 1,395 patients presented at the UDOM haemodialysis unit with history of kidney disease. Of these patients, 1244 (89.2%) enrolled into this study, 651 (52.3%) of them were female. Almost two-thirds (n=792, 63.7%) of the patients were found to have CKD, 59.1% with an estimated glomerular filtration rate of $<$60 mL/min/1.73 m^2. Among those who had CKD, 347 (43.8%) had hypertension, 241 (30.4%) had diabetic mellitus, 79 (10.0%) had chronic glomerulonephritis, 70 (8.8%) had hypertension and diabetes mellitus, 38 (4.8%) had HIV/AIDS, and 17 (2.1%) had hepatitis B. The median serum creatinine level was 222 μmol/L (interquartile range [IQR] 126 to 317), urea level was 14.5 mmol/L (IQR 5 to 24), hemoglobin was 11.0 g/dL (IQR 6.2 to 15.7), and body mass index was 27.1 kg/m^2 (IQR 17.3 to 36.8). Obesity, diabetes mellitus, and systolic hypertension were associated with developing CKD ($P<.001$). A total of 116 patients received haemodialysis during the study period.

Conclusion: CKD was common among patients presenting in our hospital and is associated with high cardiovascular risk. To that end, patients should be thoroughly evaluated to identify and correct causes of their kidney disease, and efforts should be put in place for early detection and screening as well as advocacy on risk factors for CKD development in Tanzania.

INTRODUCTION

Chronic Kidney Disease (CKD) is found in 10% of the global population, and is considered a major public health problem worldwide, due to its epidemic proportions and its association with high cardiovascular risk.[1] CKD is defined as abnormalities of kidney structure or function present or decreased estimate glomerular filtration rate (eGFR) for more than 3 months with implications for health, and classified into 5 stages according to the eGFR.[2–5] The current recommendation is to use serum creatinine concentration to estimate the eGFR and transform it using the CKD Epidemiology Collaboration (CKD-EPI) equation.[5]

CKD is associated with age-related renal function decline accelerated in hypertension, diabetes, obesity and primary renal disorders.[6] Although cardiovascular disease (CVD) is the primary cause of morbidity and mortality, CKD is regarded as an accelerator of CVD risk and an independent risk factor for CVD events.[7] Also CKD severely affects patients' health, lifestyle and well-being, and quality of life[8]; it is at least 3 to 4 times more

frequent in Africa than in developed countries.[9] Despite high demand, very few patients in some African countries receive renal replacement therapy, mainly because of resource constraints.[10]

A study conducted between 2007 and 2010 in United States found that an estimated 14.0% of the U.S adult population had CKD.[11] Although the causes vary, diabetes was the most common cause of CKD in the United States and is steadily increasing as the cause worldwide.[12] In a study conducted in a hospital in Singapore, researchers found that 87.7% of the patients had moderate to severe CKD (stage 3 and above); the majority of the patients had dyslipidemia (92.8%), hypertension (89.3%) and diabetes mellitus (64.6%).[13] In a study conducted in South Africa's black population, researchers found that primary hypertension occurred in 25.0% of the study population and was considered the cause of stage 5 CKD in 40.0% to 60.0% of these patients.[2] In another study conducted in South Africa, the authors also reported hypertension as the leading cause in the adult population and the cause of chronic kidney failure in 21.0% of patients on renal replacement therapy registry.[9] The prevalence of diabetic nephropathy is estimated to be 14.0% to 16.0% in South Africa, 23.8% in Zambia, 12.4% in Egypt, 9% in Sudan, and 6.1% in Ethiopia.[9] In a study conducted in Nigeria, the overall prevalence of CKD was 18.8%. The researchers attributed the disease to hypertension (30.0%), diabetes mellitus (3.7%), obesity (14.6%), and haematuria (3.1%.), with age, female gender, systolic blood pressure, and diabetes mellitus as predictors of CKD.[14]

In a study of outpatients in North Western Tanzania, an alarmingly high prevalence (87.3%) of CKD was identified among adult patients attending diabetes mellitus clinic.[15] In a community-based study conducted in Northern Tanzania, the overall prevalence of CKD was found to be 7%; and, of these patients, 19.3% had hypertension alone, 14.0% had both diabetes and hypertension, 7.0% had diabetes alone, 7.0% had HIV alone, and 3.5% had both HIV and hypertension. Nearly half (49.2%) of the cases of CKD were not associated with any of the measured risk factors of hypertension, diabetes, or HIV.[16] Another hospital study conducted in North Western Tanzania, reported that hypertension-related diseases were the most common cause of hospital admission and CKD, and accounted for most deaths.[17]

While CKD is associated with high mortality and morbidity, limited data is available on the burden of CKD among patients attending hospitals in Tanzania, and, to date, the country has no national registry for CKD. Very few studies on prevalence of CKD have been reported in Tanzania, those that have been conducted have focused on patients either attending diabetic clinics or seeking care in communities.[15–17] The aim of this study was to determine the prevalence and risk factors associated with CKD among patients presenting at the University of Dodoma (UDOM) haemodialysis unit in Dodoma, Tanzania.

METHODS

Study Design, Population, and Settings

Data for this retrospective study data were collected from patients who presented at the UDOM haemodialysis unit from January 2013 to June 2015. The UDOM hospital started its operations in 2007, when the university was officially launched, with the main aim of providing health-care services to the UDOM staff and students as well as the community of Dodoma. The hospital serves about 120,000 people, has a bed capacity of 100, and has specialist clinics conducted and run by medical specialists from the UDOM College of Health Sciences. In January 2013, the UDOM hospital launched its haemodialysis unit. The unit serves as a referral centre for the Singida, Morogoro, Iringa, Manyara, and Tabora regions, and for other regional referral hospitals in the central and other neighbouring zones, serving a total population of 20 million people.

All patients who required haemodialysis were treated at the unit, and all patients who presented at the haemodialysis unit but had no indications for haemodialysis were scheduled for routine medical follow up and continuous medical care.

Data Collection and Laboratory Procedures

Patient medical record files of the 1,395 patients who presented at the UDOM haemodialysis unit from January 2013 to June 2015 were reviewed. Study data were obtained from handwritten medical records and then cross-checked with the electronic records. Any discrepancies were reviewed and verified to ensure the validity of data. The data were carefully reviewed and all patients with incomplete records were excluded. Information on patient sociodemographics, clinical characteristics, and relevant laboratory investigations were extracted. The study team reviewed information about age, gender, marital status, weight in kilograms, height in centimetres, clinical signs (oedema, anuria, hypertension), and laboratory data—including urinalysis, hepatitis panel, full blood picture, urea, creatinine, electrolytes, random blood glucose, and HIV test results—were recorded.

Body mass index (BMI) was calculated using the National Health Services (United Kingdom) BMI calculator, an eGFR was calculated using the CKD-EPI equation, and bedside isotope-dilution mass spectrometry-traceable Schwartz GFR calculator for children was used to stage patients.

Acute kidney injury was defined as an acute deterioration in renal excretory function, with a serum urea >10 mmol/L and/or a rise in serum creatinine (Scr) by ≥ 0.3 mg/dL, or a percentage increase in Scr of $\geq 50\%$ from baseline using the Acute Kidney Injury Network criteria. End stage renal disease was defined as progressive CKD with eGFR ≤ 15 mL/min/1.73 m^2 with or without other indications for haemodialysis. Outcome measures were CKD and its associated risk factors.

Data Analysis

The de-identified data collected were entered into a computer using EpiData version 3.1 (CDC, Atlanta, USA) and analysed using Stata version 11.0 (StataCorp LLC, College Station, Texas, USA). Data were summarized as proportions and frequency tables for categorical variables. Depending on variable distribution, either mean with standard deviation or median with interquartile range were used to summarize continuous data. The correlation between the development of CKD and different patient parameters was determined by performing logistic regression analyses. Odds ratios (OR) were calculated to estimate the percentage change in risk of CKD development, and parameters with P values $<.05$ were considered statistically significant.

Ethical Considerations

The study was approved by the UDOM research and publications ethics review board. Waiver of consent was approved by the committees as this retrospective study analysed only de-identified data.

RESULTS

Demographic and Other Characteristics of the Study Population

A total of 1,395 patients presented at the UDOM haemodialysis unit from January 2013 to June 2015. Of these patients, 151 (10.8%) were excluded from this study due to incomplete records, and the remaining 1,244 (89.2%) patients were enrolled in this study. The median age of the study population was 36 years (interquartile range [IQR] 6 to 88), over half (n=651, 52.3%) were female, more than half (n=704, 56.6%) were classified as obese, and about a third (n=434, 34.9%) reported having a history of smoking. A small percentage of the study population was living with HIV (n=57, 4.6%) or hepatitis B (n=26, 2.1%). Of the 1,244 patients enrolled, 792 (63.7%) were found to have CKD, 59.1% with an eGFR of <60 mL/min/1.73 m^2 and 452 (36.3%) had no CKD after screening. A total of 116 patients received haemodialysis during study period, 32 (27.6%) of whom were found to have acute kidney injury (Table 1).

TABLE 1. Baseline Characteristics of Patients Who Presented at the University of Dodoma Haemodialysis Unit

Characteristic	Proportion (%) or Median [IQR]
Sex	
Male	593 (47.7)
Female	651 (52.3)
Age, in years	36 [6–88]
Marital status	
Never married	357 (28.7)
Married	479 (38.5)
Divorced	215 (17.3)
Widow	193 (15.5)
Smoking status (ever)	
No	810 (65.1)
Yes	434 (34.9)
Obesity (BMI) kg/m^2	27.1 [17.3–36.8]
Haemoglobin (g/dL)	11.0 [6.2–15.7]
Serum creatinine level (μmol/L)	222 [126–317]
Urea level (mmol/L)	14.5 [5–24]
HIV status	
Positive	57 (4.5)
Negative	1187 (95.4)
Hepatitis	
Positive	26 (2.1)
Negative	1218 (97.9)
Chronic kidney disease	
No	452 (36.3)
Yes	792 (63.7)
Classification of stage of chronic kidney disease (n=792)	
Stage one	32 (4.0)
Stage two	292 (36.9)
Stage three	220 (27.7)
Stage four	164 (20.7)
Stage five	84 (10.6)

Abbreviations: BMI, body mass index; IQR, interquartile range.

Causes of CKD Among Patients Who Presented at the Haemodialysis Unit

Of the 792 patients found to have CKD, 347 (43.8%) had hypertension, 241 (30.4%) had diabetes mellitus, 79 (10.0%) had chronic glomerulonephritis, and 70 (8.8%) had both hypertension and diabetes mellitus, while 38 (4.8%) and 17 (2.1%) were associated with HIV/AIDS and hepatitis B, respectively (Table 2).

