EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

The East African HEALTH RESEARCH JOURNAL

The basis for better health policy and practice

Volume 1 | Issue 1 **March 2017**

Published in conjunction with the 6th East African Health and Scientific Conference:

Preparedness for and Control of Disease Outbreaks, Epidemics, and Pandemics in the Context of Climate Change, Globalisation and Gaps in Health Systems

29-31 March 2017
Bujumbura, Republic of Burundi

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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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EDITORIAL

A journal to highlight health research in EAC countries

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We are pleased to present The *East African Health Research Journal* (*EAHRJ*), a new no-fee, open-access, peer-reviewed journal that focuses on research conducted in and relevant to East Africa. Established by the East African Health Research Commission (EAHRC) of the East African Community (EAC; www.eac.int), the primary aim of the journal is to present evidence that can be the basis for better health policy and practice in the countries of the EAC. The journal also aims to:

- represent the East African perspective on health and health related-issues,
- provide information that is relevant to the EAC,
- be a platform for sharing and dissemination of knowledge,
- enable scholarly recognition of professionals and institutions,
- support professionals' career development,
- provide forum for health professionals from East Africa to be more visible globally, and
- provide direction in setting up health priorities in the region.

The *EAHRJ* will promote and facilitate the application of knowledge from research to strengthen national and regional health policy and practice; development of human-resource capacities and skills; exchange and dissemination of health-research information; and advocacy of evidence generated from health research.

Issues of the journal will include original articles, reviews, short communications, surveys, commentaries, opinions, book reviews, essays, special and supplementary issues, and reports, and cover a broad range of health and related aspects, including medicine, geo-medicine, dentistry, nursing, pharmacology, toxicology, pharmaceutical science, veterinary science, food science, environment, health-related agriculture science, and public health.

The *EAHRJ* will be published three times a year online, at www.eahealth.org, and in hard copy. Hard copies will be distributed to all relevant stakeholders, such as government institutions and organisations, research institutions, academic institutions, relevant NGOs, civil society organisations, etc.

We are publishing the first issue of the journal in conjunction with the 6th East African Health and Scientific Conference (EAHSC) taking place in Bujumbura, The Republic of Burundi, 29–31 March 2017. The theme of the conference is Preparedness for, and control of disease outbreaks, epidemics, and pandemics in the context of climate change, globalisation, and gaps in health systems. We have aligned the articles published in the first issue of the EAHRJ with the theme of the conference.

We expect that this journal will add value to the various initiatives taken to improve health and wellbeing of the people of East Africa and the world in general.

Cite this article as: Kibiki GS. A journal to highlight health research in EAC countries. East African Health Res J. 2017;1(1):1. http://dx.doi.org/10.24248/EAHRJ-D-17-00074

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COMMENTARY

Beyond the Numbers: Interpreting WHO's *Global Tuberculosis Report 2016* to Inform TB Policy and Practice in the East African Community

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ABSTRACT

By 2000, 5 East African Community (EAC) member states—Uganda, Kenya, Tanzania, Rwanda, and Burundi—had adopted the World Health Organization's (WHO's) policy of directly observed treatment short-course (DOTS) for tuberculosis (TB). This policy is meant to speed up the control of TB through effective diagnosis and treatment. However, the rate of reduction of TB burden has been slow, and as of 2016, 3 EAC member states—Uganda, Kenya, and Tanzania—are still categorised as high TB burden countries. We analysed WHO's *Global Tuberculosis Report 2016* and drew key lessons to inform policy and practice for effective control of TB. From the report, we acknowledge the existence of national TB control policies operationalised through national TB control programmes in all EAC member states. However, we found persistent underfinancing of the TB control programmes; low national coverage of TB diagnostic and treatment services, meaning that many TB cases are most likely going undetected; and deaths due to lack of treatment. We also found poor reporting practices; for example, there was no data on the number of cases detected with rapid diagnostics in Uganda and Tanzania, which was unexpected since there are more than 170 Xpert MTB/RIF machines for rapid diagnosis of TB in the 2 countries. We recommend comprehensive implementation of existing TB policy, including adequate financing, universal access to diagnosis and treatment, and socioeconomic empowerment of affected communities, all of which are critical for ending TB in East Africa and the world at large.