Risk Factors Associated With CKD Among Patients Who Presented at the Haemodialysis Unit

A total of 15 potential risk factors were identified after performing univariate logistic regression analyses. Backward

TABLE 2. Causes of Chronic Kidney Disease Among Patients Who Presented at the Haemodialysis Unit

Medical Condition	Patients with CKD n (%)
Hypertension	347 (43.8%)
Diabetes mellitus	241 (30.4%)
Chronic glomerulonephritis	79 (10.0%)
Both hypertension and diabetes mellitus	70 (8.8%)
HIV/AIDS	38 (4.8%)
Hepatitis	17 (2.1%)

Abbreviation: CKD, chronic kidney disease.

elimination reduced this to 4 parameters. The potential predictors identified were gender, age, obesity, history of smoking, and causes of CKD. Gender, age, and smoking were found to be significant in univariate logistic regression analyses. However, this significance was lost after multivariate logistic regression analyses (Table 3).

Obesity (BMI ≥30) was predictive of CKD development (OR 8.7; 95% confidence interval [CI], 3.5 to 15.9; $P<.001$). In addition, the multivariate adjusted odds of CKD development were 9.2 times higher for a patient with diabetes mellitus (OR 9.23; 95% CI, 4.64 to 19.57; $P<.001$). Patients with systolic hypertension (OR 7.5; 95% CI, 3.94 to 11.68; $P<.001$) also had higher odds of developing CKD.

TABLE 3. Adjusted Odds Ratios for Risk Factors Associated With Chronic Kidney Disease

Parameters	Adjusted OR (95% confidence interval)	*P* value
Gender		.516
Male	1.00	
Female	1.3 (1.1–1.8)	
Age		.135
<50 years	1.00	
≥50 years	1.8 (0.9–3.7)	
Smoking status (ever)		.763
No	1.00	
Yes	2.6 (1.6–4.3)	
Obesity		<.001
<30	1.00	
≥30	8.7 (3.5–15.9)	
Causes of CKD		
Hypertension (systolic)	7.5 (3.94–11.68)	<.001
Diabetes mellitus	9.23 (4.64–19.57)	<.001

* $P<.05$

Abbreviations: CKD, chronic kidney disease; OR, odds ratio.

DISCUSSION

This study evaluated the prevalence and risk factors associated with CKD at the UDOM hospital. A total of 792 (63.7%) patients enrolled in the study were found to have CKD, of whom 59.1% had an eGFR of <60 mL/min/1.73 m^2. These findings are lower than an earlier study conducted by Janmohamed et al in North Western Tanzania, that showed the prevalence of CKD to be 80.0% among adults with diabetes mellitus, with nearly 25.0% having an eGFR of <60 mL/min/1.73 m^2.[15] These findings are also contrary with a community-based study conducted in Northern Tanzania, where the overall prevalence of CKD was found to be 7.0%.[16] The vast differences in the findings could be attributed to the difference in study populations.

In a hospital study conducted in Singapore, researchers found that 87.7% of patients had moderate to severe CKD[13]; while in another hospital study conducted in Nigeria, found the overall prevalence to be 18.8%.[14] CKD was at least 3 to 4 times more frequent in Africa than in developed countries.[9,11] In a country like Tanzania, where there is no registry or advocacy for CKD prevention or early detection of kidney diseases, more effort is needed to fight the growing epidemic of noncommunicable diseases.

Of all the patients in our study who had CKD, 347 (43.8%) had hypertension, 241 (30.4%) had diabetic mellitus, 79 (10.0%) had chronic glomerulonephritis, and 70 (8.8%) had both hypertension and diabetes mellitus. Similar findings have been reported from other studies conducted in low- and middle-income countries, whereby hypertension has been reported to be the leading cause of CKD.[2,9,10,13,14] However, these findings are contrary to a community-based study conducted in Northern Tanzania that showed that nearly half (49.2%) of the cases of CKD were not associated with any of the measured risk factors of hypertension, diabetes, or HIV.[16] This discrepancy calls for more research into other causes of CKD, including the use of local herbs and medicines, which is very rampant in this area where this study was conducted. Different findings have also been reported from the United States and other developed countries, whereby diabetes mellitus has been reported as the leading cause of CKD and is steadily increasing as the cause worldwide.[4,11,12,18]

Our study found that 704 (56.6%) patients were found to be obese and 434 (34.9%) patients reported having had a history of smoking. Similar findings have been reported from other studies whereby obesity and positive history of smoking were reported as risk factors for CKD.[14,19–21] In this study obesity (BMI ≥30), diabetes mellitus and systolic hypertension were strongly associated with development of CKD ($P<.001$). Similar findings have been reported from other studies.[6,14,19–21]

Limitations and Strengths

This study was conducted in a hospital in central part of Tanzania and, as such, the results are limited to patients in the central part of Tanzania. Despite the limited generalizability, this information could provide insight to strategies for improving the management of patients who present with CKD in hospitals. The strengths of this study were that our hospital uses both paper-based and electronic medical records, which allowed the recording and collection of patients' health related information in real time. The study data were obtained from handwritten medical records and then cross checked with the electronic records. Any discrepancies were reviewed and verified to ensure the validity of data.

CONCLUSION

CKD was common among patients presenting in our hospital and is associated with high cardiovascular risk. To that end, patients should be thoroughly evaluated to identify and correct the causes of their disease. As CKD is regarded as an accelerator of cardiovascular risks and an independent risk factor for cardiovascular events and eventually death, efforts should be put in place for early detection and screening as well as advocacy on risk factors for CKD development in Tanzania.

Acknowledgements: Special thanks to staff working at the UDOM hospital for the services they are providing to patients and for proper keeping of data which made this study possible. Additional thanks to the members of the Department of Internal Medicine of the College of Health Sciences for their excellent technical assistance and to the UDOM management for continuous material and moral support to the hospital.

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Peer Reviewed

Competing Interests: None declared.

Received: 31 Oct 2016; **Accepted:** 19 Dec 2017

Cite this article as: Meremo AJ, Masalu MB, Sabi I, Ngilangwa DP, Kapinga J, Tagalile R, et al. Prevalence and Risk Factors Associated With Chronic Kidney Disease Among Patients Presenting at a Haemodialysis Unit in Dodoma, Tanzania. East African Health Res J. 2018;2(1):53-57. https://doi.org/10.24248/EAHRJ-D-16-00367

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Comparison of Indoor Mosquito Collection Methods in the Assessment of Lymphatic Filariasis Transmission Dynamics in Mosquito Vectors in Tana River County, Kenya

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ABSTRACT

Background: Lymphatic filariasis (LF) is a parasitic infectious disease that is transmitted by several species of mosquitoes. Diagnosis of LF is done in both human hosts and vectors. Effective mosquito collection method(s) is/are required in order to collect large numbers of mosquitoes with high chances of infectivity.

Methods: In this study, 3 mosquito sampling methods were compared. Mosquitoes were collected from 6 randomly selected villages of Tana River County, Kenya. The effectiveness of CDC light traps, gravid traps, and pyrethrum spray methods in collecting mosquitoes were compared. Mosquitoes were morphologically identified into genera and species level, and mosquito dissection was done in search of microfilariae larvae to assess the infection and infectivity rates. Data was analysed by SPSS version 15.0 and analysis of variance (ANOVA).

Results: A total of 1632 female mosquitoes were collected belonging to 5 mosquito genera: *Culex, Anopheles, Aedes, Mansonia*, and *Ficalbia*. The most abundant mosquito genera was *Culex*. Light traps obtained the most blood-fed mosquitoes.

Conclusion: Light traps were found to be the most effective method of mosquito collection in terms of high catches and high infectivities.

INTRODUCTION

Lymphatic filariasis (LF) is a chronic parasitic disease of public health and socioeconomic significance in tropical and subtropical countries. More than 128 million people are estimated to be infected in 83 countries worldwide, with nearly 1.2 billion people at risk. The Global Programme to Eliminate Lymphatic Filariasis was launched in 2000[1] with the aim of interrupting LF transmission through chemotherapy and vector control. Mosquitoes are the main vectors of lymphatic filariasis parasites: the *Culex, Anopheles,* and *Aedes* species transmit *Wuchereria bancrofti* and the *Mansonia* and *Anopheles* species are involved in the transmission of *Brugia malayi*.[2]

The development and transmission of the LF parasite follows this cycle. Upon feeding from infected blood, mosquitoes acquire microfilariae (L1) from host circulation system; development of the parasite takes place in the mosquito (L2) to the infective stage (L3). The monitoring of a control intervention strategy involves assessment of mosquitoes carrying microfilariae developing larvae stages (L1 to L3) or having the human infective stage (larvae L3).[3,4] A key metric used for quantifying the risk of infection with mosquito-borne pathogens is the human-biting rate, which estimates the number of mosquito bites per person per day or night. When LF infections are at low levels, large numbers of mosquitoes must be dissected in order to determine infection rates.[5] Various mosquito-sampling methods are used in entomological studies to ensure large mosquito catches, however, the methods differ in their effectiveness. Until recently, human landing catches (HLC) have been the gold standard method for effective disease transmission index assessment,[2] but due to the ethical problem of using human subjects to bait the mosquitos, it is no longer recommended for use in most field mosquito surveillance studies.[6] U.S. Centers of Disease Control and Prevention (CDC) light traps have been also used, baited with different attractants, or placed near occupied untreated bed nets. The

trap's light bulb attracts mosquitoes from a distance and draws them in to be trapped. Other methods, such as pyrethrum spray catches, direct aspiration, and CDC gravid traps are also used in mosquito sampling.[2–5] Each method has shortcomings and is subject to bias, which may influence results.[6] Gravid traps are useful tools in entomological surveillance, as they target gravid mosquitoes, those that have already fed on blood, providing an opportunity for researchers to acquire the parasite from an infected individual. In contrast, pyrethrum spray is able to knock down mosquitoes within the house where the spraying is done. Mosquito species differ in their feeding, resting, and breeding behaviours as well as their ecological requirements. As such, mosquito collection for transmission dynamics assessment requires a method or methods that can take into consideration the level of prevalence in the area and the ability of that method(s) to obtain mosquitoes of different species and physiological status. The targeted method should be the one that can capture a large number of mosquitoes with high infection and infectivity rates.

The choice of the method depends on the objectives of the study, the environment, and the available means.[2] This study compares three methods – CDC light traps and gravid traps (John W. Hock Co., Gainesville, FL, U.S.A.) and pyrethrum spray catches (Knockdown) – to determine the most effective for vector collection in Kenya.

METHODS

Study Site

Mosquitoes were collected from 6 villages: Kilelengwani, Hewani, Idsowe, Kisiwani farm, Onindo, and Chakamba villages of Tana River County, Coastal, Kenya (Figure 1). The villages were chosen based on infection prevalences and abundance of mosquitoes from previous studies.[7] The houses were made of grass thatch both on the roof and walls, mud walled and grass-thatched houses, or block walled and galvanise iron sheet roofs with window screens. In Hewani village, most of the houses were built of burned bricks with iron sheet roofs and window screens. In Kilelengwani and Kisiwani farm, most of the houses were made of bricks with iron sheet roofs and windows without wire mesh screens. Other houses were made of bricks with a grass-thatched roof. In the rest of the villages, most of the houses were made of mud walls or sticks smeared with cow dug and grass-thatched roofs (Photo 1). The rainfall in this region ranges between 220 and 900 mm per year, which falls in 2 rain seasons: long rains between March and May and short rains between October and December. The major ethnic groups are the Pokomo, who practise farming and fishing, and the Orma and Wardey, who are predominantly nomadic. These farming and fishing practices create favourable mosquito breeding areas for transmission of LF.

Study Design and Mosquito Collection

This was a cross-sectional study design. A total of 60 houses – 10 houses in each of the 6 study villages – were selected using simple random selection whereby the villages were assigned a number and 6 numbers were selected for mosquito sampling. Oral consent to collect mosquitoes was obtained from the area chief, household heads, and the occupants of the selected houses. Mosquitoes were collected between April and August 2010, during the long rain season, which coincides with high transmission intensity of *W. bancrofti* due to high mosquito densities.[8] Indoor collection of mosquitoes was done using CDC light traps, gravid traps, and pyrethrum spray catches (Photos 2, 3, 4). Light traps and gravid traps were set from 7:00 pm to 7:00 am in the same houses for better comparison. Pyrethrum spray catches were done 5 days after use of the traps in the same houses. Alternation was done whereby spray catches would be done 5 days before the traps to avoid bias. Spraying was done in the evening between 7:00 pm and 10:00 pm and in the early morning between 5:00 am and 7:00 am.

Mosquito Processing

To analyse the mosquitoes, researchers first used chloroform to kill them and then removed them from the trapping nets. All mosquitoes were counted and recorded as per the method used. The village of collection and household location were recorded on Eppendorf tubes. The mosquitoes were morphologically identified up to genera level using entomological keys.[9] The abdominal statuses of the mosquitoes were determined as unfed, blood fed, half gravid, or gravid. All the mosquitoes were dissected to determine if microfilaria were present.

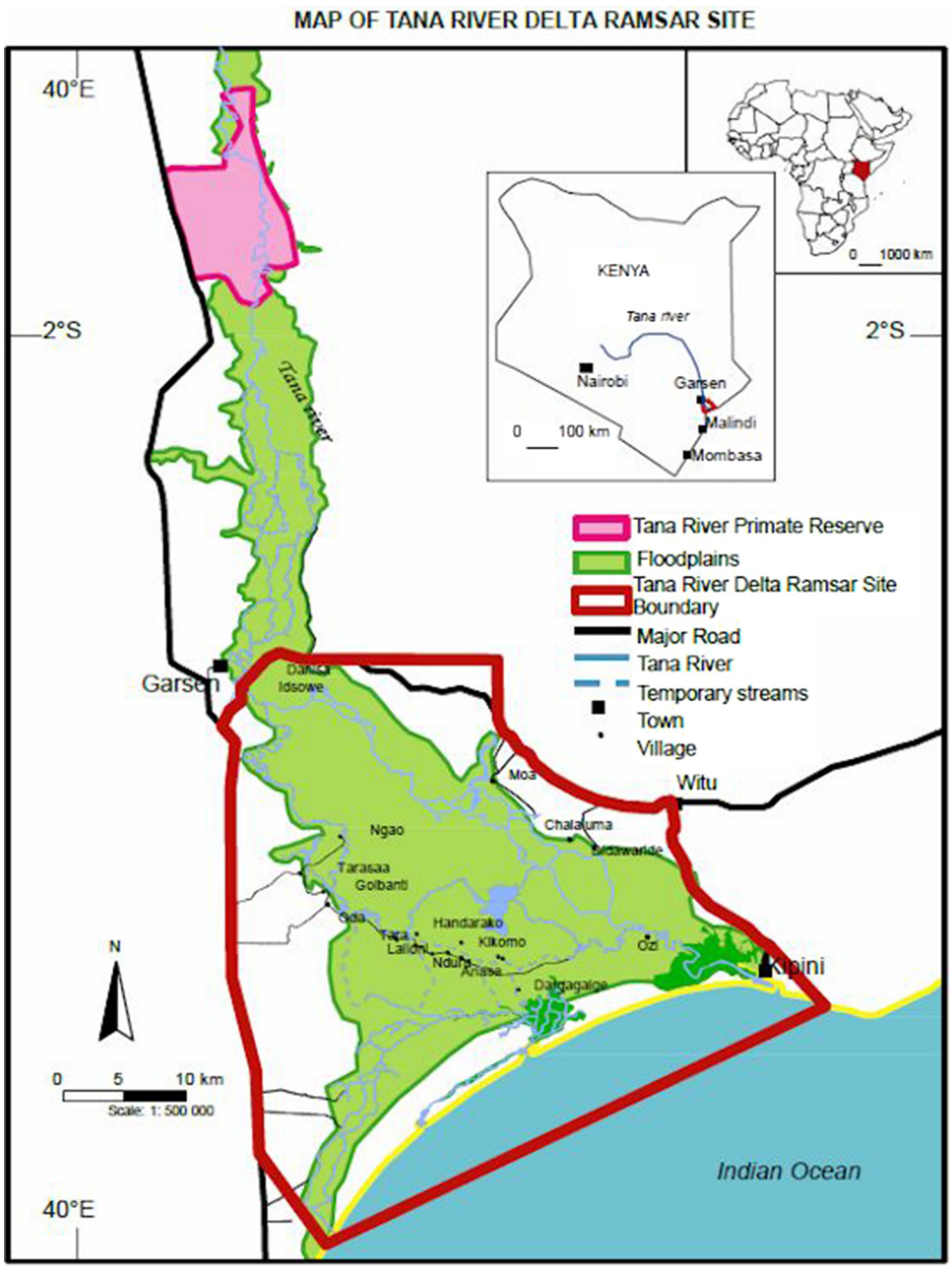
Deoxyribonucleic Acid Amplification By Polymerase Chain Reaction Assays

Deoxyribonucleic acid (DNA) extraction was done as described by Ramzy et al[10] with few modifications. The extracted DNA was amplified in a polymerase chain reaction (PCR) thermo cycler (GeneAmp PCR system 9700), detection and analysis of the PCR products were done through gel electrophoresis. PCR was used to amplify Ssp1 repeat of the DNA, the primer sequences were NV1 forward 5′ CAACCAGAATACCATTCATCC 3′ and NV2 reverse 5′ CGTGATGGCATAAAGTAGCG 3′ to amplify a 188-bp product in gDNA of *W. bancrofti* and the product confirmed through agarose gel electrophoresis.[10,11] Nested PCR was carried out on a few randomly selected PCR products for results confirmation. A positive and a negative control were included in each reaction, together with a molecular weight marker for size determination.

Data Analysis

Data were entered in record books and then transferred to a Microsoft excel spreadsheet. The data were analysed

FIGURE 1. Map of Tana River/Delta, Kenya



Source: Tana River Delta added to the Ramsar List. Ramsar Secretariat Website.

https://www.ramsar.org/news/tana-river-delta-added-to-the-ramsar-list. 12 October 2012. Accessed: 26 January 2018.

PHOTO 1. A typical house in the study area with high mosquito contact. The house is made of stick walls and grass-thatched roof.

PHOTO 2. A CDC light trap set inside a house to trap mosquitoes seeking a host indoors.

using SPSS Version 15.0 (SPSS Inc., Chicago, IL, USA). Generalized linear model univariate analysis was used to test statistical differences of the 3 methods used in collection and any other factors affecting the density of the collected mosquitoes.

Ethical Approval

Ethical approval for this study was obtained from Kenya Medical Research Institute Scientific Steering Committee (SSC) and the National Ethical Review board (SSC protocol No. 1692).

PHOTO 3. A CDC gravid trap set inside a house to trap mosquitoes seeking a host indoors.

RESULTS

Mosquito Collection Methods and Abdominal Status Analysis

A total of 1632 female mosquitoes were collected: 1265 (77.55%) by light traps, 311 (19.1%) by pyrethrum sprays, and 56 (3.4%) by gravid traps (Table 1). The mean difference of number of mosquitoes collected by the 3 methods was significant, ($P<.0001$) at 95% confidence interval (CI). While light traps were significantly different to pyrethrum spray and the gravid methods, there was no significant difference between pyrethrum spray and gravid traps by post hoc test ($P>.01$).

Out of the total number of mosquitoes collected, 1460 (89.5%) were unfed, 82 (5%) were blood fed, 68 (4.2%) gravid, and 22 (1.3%) half gravid (Table 1). Blood-fed mosquitoes were obtained by light traps (n=75, 91.5%), pyrethrum spray (n=6, 7.3%), and gravid traps (n=1, 1.2%) (Table 1). Most of the gravid mosquitoes were obtained by gravid traps (n=54, 79.4%), followed by light traps (n=13, 19.1%) and pyrethrum spray (n=1, 1.5%) (Table 1). Almost two-thirds of half-gravid mosquitoes were obtained by light traps (n=14, 63.6%), followed by pyrethrum spray (n=7, 31.8%) and gravid traps (n=1, 4.6%) (Table 1). The abdominal status of the mosquitoes obtained by different methods were significantly different ($P<.05$).

PHOTO 4. Pyrethrum spraying of the knocked down mosquitoes on a white sheet inside a house.

Mosquito Identification

Five mosquito genera were identified from the collected mosquitoes. Almost two-thirds (n=1048, 64.2%) were *Culex,* followed by *Mansonia* (n=236, 14.5%), *Aedes* (n=188, 11.5%), *Anopheles* (n=148, 9.1%), and *Ficalbia* (n=12, 0.7%) (Table 2). The species collected from the *Anopheles* genera included *An. gambiae sensu lato* (n=83, 56.0%), *An. Arabiensis* (n=33, 22.3%), *An. funestus* (n=30, 20.3%), and *An. sinensis* (n=2, 1.4%) (Table 2). Under *Culex* genera the species identified were *Cx. quinquefasciatus* (n=993, 94.8%) and *Cx. Pipiens* (n=55, 5.2%). The mosquitoes of *Mansonia* genera belonged to the *Mn. africanus* (n=150, 63.6%) and *Mn. Unformis* (n=86, 36.4%) species, while those of *Aedes* genera belonged to *Ae. Aegypti* (n=98, 52.1%), *Ae. Polynesiensis* (n=70, 37.2%), *Ae. Scapularis* (n=17, 9.1%), and *Ae. Mucedual sudaneses* (n=3, 1.6%) (Table 2).