INTRODUCTION

The World Health Organization (WHO) released its most recent global tuberculosis (TB) report in October 2016.[1] This annual report outlines achievements and challenges in the global control of TB in the preceding year and sets out goals and strategies for TB control programmes.[2] The 2016 report reiterates the vision for global elimination of TB by 2035, but also sets out a phased progress evaluation programme focusing on reducing incidence of TB by 20% in 2020, then 50% in 2025, 80% in 2030, and finally 90% (with 95% reduction in TB deaths) in 2035.[3]

The global rate of reduction in TB burden was slow and did not change between 2014 and 2015, which has raised concerns about achieving the 2035 vision. The 2016 report listed 30 high TB burden countries that will be closely monitored for progress and achievement of the end TB targets. Three of the 6 member states of the East African Community (EAC)—Uganda, Kenya, and Tanzania—are on this high TB burden list. (As of 2016, the six member states of the EAC are Burundi, Kenya, Rwanda, South Sudan, Tanzania, and Uganda.) The period from 2016 to 2020 will mark the first phase during which these countries will be monitored for progress towards achieving a 20% reduction of non-HIV-associated, HIV-associated, and drug-resistant TB. We analysed WHO's *Global Tuberculosis Report 2016* and recommend policy action points that could be taken by the EAC to accelerate the end of TB in the region.

TB BURDEN, INCIDENCE, AND MORTALITY IN THE EAC

The *Global Tuberculosis Report 2016* shows that the EAC has a combined TB burden of 388,600 cases

TABLE 1. Tuberculosis Burden in EAC Member States, 2015

Country	Population (Millions)	TB Cases (includes PLHIV)	Males as % of Total TB Cases	Annual TB Mortality (includes PLHIV)
Uganda	39	79,000	66%	11,900
Kenya	46	107,000	62%	16,200
Tanzania	53	164,000	61%	55,000
Rwanda	12	6,600	65%	720
Burundi	11	14,000	63%	3,360
South Sudan	12	18,000	72%	4,160
Total	*173*	*388,600*	—	*91,340*

Abbreviations: EAC, East African Community; PLHIV, people living with HIV; TB, tuberculosis.
Note: Data from the World Health Organization's *Global Tuberculosis Report 2016*.

(see Table 1). This represents 0.23% of the total EAC population of 173 million. There were 91,340 deaths in 2015, which is 24% of the TB cases across the EAC. This rate of mortality is unacceptably high, and it has never been more urgent to find more effective interventions to prevent such deaths. In addition, 32% (124,800) of the TB cases were among people living with HIV (PLHIV). On average, TB prevalence among PLHIV was 25% across the EAC, with a prevalence of more than 30% in Uganda, Kenya, and Tanzania. This means that HIV infection remains the highest risk factor for developing TB disease in this region, and thus efforts to defeat TB must include defeating HIV too.

Important to note also is that more than 60% of the TB patients in each of these countries were men (Table 1). This observation concurs with reports from clinical trials, in which the majority of TB cases are men.[4,5] On a global scale, however, more women than men die of TB. Further research should investigate the biological factors that may underlie different responses to TB infection among men and women, as well as behavioural and cultural practices that increase the risk of contracting TB or limit access to health care.[5–7] The gender-based disparities associated with TB are comprehensively discussed in a 2015 United Nations Development Programme discussion paper, which also highlights actions to be undertaken.[7]

Apart from South Sudan, which came into existence as a nation in 2011, the other 5 EAC member states have seen a decrease in TB incidence and rate of mortality in the last 15 years. In 2000, the average TB incidence across the EAC was 290 per 100,000, and this number fell to 184 per 100,000 population in 2015. This represents a fall of 7.1 cases per 100,000 population per year over 15 years (Table 2). The slow decrease in incidence perhaps explains why Uganda, Kenya, and Tanzania are still on WHO's list of countries with high TB burden. Likewise the average rate of mortality due to non-HIV-associated TB fell from 41 to 24 per 100,000 population in 2015 across the original 5 EAC states. Although the general average shows a decrease in mortality rate, however, Kenya had a 2-point rise, from 18 to 20 deaths per 100,000 population in mortality due to non-HIV-associated TB (Table 2). The WHO report excludes HIV-associated TB mortality, which implies that the mortality rate may have been even higher than reported.