Mosquito Genera Obtained By Each of the Collection Method

Each mosquito genera appeared to have a preference for a specific collection method. Out of the 1048 (64.2%) *Culex* mosquitoes obtained, the light trap caught the highest number (n=970, 92.6%) compared to the pyrethrum spray method (n=45, 4.3%) or the gravid traps (n=33, 3.1%) (Figure 1). Most of the *Aedes* mosquitoes were caught by the spray method (n=176, 93.6%) compared to almost two-thirds of the *Mansonia* mosquitoes were obtained by light traps (n=153, 64.8%), compared to pyrethrum spray (n=71, 30.1%) and gravid traps (n=12, 5.1%). Almost all of the *Anopheles* mosquitoes were obtained by light traps (n=130, 87.8%), compared to the gravid traps (n=11, 7.4%) and pyrethrum spray (n=7, 4.8%). The mosquito genera obtained by each method were statistically different *(P*<.0001) at 95% CI.

Mosquito Composition and Distribution in the Study Area

The numbers of mosquitoes obtained from each village were different. Kilelengwani village had the highest catch (n=951, 58.3%), followed by Kisiwani farm (n=320, 19.6%), Chakamba (n=225, 13.8%), Onindo (n=105, 6.4%), Idsowe (n=19, 1.2%), and Hewani (n=12, 0.7%) (Table 3). The villages had a significant effect on the mosquito density caught *(P*<.0001) due to different ecological factors and farming activities. For example, Kilelengwani village, located near swamps and rice-growing pads, had the highest catch, representing 58.3% (n=951) of all the mosquitoes collected. In contrast, Hewani village had the lowest

TABLE 1. Number and Abdominal Status of the Mosquitoes Collected by Each Method

	Blood Fed				
Collection Method	**Fed n (%)**	**Unfed n (%)**	**Gravid n (%)**	**Half Gravid n (%)**	**Total n**
Light traps	75 (91.5)	1163 (79.6)	13 (19.1)	14 (63.6)	1265
Gravid traps	1 (1.2)	0 (0.0)	54 (79.4)	1 (4.6)	56
Pyrethroid spray	6 (7.3)	297 (20.4)	1 (1.5)	7 (31.8)	311
Total	82	1460	68	22	1632

TABLE 2. Mosquito Species Composition Collected in the Study Area

Anopheles		Culex		Mansonia		Aedes		Ficalbia	
Species	n (%)	Species	n (%)	Species	n (%)	Species	n (%)	Species	n (%)
An. gambiae sl.	83 (56.0)	*Cx. quinquefasciatus*	993 (94.8)	*Mn. africanus*	150 (63.6)	*Ae. egypti*	98 (52.1)	*Fi. uniformis theobalb*	12 (100.0)
An. arabiensis	33 (22.3)	*Cx. pipiens*	55 (5.2)	*Mn. unformis*	86 (36.4)	*Ae. mucedual sudaneses*	3 (1.6)		
An. funestus	30 (20.3)					*Ae. polynesiensis*	70 (37.2)		
An. sinensis	2 (1.4)					*Ae. scapularis*	17 (9.1)		
Total genera	148 (9.1)		1048 (64.2)		236 (14.5)		188 (11.5)		12 (0.7)

Abbreviations: *Ae.*, *Aedes*; *An.*, *Anopheles*; *Cx.*, *Culex*; *Mn.*, *Mansonia*; *W.*, *Wucheria*.

TABLE 3. Mosquito Genera Obtained in Each of the Collection Villages in the Study Area

	Mosquito Genera					
Collection Villages	*Culex* n (%)	*Aedes* n (%)	*Mansonia* n (%)	*Anopheles* n (%)	*Ficalbia* n (%)	Total n
Chakamba	142 (13.5)	2 (1.1)	61 (25.8)	20 (13.5)	0 (0.0)	225
Kisiwani Farm	259 (24.7)	6 (3.2)	45 (19.1)	9 (6.1)	1 (18.3)	320
Kilelengwani	542 (51.7)	177 (94.0)	120 (50.8)	101 (68.2)	11 (91.7)	951
Onindo	84 (8.0)	2 (1.1)	5 (2.1)	14 (9.5)	0 (0.0)	105
Idsowe	14 (1.3)	0 (0.0)	5 (2.1)	0 (0.0)	0 (0.0)	19
Hewani	7 (0.6)	1 (5.5)	0 (0.0)	4 (2.7)	0 (0.0)	12
Total	1048	188	236	148	12	1632

catch, representing 0.7% (n=12) of the caught mosquitoes, since there were no bodies of water around the homesteads in Hewani. Regarding the 5 mosquito genera collected from the study area (Table 2), the *Culex* species was the most prevalent, with the highest number (n=542, 51.7%) obtained from Kilelengwani village and the least (n=7, 0.6%) from Hewani (Table 3). There were no *Aedes*, *Anopheles*, or *Ficalbia* species obtained from Idsowe village. Hewani village had no *Mansonia* and *Ficalbia* species. *Ficalbia* species mosquitoes were obtained from Kisiwani farm (n=1, 18.3%) and Kilelengwani village (n=11, 91.7%). The villages had a significant effect on mosquito species ($P<.0001$), depending on environmental factors and human activities.

Mosquito Infection Status

Upon mosquito identification and dissection, microfilariae larvae L1, L2, and L3 were found in mosquitoes of the *Anopheles* and *Culex* genera. In *An. gambiae sl.*, 2 mosquitoes had L2 larvae and 3 had L3 larvae; there were no L1 larvae in this species. In *An. funestus*, 2 mosquitoes had L2 larvae, no L1 or L3 larvae were present. In the *Cx. quinquefasciatus* species, 4 mosquitoes had L1 larvae, 2 had L2 larvae, and 21 mosquitoes had L3 larvae. On *W. bancrofti* DNA detection by PCR and agarose gel electrophoresis, 7 (0.4%) mosquitoes within *An. Gambiae sl,* 2 (0.1%) of *An. funestus*, and 30 (1.8%) of *Cx. quinquefasciatus* tested positive for *W. bancrofti* DNA by PCR, representing 2.3% infection rate (Table 4). The infection and infectivity rates by microscopy was 2.1% and 1.5% respectively, calculated as follows:

$$\text{Infection rate} = \frac{\text{Number of mosquitoes carrying L1} + \text{L2} + \text{L3}}{\text{Number of dissected mosquitoes}} \times 100$$

$$34/1632 = 2.1\%$$

TABLE 4. Prevalence of Microfilariae in Dissected Mosquitoes and *Wucheria bancrofti* DNA Detection by Polymerase Chain Reaction

		Microscopy				PCR	
Mosquito Genera	Species	Larvae L1 n	Larvae L2 n	Larvae L3 n	% Infection	*W. bancrofti* DNA n	% Infection
Anopheles	*An. gambiae*	0	2	3	0.3	7	0.4
	An. funestus	0	2	0	0.1	2	0.1
Culex	*Cx. quinque-fasciatus*	4	2	21	1.7	30	1.8
			Total microfilaria observed: 34 Microfilaria prevalence: 34/1632 = 2.1%			Total microfilaria DNA: 39 Microfilaria prevalence: 39/1632 = 2.3%	

Abbreviations: *An.*, *Anopheles*; *Cx.*, *Culex*; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; *W.*, *Wucheria*.

$$\text{Infectivity rate} = \frac{\text{Number of mosquitoes carrying L3}}{\text{Number of dissected mosquitoes}} \times 100$$

$$24/1632 = 1.5\%$$

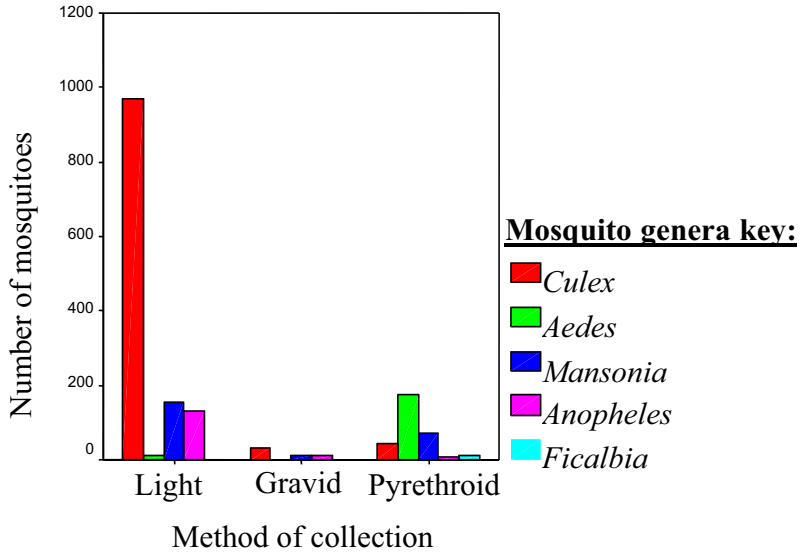
DISCUSSION

Comparison of Mosquito Collection Methods

The results of this study have demonstrated the use of the 3 methods: light traps, gravid traps, and pyrethrum spray for sampling disease vectors. The field evaluation of light traps, gravid traps, and pyrethrum sprays in the same ecological settings enabled the efficient comparison of these sampling methods in mosquito collection. In this study, the 3 methods were found to be significantly different, with the light trap being more significant ($P<.0001$) compared to pyrethrum spray and gravid traps using the ANOVA univariate post hoc analysis. Differences between pyrethrum spray and the gravid traps were not significant ($P>.05$). Light traps were able to obtain large numbers of mosquitoes in areas where there were large or small numbers of mosquitoes, followed by pyrethrum sprays and then gravid traps. This suggested that light traps would be the most suitable method for capturing large numbers of mosquitoes.

The results of this study were in agreement with the results of a study in Tanzania by Mboera et al,[12] who found out that light traps collect a lot of mosquitoes because light from the bulb could attract mosquitoes from a distance. The traps in this study were set in sleeping areas/rooms to use humans as bait for attracting mosquitoes. This was done in reference to observations of Mboera et al[12] who reported that human baits are the most efficient in attracting mosquitoes as compared to other attractants used in traps. Different odours and carbon dioxide produced by humans have attractant effect to the mosquitoes.[12] Light traps were able to obtain mosquitoes of different abdominal status: unfed, blood fed, half gravid, and gravid. This difference in abdominal status reflected the number of mosquitoes seeking a blood meal, those that have taken a blood meal and resting for blood digestion and egg development, and those seeking for oviposition sites.[12] The fed and half-gravid mosquitoes have high chances of having microfilaria picked during blood meal. Gravid traps collected gravid mosquitoes seeking for oviposition sites. Hay infusions (oviposition medium) used in gravid traps only attracted gravid mosquitoes, as reported by Reiter,[13] and this limited the captured mosquitoes only to female gravid mosquitoes, which were attracted for oviposition. Bad odour from the hay infusion used in gravid traps was a problem for people sleeping in the rooms where the traps were set. New attractant media was used each day since the rotten hay infusion produced an odour that acted as a repellent to the mosquitoes (personal observation) in the study during mosquito collection. Pyrethrum sprays were capable of obtaining unfed, fed, half-gravid, and gravid mosquitoes as long as they were in reach of the sprays. The number of blood-fed mosquitoes trapped was different for each collection method, with gravid traps having the least number (1). Gravid traps attract only gravid mosquitoes ready for oviposition and, thus, the blood meal had been digested for egg development. This means that mosquitoes obtained by gravid traps had a higher chance of being infected since they had taken at least 1 blood meal. There were no unfed or male mosquitoes obtained by gravid traps. This suggests that mosquitoes obtained by gravid traps have a higher chance of being infected as compared to pyrethrum sprays, hence are suitable for assessing disease dynamics in the vectors as it has been suggested by Reiter.[13] However the large numbers of mosquitoes required when the infection rates are low are

FIGURE 2. Mosquito Genera Collected by Each Sampling Method



not achievable by gravid traps due to selection bias of only gravid mosquitoes. Different sampling methods have shown varying ability in collecting mosquitoes of different abdominal conditions, which can be more informative in disease epidemiology.[14] The methods were significantly different in obtaining mosquitoes of different abdominal status ($P<.05$). However, most of the available sampling methods for mosquito vectors have limitations associated with their use because the different species are attracted differently because of their different behaviour. Thus, in areas with different mosquito species, it is difficult to recommend a single method as the appropriate tool for trapping host-seeking mosquitoes.

Different numbers of mosquitoes were obtained from each village due to differences in ecological factors of the villages sampled. For example, more mosquitoes were obtained in villages near swampy and marshy areas compared to villages not surrounded by water bodies. The villages where the sampling of mosquitoes was carried out had a significant effect on the number of mosquitoes obtained ($P<.0001$). These findings suggest that there are more attractive ecologic niches that favour breeding of filarial vectors in villages with highest mosquito catches than the villages with few catches. This means that understanding the ecological requirements of mosquitoes is important for vector control and human-vector contact control efforts. For example, *Mansonia* species are found in submerged vegetation and the larvae attach themselves to plants.[15] Removal of such plants through mechanical, biological, or chemical control would effectively prevent breeding of *Mansonia* species. The numbers of mosquitoes obtained by the different sampling methods were also significantly different between houses in the same villages. This suggested that the nature of houses and housing materials influenced the mosquito density. More mosquitoes were obtained from grass-thatched houses, most of which had open windows and many eaves into the houses.

There was a significant difference in mosquito species obtained from the collection villages ($P=.046$). *Culex* species had the highest number representing 64.2% (n=1048) of all the obtained mosquitoes and *Ficalbia* species was the least representing 0.7% (n=12). According to this study, the most prevalent mosquito species in Tana Delta district were *Culex*. This is in agreement with reports by Mwandawiro et al[16] who found that *Culex* species were the main LF vectors in both urban and rural areas. Increasing urbanization, inadequate disposal, sanitation facilities, and wet season lead to increased breeding sites for LF vectors.[17] *Culex* species breed in the foulest waters, especially in wet pit latrines,[16] which were common in the study area. *Mansonia* species breed in submerged vegetation, which were common around swampy and marshy areas; most of the *Mansonia* species were caught in the houses near the swamps. This suggests that different mosquito species prefer different types of breeding sites. Increases in mosquito breeding coincides with a high transmission rates as reported by Kasili et al,[18] especially during and after the long and short rain seasons. Few mosquitoes are found during the dry season with very low transmission rates.[18]

Different genera of mosquitoes were collected by different methods. Light traps captured most of the *Culex* mosquitoes (n=970, 92.56%) (Figure 1), almost two-thirds of the

Mansonia mosquito species (n=153, 64.83%), and most of the *Anopheles* mosquito species (n=130, 87.8%). The pyrethrum spray method caught most of the *Aedes* species (n=176, 93.61%) and all the *Ficalbia* mosquitoes (Figure 2). No *Aedes* or *Ficalbia* mosquito species were obtained by gravid traps. The results of the mosquito species obtained by each method suggest that different methods are suitable for different mosquito species. The mosquito genera obtained by each method were significantly different ($P<.018$) at 95% CI. This information can help guide people working on mosquito vectors for different diseases, to choose the method(s) most appropriate for their specific species. For example, from this study we have determined that the spraying method would be more suitable for *Aedes* and *Ficalbia* species (Figure 2) and light traps are the most suitable for all species.

The results of *W. bancrofti* infection status in mosquitoes showed that mosquito species of *An. gambiae sl.*, *An. funestus*, and *Cx. quinquefasciatus* had the capacity for transmitting *W. bancrofti* in the study area, according to dissection and *W. bancrofti* DNA detection. While the microfilaria infection rates were not significantly different using the PCR and dissection methods, however, the dissection method was labour intensive, time consuming, and tiresome as compared to PCR method.

Study Limitations

This study did not consider comparing different seasons of the year. Data on amount of rainfall and the number of mosquitoes obtained was not gathered.

CONCLUSIONS

In this study, light traps, were found to be the most appropriate for indoor mosquito collection, since they were capable of obtaining most mosquitoes within various genera with different abdominal status (fed, half gravid, and gravid), which have a high chance of having microfilariae. Indoor collection of mosquitoes by light traps using humans under bed nets as bait was sufficient for collecting mosquitoes needed for accurate estimation of disease transmission indices. However, combining 2 or more collection methods is ideal for accurate estimation of the disease dynamics.

Acknowledgements: We acknowledge the support we received from Gideon Maume and Festus Kalama for their great help in fieldwork. We are thankful for Dunstone Bett and Ruben Lugaria who assisted with mosquito identification. We are grateful to Ahmed Muhamud and Jacinta Muli for their assistance in mosquito dissection and molecular assays. We are also grateful to Karimi Njoki for her assistance in statistical data analysis by SPSS. We also acknowledge the chief and the village elders of the villages in Tana River/Delta County and the entire community for their cooperation and support. The funding for this study was part of the International Atomic Energy Agencies funded projects under KEN 6014 TC project. This paper is published with the permission of the director of the Kenya Medical Research Institute.

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Peer Reviewed

Competing Interests: None declared.

Received: 1 Dec 2016; **Accepted:** 3 Nov 2017

Cite this article as: Mutanu Kinyatta N, Wangui Ng'ang'a Z, Kamau L, Mwaniki Kagai J. Comparison of Indoor Mosquito Collection Methods in the Assessment of Lymphatic Filariasis Transmission Dynamics in Mosquito Vectors in Tana River County, Kenya. East African Health Res J. 2018;2(1):58-66. https://doi.org/10.24248/EAHRJ-D-16-00401

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Occupational Practices and Hazards of Rural Livestock Keepers in Uganda: A Cross-Sectional Study

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ABSTRACT

Objective: In Uganda, 70% of rural poor rely on livestock for subsistence, to meet social obligations, and to insure against disaster. Livestock farming in Africa is in a state of transition from traditional management systems toward intensified modern systems, calling into question the future of traditional systems. To inform this debate, we conducted a survey in Moyo District, Uganda, to describe occupational practices and hazards of agropastoralist livestock keepers.

Methods: Household surveys were administered to heads of household (N=49) from July to September 2016. Cross-sectional data were used to generate descriptive statistics for livestock-associated practices and exposures. Logistic regression was used to estimate odds ratios and Wald-type 95% confidence intervals for risk factors for injury, defined as any animal-related injury in the household in the past year. Risk factors studied were total number of male animals; number of male cattle, sheep/goats, and pigs; proportion male by herd size; herd size; and castration practices.

Results: Adult men perform most livestock-associated tasks, while women, girls, and boys prepare meat, milk cattle, care for poultry, and dispose of waste. While 31 (63%) of households use professional veterinary services and most (n=28, 57.2%) are familiar with zoonoses, 25 (53.2%) do not believe sick animals may look healthy. Over 85.0% (n=41) of respondents routinely wash their hands, while only 31 (64.6%) use soap. Twenty-eight (57.0%) reported using personal protective equipment, while none used gloves or face protection. Most respondents had contact with animal waste "often", and had contact with urine and blood "sometimes". Six (12%) reported a needlestick injury while treating an animal, and 22 (45%) reported at least 1 injury from an animal. No significant association was found between the risk factors studied and animal injury, after adjustment for confounders.

Conclusions: Occupational risks for female and young agropastoralists are distinct from those of men. Contact with potentially infectious material is common and current practices – handwashing without soap and low glove use – do little to prevent zoonotic transmission. While agropastoralists are familiar with zoonoses, subclinical infections may be missed. While no significant risk factors were identified for animal injury, both animal and needlestick injuries are common. As livestock agriculture intensifies, these hazards will become more pronounced; drivers of risk behavior and animal injury must be identified to inform interventions to improve the occupational health of rural livestock keepers in Uganda.

BACKGROUND

As many as 400 million individuals are engaged in rural livestock keeping in Africa.[1] These keepers rely on livestock for subsistence, to meet social obligations, and to insure against disaster. In Uganda, over 70% of households are engaged in livestock rearing,[2] many as members of rural pastoralist and agropastoralist communities. In response to a rapid increase in demand for animal-source food, it has been proposed that livestock farming in Africa transition toward intensified management systems to more efficiently use land and resources,[3] calling into question the role that pastoralism will play in the future of agriculture in Africa.

Livestock keepers are routinely exposed to a variety of hazards. While the hazards of animal husbandry in industrialized settings have been well-documented, including zoonotic diseases[4,5] and injuries,[6,7] the risks for rural livestock keepers in Africa remain largely unstudied.

Little has been documented about the hazards traditional livestock keepers face. To better understand the

risks and benefits of traditional livestock-keeping systems and describe the occupational practices and hazards of agro-pastoralist livestock keepers, we conducted an occupational health questionnaire in rural livestock-keeping communities in Moyo, Uganda, from July through September of 2016. The Moyo district has a population of approximately 140,000, 50.4% of whom are female and 55.5% under 17 years of age. Over three-quarters (76.3%) of the population is engaged in livestock farming and two-thirds (68.4%) of the population over 18 years old is literate.[8]

METHODS

Study Design and Setting

This study was nested within the larger Syndemic Relationships Among Human Diets and Livestock Associated Zoonotic Diseases study implemented by Veterinarians Without Borders, a cross-sectional study that evaluated the risk factors for and frequency of zoonotic disease transmission in rural livestock keeping communities in Iganga, Arua, Adjumani, and Moyo districts in Uganda. The parent study selected the Iganga district as it was thought to be representative of the Busoga region – a region of interest to local collaborators – while the Arua, Adjumani, and Moyo districts were thought to collectively represent the West Nile Region, a region with high endemicity of the diseases of interest to this study. The parent study collected household survey data relevant to livestock keeping and nutrition as well as human morphometric data and human and cattle tuberculosis skin test data. This survey was administered while parent study data collection was in Moyo District in northwestern Uganda.

Recruitment of Households

In each district, subcounties, parishes, and villages were identified for sampling by discussion with local government officials, typically district veterinary officers (DVOs), district health officers (DHOs), and/or animal health officers (AHOs). The number of selected subcounties, parishes, and villages varied with the size of the corresponding district and study resources at the time of sampling.

Sampling in Moyo district was conducted in 2 phases, with 2 subcounties sampled in the first phase and 3 in the second. Larger subcounties containing more livestock were preferentially selected in both phases. In the first phase, households within selected subcounties were mobilized to bring their animals to a central location, without specific selection of parishes or villages. A total of 5 parishes and 23 villages were sampled in this phase. In the second phase, 5 parishes and 10 villages were selected, prior to mobilization of households within these villages. Within the selected villages, households were selected and mobilized by the same government official or a colleague, with study administration beginning within a few days of recruitment. All livestock-owning households within a selected village were eligible for participation.

Data Collection

An animal worker health questionnaire was developed by the authors as a component of the Global Assessment of Zoonotic Exposure Risks (GAZER) study, a multicomponent survey for populations with close contacts with animals. Many of the individual survey items were drawn from previously validated surveys including the National Health and Nutrition Examination Survey[9] and Patient Health Questionnaire-9.[10] This component was reviewed by experts prior to piloting (Appendix A), and these analyses include several variables from the parent study (Appendix B). The tool was piloted in 6 households in Metu subcounty in July 2016. Modifications were made to improve understanding and reduce sensitivity of questions. Questionnaires were prepared in English and administered in Ma'di.

Surveys were completed by in-person interviews with heads of household; all data was self-reported. Administration of the interviews was performed by trained bilingual local members of the study team who simultaneously translated the survey into the local language. We performed all study procedures at participant homesteads after administration of the parent study's survey.

While the questionnaire was primarily comprised of questions that provided only coded check/tick box answers, several questions allowed text entry if the box for "other" was selected. For questions pertaining to delegation of livestock-associated tasks, respondents completed a table identifying tasks commonly performed, the household member usually performing that task (identified by gender and age), and the frequency that the task is performed within the household. With regards to injury variables, respondents were asked about their personal history of needlestick injury and animal-related injuries among household members and in the respective village. Animal injury was defined as any injury caused directly by an animal, such as a bite, kick, or gore, that occurred in the household within the past year.

Livestock keepers preparing to cast a cow in Dufile Subcounty, Moyo District. © 2016. J Meisner

Analyses

We entered completed surveys into Excel (Microsoft Cooperation, Redmond, Washington, USA) and used R v3.2.2 for all further analyses. Univariate analyses were used to generate descriptive statistics for household demographics, herd management practices, delegation of livestock-associated tasks, veterinary care practices, zoonotic disease awareness, personal protective equipment (PPE) use, exposure to animal excreta, and history of needlestick or animal injury. Correlation coefficients were then generated to describe the association between handwashing practices and animal contact as an exploratory analysis.

Logistic regression was used to estimate odds ratios and Wald-type 95% confidence intervals (CI) for risk factors for animal injury, defined as any injury to a household member in the past year that was caused directly by an animal, and adjusted for confounders. Risk factors studied were total number of male animals; number of male cattle, sheep/goats, and pigs; proportion of herd comprised of males; herd size; and castration practices. Potential confounders evaluated – categorized as in Table 1 – were animal breeds kept (local, local-exotic cross, exotic, or both), household size (number of individuals residing in the household), management system (communal grazing, tethering, combination, or other), addition of new stock within the past year, distance between residence and kraal as perceived by respondent (close, far or very far), respondent education (none, primary, secondary, tertiary, or diploma), respondent occupation (free text), co-housing with animals at night (yes or no), and frequency of animal-associated tasks (mean frequency score over the 9 tasks studied). Confounders were identified via an *a priori* approach. Variables identified *a priori* as independent causes of the outcome of interest – animal injury – were considered as possible confounders, and a directed acyclic graph (DAG) was constructed using DAGitty.net to determine the minimal sufficient adjustment set and avoid over-adjustment[11]; this DAG can be provided on request. Confounders identified in this way were then examined for association with the exposure of interest in the dataset on the basis of correlation coefficients for continuous variables, bivariate frequency tables for nominal categorical variables, and logistic regression coefficients for binary variables.

Ethical Approvals

The parent study was approved by the Mildmay Uganda Research and Ethics Committee (REC REF 0406-2015) and registered with the Uganda National Council for Science and Technology (approval #1830). Written informed consent was obtained from all participants of the parent study. As completed surveys did not contain any participant identifiers, the Human Subjects Division of the University of Washington determined this activity to constitute "non-engagement" with human subjects.

RESULTS

Household Demographics and Herd Management Practices

Questionnaires were administered to 49 households, with a mean size of approximately 10 individuals and a mean herd size of 72 head. Almost all respondents (n=46, 97.9%) were peasant farmers, and most had primary school education (n=36, 76.6%). Local cattle breeds were the most commonly kept (n=41, 87%) and communal grazing was the most commonly-used management system (n=40, 85%). Most (n=41, 87%) of households live "far" from the used kraal, and almost all households (n=45, 98%) did not keep livestock in the home at night. Most respondents (n=33, 70%) have not purchased new stock in the past year. All respondents owned at least 1 male animal, and 45 (97.8%) of respondents owned at least 1 intact (non-castrated) male animal (data not shown). The mean number of male animals kept was 13, or 23% of the herd. The predominant male species kept was cattle, followed by sheep and goats, then poultry, and lastly pigs. On average, survey respondents spend 6.5 hours per day caring for their livestock (Table 1).

Livestock-Associated Tasks

Most livestock-associated tasks were performed by adult men, including herding (n=47, 95.9% of households that commonly perform this task), assisting in animal birthing (n=27, 100%), treating injured or ill animals (n=26, 100%), milking animals (n=38, 82.6%), and ploughing crops with oxen (n=27, 93%). Women and girls more commonly provided care for poultry, while adult women also prepared meat for home consumption, disposed of animal and human waste, and provided supplementary feeding to livestock. Male children were reported to herd livestock to grazing sites, provide care for poultry, milk cattle, perform crop ploughing using animal traction, and dispose of human and animal waste (Figure 1).

Most households reported they care for poultry and milk cattle daily, move herd livestock to grazing areas weekly, and prepare meat for home consumption less than once per month. Almost a quarter (n=22, 45%) of households reported they do not assist in animal birthing, while almost two-fifths (n=19, 39%) perform this task less than once per month. More than a third (n=17, 36%) of households reported they do not plough crops with animal traction, while less than a third (n=15, 32%) perform this task weekly. Almost half (n=21, 45%) of households do not dispose of waste, while over a third (n=17, 36%) perform this task daily. Most households did not provide supplementary feeding (n=40, 82%), treat animal injury or illness (n=23, 47%), drive animals to market (n=49, 100%), or butcher animals (n=48, 98%) (Table 2).

TABLE 1. Demographic and Herd Management Variables From 49 Respondents Corresponding to 49 Households

Variable (missing)	All (N=49) n (%)	Injury Households[a] (N=22) n (%)	Non-injury Households[a] (N=27) n (%)
Individuals			
Occupation of respondent (2)			
Peasant farmer	46 (97.9)	21 (100)	25 (96.2)
Schoolteacher	1 (2.2)	0 (0)	1 (3.8)
Education level of respondent (2)			
None	4 (8.5)	1 (4.8)	3 (11.5)
Primary	36 (76.6)	16 (76.2)	20 (76.9)
Secondary	4 (8.5)	3 (14.3)	1 (3.8)
Tertiary	2 (4.3)	1 (4.8)	1 (3.8)
Diploma	1 (2.1)	0 (0.0)	1 (3.8)
Hours per day spent caring for livestock (2)	6.5 (2.1)*	6.7 (1.4)*	6.3 (2.6)*
Households			
Household size (5)	9.7 (4.7)*	9.3 (4.0)*	10.0 (4.5)*
Herd size (0)	72 (65)*	70.5 (65.6)*	73.3 (65.6)*
Male animals (0)	12.9 (11)*	12.1 (11.9)*	13.6 (11.0)*
Percent of herd comprised of male animals (2)	23 (19)*	22.3 (18.7)*	23.4 (18.7)*
Cattle (3)	41 (46)*	47.1 (57.9)*	36.7 (34.0)*
Male cattle (13)	7.7 (9.9)*	9.4 (14.5)*	6.6 (5.4)*
Sheep and goats (2)	20.3 (32)*	17.1 (18.4)*	23.0 (39.6)*
Male sheep and goats (9)	4.9 (7.4)*	4.5 (5.3)*	5.1 (8.8)*
Pigs (2)	1.8 (2.4)*	1.9 (3.0)*	1.6 (1.7)*
Male pigs (2)	0.9 (1.4)*	1.0 (1.8)*	0.8 (1.1)*
Poultry (2)	12.6 (13)*	10.0 (8.3)*	14.8 (15.6)*
Male poultry (12)	3.3 (3.8)*	2.5 (2.0)*	3.9 (4.6)*
Management (2)			
Communal grazing	40 (85.0)	20 (95.2)	20 (76.9)
Combination	5 (11.0)	1 (4.8)	4 (15.4)
Tether	2 (4.3)	0 (0.0)	2 (7.7)
Breed (2)			
Local	41 (87.0)	20 (95.2)	21 (80.8)
Local–exotic cross	4 (8.5)	1 (4.8)	3 (11.5)
Local and crosses	2 (4.3)	0 (0.0)	2 (7.7)

Continued

TABLE 1. Continued

Variable (missing)	All (N=49) n (%)	Injury Households[a] (N=22) n (%)	Non-injury Households[a] (N=27) n (%)
New stock (2)			
No	33 (70)	13 (61.9)	20 (76.9)
Yes	14 (28)	8 (38.1)	6 (23.1)
Kraal distance (2)[b]			
Far	41 (87)	19 (90.5)	22 (84.6)
Close	6 (13)	2 (9.5)	4 (15.4)
Co-housing with animals at night (3)			
No	45 (98)	21 (100)	24 (96)
Yes	1 (2)	0 (0)	1 (4)
Mean frequency score of livestock-associated tasks[c]	3.4 (0.5)*	3.3 (0.4)*	3.5 (0.5)*

Notes: "Missing" refers to the number of observations for which this variable was not recorded.
* Mean (standard deviation).
[a] Injury households are those reporting an animal injury to a household member in the past year, as reported by one respondent per household; non-injury households are those reporting no such history.
[b] Qualitative difference, as perceived by household respondent.
[c] 1=every day, 2=every week, 3=every month, 4=less than once per month, 5=never.

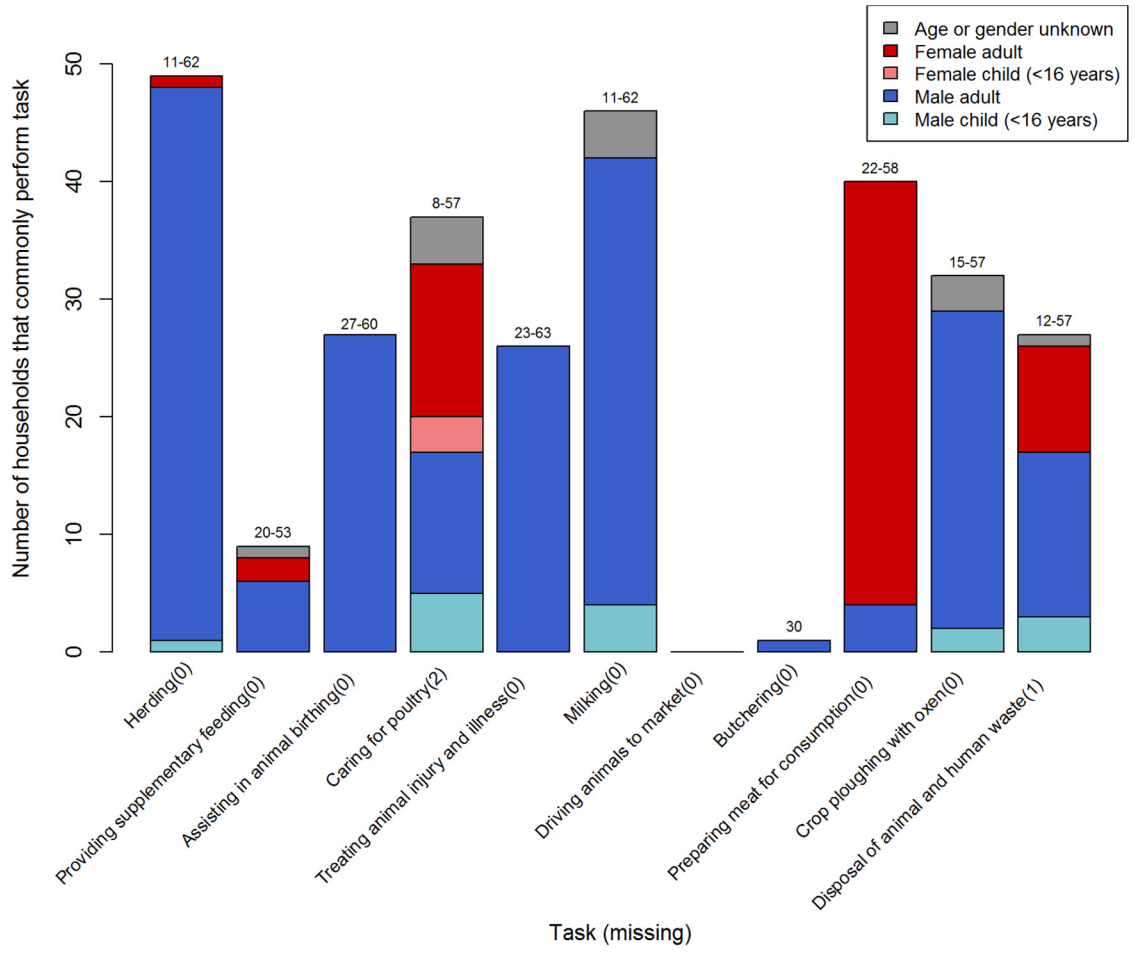
Hygiene Practices and Exposure to Potentially Infectious Material

Most respondents (n=28, 57%) commonly use PPE – typically gumboots. With regard to handwashing, 41 (85.4%) respondents reported they routinely wash their hands; of this group, 31 (75.6%) using soap and water, 8 (19.5%) using water only, and 2 (4.9%) using another substance, such as ash (Table 3). All respondents reported they "often" wash their hands before eating (data not shown). Most respondents report handwashing as "often" after milking (n=41, 89%), before handling meat (n=29, 63%), after processing meat (n=21, 62%), after assisting with animal birth (n=18, 58%), and after handling blood, urine, or manure (n=24, 57%). Respondents less commonly washed hands before drinking (sometimes/rarely: n=22, 45%; never: n=12, 25%), after butchering animals (sometimes/rarely: n=3, 33%; never: n=2, 22%), and after touching animals (sometimes/rarely: n=32, 70%; never: n=3, 6.5%). Note, all frequency measures such as "routinely" and "often" were defined by the respondent.

Respondents reported they "often" had contact with faeces (n=38, 78%), and "sometimes/rarely" had contact with blood (n=25, 76%), urine (n=30, 61%), animal flesh (n=39, 81%), and/or animal fluids (n=31, 63%) (data not shown). Both general handwashing practices (commonly performed yes/no) and frequency of handwashing after animal contact were positively and significantly associated with frequency of contact of blood (general handwashing: correlation coefficient [r]=0.32; 95% CI, 0.03 to 0.55 and handwashing after animal contact: r=0.36; 95% CI, 0.09 to 0.57) and animal fluids (general handwashing: r=0.33; 95% CI, 0.05 to 0.56 and handwashing after animal contact: r=0.36; 95% CI, 0.09 to 0.59), although this association was not strong (Table 4).

Most respondents (n=28, 57.2%) believed diseases could be transmitted from animals to humans, however, only half of these 28 believed transmission could go in the reverse – from humans to animals. Most did not believe that sick animals can look healthy (n=25, 53.2%), and almost all believed they can tell when their animals are sick (n=48, 98%). In 31 (63%) households, respondents reported that a veterinary professional provides care for their livestock, while in the remaining households, a household member provides veterinary care (n=15, 31%) or both household members and veterinary professionals provide veterinary care (n=3, 6%). It is important to note that these data did not distinguish between preventative care versus care for sick animals, nor frequency at which such care was provided. In households where a household member provides veterinary care, all reported that an adult male household member (mean age 44.3 years old, range 23 to 76 years) performs this task (data not shown).

FIGURE 1. Division of Livestock-Associated Tasks Within Households (N=49)



Division of livestock-associated tasks within households by gender and age of household member. Numbers in parentheses represent the number of observations for which this variable was not recorded. Numbers at the top of each bar represent the age range (across households) of the household member that typically performs that task. The height of each bar represents the total number of households that commonly perform a given task, while the color of each bar represents demographic characteristics of the household member who most commonly performs that task.

Needlestick and Animal Injury

Six (12%) respondents – or 40% of the 15 respondents residing in a household in which a household member provides veterinary care – reported any history of a needlestick injury to themselves (Table 5), without time bounds. Twenty-two (45%) households report injury to household members caused by animals within the past year, with most injuries being identified as gores (54.5%) (Table 5). Four households (8.2%) reported more than 1 injury within the past year: 2 muscular strains, 1 gore, and 1 scratch; and 1 household reported 4 injuries in the past year. In all households with a history of injury, the injured household member was male, with mean age of 38 years old and age range of 10 to 60 years. Most injuries were caused by cattle (86.0%) and female animals (77.8%) (Table 5). A total of 7 respondents reported any household history of serious injury, with 6 of these being open wounds, all caused by cattle, and most caused by female animals (n=4, 80%) (Table 5). Sixteen households reported knowledge of serious animal injury in the village, with 12 of these being open wounds, and most caused by cattle (n=11, 78.6%); however, only half of those injuries were caused by female animals (Table 5).

TABLE 2. Frequency at Which Households Report Performing Livestock-Associated Tasks

	Number of Households (%)				
Task (missing)	**Not Performed**	**Daily**	**Weekly**	**Monthly**	**Less Than Once per Month**
Herding (0)	0 (0.0)	17 (35.0)	25 (51.0)	6 (12.0)	1 (2.0)
Supplementary feeding (0)	40 (82.0)	3 (6.1)	3 (6.1)	2 (4.1)	1 (2.0)
Assist in animal birthing (0)	22 (45.0)	1 (2.0)	0 (0.0)	7 (14.0)	19 (39.0)
Caring for poultry (2)	10 (23.0)	34 (77.0)	0 (0.0)	0 (0.0)	0 (0.0)
Treating animal injury and illness (0)	23 (47.0)	1 (2.0)	5 (10.0)	8 (16.0)	12 (24.0)
Milking (0)	4 (8.3)	26 (54.0)	14 (29.0)	3 (6.3)	1 (2.1)
Driving animals to market (0)	49 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Butchering (0)	48 (98.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)
Preparation of meat for home consumption (0)	9 (19.0)	4 (8.5)	7 (15.0)	11 (23.0)	16 (34.0)
Crop ploughing with oxen (0)	17 (36.0)	8 (17.0)	15 (32.0)	6 (13.0)	1 (2.2)
Disposal of human and animal waste (1)	21 (45.0)	17 (36.0)	5 (11.0)	0 (0.0)	4 (8.5)

Notes: "Missing" refers to the number of observations for which this variable was not recorded. Printed surveys defined these tasks as detailed in Appendix A. Columns denote the number and percent of households that perform a given task at a given frequency.

Regression

After adjustment for confounders, the odds of animal injury in the household in the past year was higher in households that kept a greater number of male pigs (OR 1.05; 95% CI, 0.94 to 1.17), in households that kept a herd with a greater proportion of males (OR 1.33; 95% CI, 0.25 to 7.02), and in households that ever versus never castrated male animals (OR 1.15; 95% CI, 0.72 to 1.83), however, none of these associations were statistically significant (Table 6). These data did not provide any other evidence of association between the exposures studied and animal injury.

DISCUSSION

Our study found animal injury and needlestick injury to be common, task delegation to be distinct between men and women and adults and adolescents, contact with potentially infectious material to be common, and both handwashing with soap and glove use to be uncommon. Furthermore, while rural livestock keepers in Uganda appear to be generally familiar with zoonoses, most do not recognize the zoonotic risk of subclinical infections.

Most of the previous studies on rural livestock-keeping communities in Africa have focused their research on zoonotic diseases,[12] with minimal attention paid to work practice-related risks. An exception to this is a 2-part review of the occupational risks of livestock and crop farmers in The Gambia by Kuye et al.[13,14] In their report, 80% of farmers reported a work-related injury in the past year, which was far higher than our results. While our study did not ask specifically about whether animal injuries were incurred in the context of work, we considered all animal keepers as workers, and it is reasonable to assume animal injuries did not occur outside of the context of animal keeping. While our survey asked specifically about animal-origin and needlestick injury – rather than all work-related injuries – our animal-injury question pertained to the entire household and our needlestick-injury question did not define time bounds, suggesting questionnaire differences should result in higher reporting in our study. It is not possible to conclude if this discordance can be explained by questionnaire structure, by biases in 1 or both studies, or by differences between Gambian and Ugandan farmers.[12–14]

To our knowledge, there have been no previous efforts to describe the frequency with which livestock-associated tasks are performed in this setting, though limited prior efforts have been made to describe the delegation of livestock-associated tasks within households in rural African communities. A review of literature from Zimbabwe found that men are usually responsible for outside work and women are responsible for inside work and the feeding of animals; domestic fowl are mainly owned by women and boys are responsible for milking and herding animals.[18] This is largely consistent with our finding that the majority of livestock-

TABLE 3. Handwashing and PPE Practices As Reported By One Respondent Per Household

Behavior (missing)	n (%)
PPE use (0)	
Commonly used	28 (57)
Gumboots	14 (50.0)
Gumboots and raincoat	5 (21.4)
Gumboots and overalls	4 (14.3)
Raincoat	3 (10.7)
Gumboots, overalls, and raincoat	1 (3.6)
Not commonly used	21 (43)
Handwashing (1)	
Typically done	41 (85.4)
Soap and water	31 (75.6)
Water only	8 (19.5)
Other (typically with ash)	2 (4.9)
Typically not done	7 (14.6)

Note: "Missing" refers to the number of observations for which this variable was not recorded.

associated tasks are completed by men, with women and girls caring for poultry, feeding livestock, and performing household-related tasks, and with boys herding livestock and milking cattle. However, our study also found that boys may also perform crop ploughing, provide care for poultry, and dispose of human and animal waste, suggesting there is not a clear delineation between tasks commonly performed by women and girls and those performed by boys. Notably, our study found caring for poultry, milking cattle, and herding livestock to be the most commonly performed tasks, suggesting that women and children frequently contact livestock. While these data do not allow conclusions to be drawn regarding specific hazards arising from this contact, they do suggest that future efforts to enumerate exposure to animal-related hazards in this setting should include women and adolescents.

Despite the fact that all respondents keep male animals, and almost all keep intact/non-castrated male animals, most injuries were caused by female animals and cattle. This may be due either to greater contact with female cattle than other animals, or to behavioral differences between male and female animals. No evidence was found to suggest that keeping of male animals, castration practices, and herd size are risk factors for animal injury, in contrast with findings from the United States.[6] This may be the result of temperamental differences in Ugandan vs. U.S. male livestock – due to breed or husbandry differences – or biases in study design. It is not unreasonable to hypothesize that in a setting where male animals are kept with the female and juvenile herd and handled frequently, they are more socialized and less dangerous, although, to our knowledge, no studies exist on male animal behavior in a rural African livestock keeping setting. While this study found that most victims of injury were men, livestock development interventions are increasingly targeting women to alleviate gender disparities[15] and promote agricultural development; as livestock keeping transitions towards women, women may be at greater risk of animal injury.

This study showed that rural livestock keepers in Uganda are commonly exposed to animal faeces, and that while most respondents wash their hands regularly, nearly one-quarter of respondents do not use soap and most do not wash their hands after touching animals, putting them at

TABLE 4. Correlation Between Handwashing Practices and Frequency of Contact With Animal Excreta, Expressed As Pearson's Product Moment Correlation Coefficient

	Blood (r, 95% CI)	Urine (r, 95% CI)	Faeces (r, 95% CI)	Flesh (r, 95% CI)	Fluids (r, 95% CI)
General handwashing	0.32 (0.03, 0.55)	0.24 (-0.05, 0.50)	-0.07 (-0.34, 0.22)	-0.10 (-0.38, 0.19)	0.33 (0.05, 0.56)
After animal contact	0.36 (0.09, 0.58)	0.17 (-0.11, 0.43)	0.21 (-0.07, 0.47)	-0.21 (-0.40, 0.07)	0.36 (0.09, 0.59)

Notes: General handwashing defined by yes (0) vs. no (1) answer to the question "Do you routinely wash hands after touching your animals". Handwashing after animal contact defined by categorical answer to the question "How often do you wash your hands after touching an animal": often (1), sometimes (2), rarely (3), never (4), not applicable (5). Abbreviations: CI, confidence interval; r, correlation coefficient.

TABLE 5. History of Needlestick Injury or Animal Injury, as Reported by One Respondent Per Household

Injury (missing)	n (%)
Needlestick (0)	
Never	32 (65.3)
Do not give injectable medications to animals	10 (20.4)
Ever	6 (12.2)
Don't know	1 (2.0)
Animal: any, household, this year (0)	
No	27 (55)
Yes	22 (45)
Injury type (0)	
Gore	12 (54.5)
Muscular strain	6 (27.5)
Bite	2 (9.1)
Scratch	1 (4.5)
Crush	1 (4.5)
Animal species (0)	
Bovine	19 (86.0)
Swine	2 (9.1)
Canine	1 (4.5)
Animal sex (4)	
Female animal	14 (77.8)
Male animal	4 (22.2)
Animal: serious, household, ever	
Injury (4)	
None	38 (84.4)
Open wound/no death	6 (13.3)
Fracture/no death	1 (2.2)
Death	0 (0.0)
Animal species (2)	
Bovine	5 (100)
Animal sex (1)	
Female	4 (80)
Male	2 (20)
Animal: serious, village, ever	
Injury (7)	
None	28 (61.9)
Open wound/no death	12 (28.6)
Fracture/no death	3 (7.1)
Other	1 (2.4)
Death	0 (0.0)
Animal species (2)	
Bovine	11 (78.6)
Canine	3 (21.4)
Animal sex (2)	
Female	7 (50)
Male	7 (50)

Note: "Missing" refers to the number of observations for which this variable was not recorded.

risk for zoonoses transmitted through the animal faeces. While over half report PPE use, none of the PPE types listed provide respiratory and mucous membrane protection, and gloves are not worn. Compounding this lack of rigorous PPE use and hygiene standards is the finding that over half of our respondents did not believe that sick animals may look healthy. While most respondents were aware of zoonoses, and almost two-thirds use professional veterinary services to provide care for their livestock, this knowledge and practice will not prevent transmission of zoonoses, such as brucellosis, from animals that have no clinical signs.

Limitations

There are several limitations to this study. The sample size was small, with only 49 households surveyed. All analyses were complete case, meaning that observations or variables with missing data were dropped from analyses. This could introduce bias if missingness is not completely at random, that is if missingness is the result of observed or unobserved variables. While less than 5% of observations were missing for most variables, higher multivariate missingness is a concern in the regression models. Furthermore, even if missingness is completely at random, the loss of observations with missing data compromises the already limited precision of this study. Additionally, households were selected by convenience sampling. This may have introduced selection

TABLE 6. Association Between Herd Management Variables and Any Animal-Associated Injury in the Household in the Past Year

	Adjusted	
	OR	**95% CI**
Number of males kept	1.00[a]	0.99, 1.02a
Number of male cattle	0.99[b]	0.96, 1.03[b]
Number of male sheep/goats	0.99[c]	0.96, 1.02[c]
Number of male pigs	1.05[d]	0.94, 1.17[d]
Proportion male	1.33[e]	0.25, 7.02[e]
Herd size	1.00[f]	1.00, 1.00[f]
Castration	1.15[g]	0.72, 1.83[g]

[a] Adjusted for herd size, number of household residents, breed
[b] Adjusted for herd size, breed
[c] Adjusted for herd size, number of household residents, co-housing with animals at night
[d] Adjusted for new stock
[e] Adjusted for number of males, herd size, co-housing with animals at night
[f] Adjusted for number of male animals, number of household residents
[g] Adjusted for proportion males, breed, management system, occupation
Abbreviations: CI, confidence interval; OR, odds ratio.

bias if the households selected to participate differed in their practices or exposures than those not selected. It should be noted that household recruitment was not specific to this questionnaire, but to the parent study, and, as a result, potential bias arising from this is difficult to quantify.

Information bias may arise due to the self-reported nature of these data and the need to administer this questionnaire in the Ma'di language. While the questionnaire was reviewed by Ugandan and U.S. experts in occupational health and veterinary medicine, validation of translation was not performed. While an author was present for questionnaire administration and able to answer translator questions as they arose, formal cognitive interviewing of respondents or translators was not performed. Information bias may also result from the household-level nature of questionnaire administration, as only 1 member of each household – typically the head of household – completed the questionnaire. This imparts a multilevel quality to these data, as some questions pertained to household practices and exposures while others pertained to the individual's exposures and practices. Where the individual is answering for the household, potential biases may arise if the individual's understanding of other household members' practices or exposures is inaccurate. Typically, other household members were present while the questionnaire was administered, but we are not certain to what extent these individuals were consulted for answers. It is difficult to predict the possible direction of these resultant biases, thus bias away from the null or to the other side of the null cannot be ruled-out.

Finally, the regression analyses conducted inherit the limitations of any cross-sectional study, which is an uncertain temporal relationship between outcome (animal injury) and the exposures (herd composition and castration practices). As it seems unlikely that a household's history of animal injury causes changes in herd composition or castration practices, we do not think this is an important limitation.

CONCLUSION

Currently, traditional livestock-keeping systems predominate in Uganda, with 16 times more workers engaged in the subsistence agricultural sector than the commercial sector.[2] However, agriculture in Africa is trending toward intensified management systems – where more animals are kept per unit area and per worker – and the inclusion of women, and away from male-dominated traditional livestock-keeping systems. With this change will likely come an intensification of the hazards already present and extension of these hazards into previously unexposed demographic groups. In Uganda, the agricultural sector is within the purview of the Occupational Safety and Health Act, 2006 (No 9).[16] This study demonstrates that despite this appropriate legislative framework, agricultural injury and illness are common. These findings should motivate future research efforts to identify reasons for injury and illness and predict the impact of intensification on the health of rural livestock keepers, so that effective interventions can be developed and targeted to appropriate worker groups. Collaboration with qualitative researchers should be sought to better understand what tasks performed, describe how they are performed, and understand the drivers for task delegation and risky behaviors, such as low PPE use. Livestock keeping brings wealth, means of transport, financial security, and animal-source nutrition to rural communities. However, with these benefits come the necessity of providing animal husbandry and the attendant risks of this work. When considering the future of agriculture in Africa, attention must not be solely focused on food security and economic development at the expense of the health of communities that would otherwise benefit from these gains.

Acknowledgements: Nyolimati Ceaser Adibaku, DVM, for his assistance in survey development, pretesting, and administration. This research was supported by The Stergachis Endowed Fellowship for International Exchange, Department of Global Health, University of Washington, Seattle, Washington, USA; grant 2T42OH008433-11, Centers for Disease Control and Prevention; Farmer to Farmer Cooperative Agreement No. AID-OAA-A-13-00053 Sub-Award Number: PDP 2014-3, USAID;

Veterinary Consulting Services Inc., 1124 Pistachio Court, Davis, California, USA. These sources had no role in study design, analysis, interpretation of data, or decision to submit this manuscript for publication. The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of their respective institutions or funding sources.

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Peer Reviewed

Competing Interests: Dr. Graham reports personal fees and non-financial support from Veterinary Consulting Services Inc., during the conduct of the study; and non-financial support from Ellie Laboratory, DairyQuality, and DeLaval Inc., outside the submitted work.

Received: 14 Apr 2017; **Accepted:** 26 Oct 2017

Cite this article as: Meisner J, Curtis K, Graham T, Rabinowitz P. Occupational Practices and Hazards of Rural Livestock Keepers in Uganda: A Cross-Sectional Study. East African Health Res J. 2018;2(1):67-77. https://doi.org/10.24248/EAHR-D-17-00139

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