Drug-Resistant TB

The burden of drug-resistant TB is generally low within the EAC. Drug-resistant TB can be categorised as monoresistant—resistance to one anti-TB drug such as rifampicin; multidrug resistant (MDR)—resistance to both rifampicin and isoniaizid; polydrug resistant—resistance to more than one first-line anti-TB drug other than both isoniaizid and rifampicin; and last but not least, extensive drug resistant (XDR)—resistance to any fluoroquinolone and at least one of the 3 second-line injectable anti-TB drugs.[8] The estimated burden of rifampicin-resistant (RR) TB is 2% (7,920/388,600) of the total TB burden in the EAC.

Only 48% of the estimated 7,920 MDR/RR-TB cases in the EAC were reported in 2015, and on average only 30% were confirmed by a laboratory test (Table 3). The low rates of MDR/RR-TB case notification and laboratory confirmation are most likely due to inadequate laboratory capacity for drug sensitivity testing in the EAC states. For example, there are only 3 laboratories with drug sensitivity testing capacity in Tanzania, 4 in Kenya (3 of which are in the Nairobi area), and 3 in Uganda (all of which are in

TABLE 2. Change in TB Incidence and Mortality Rate in EAC Member States, 2000–2015

	TB Incidence			TB-related Mortality		
Country	**2000[a]**	**2015**	**Annual Decrease in Incidence**	**2000[a]**	**2015**	**Annual Decrease in Mortality**
Uganda	300	202	6.5	50	14	2.4
Kenya	300	233	4.5	18	20[b]	−0.1
Tanzania	450	306	9.6	70	56	0.9
Rwanda	100	56	2.9	5	4	0.1
Burundi	300	122	11.9	60	24	2.4
South Sudan	150[a]	146	1.0	30[a]	28	0.5
Average	*290*	*184*	*6.1*	*41*	*24*	*1.0*

Notes: Numbers are per 100,000 population, excluding cases among people living with HIV. Decrease in incidence and decrease in mortality is the change divided by 15 years (4 years for the case of South Sudan). Data from the World Health Organization's *Global Tuberculosis Report 2016*.
[a] South Sudan's estimated numbers are from 2011, not 2000. 2001 is the year South Sudan entered the EAC.
[b] Kenya was the only EAC state to show an increase in mortality from 2000 to 2015.

TABLE 3. Burden of Drug-Resistant TB in EAC Member States, 2015

Country	**Estimated Burden of MDR/RR-TB**	**New MDR/RR-TB Cases Notified**	**New Cases (% of Total)**	**Previously Treated Cases (% of Total)**	**Laboratory-Confirmed Cases (% of Total)**
Uganda	1,900	1,000	2%	12%	25%
Kenya	2,000	1,400	1%	9%	26%
Tanzania	2,600	730	1%	30%	24%
Rwanda	160	120	2%	11%	78%[a]
Burundi	500	190	3%	14%	23%
South Sudan	760	370	7%	8%	5%
Total	*7,920*	*3,810*	*3%[b]*	*14%[b]*	*30%[b]*

Abbreviations: EAC, East African Community; MDR-TB, multidrug-resistant TB; RR-TB, rifampicin-resistant TB; TB, tuberculosis.
Notes: Data from the World Health Organization's *Global Tuberculosis Report 2016*. Previously treated patients are at higher risk of developing drug-resistant tuberculosis.
[a] Rwanda was the only EAC state with a rate of laboratory-confirmed drug-resistant TB greater than 30%.
[b] Values are average percentages.

Kampala). This could be a recent improvement, however, as the 2015 version of the WHO global TB report shows only 1 drug sensitivity testing laboratory in Kenya and Uganda.[3] Also important to note is that in all 6 EAC states, the ratio of MDR/RR-TB to total TB cases, although low among new cases, was high among patients with previous exposure to anti-TB drugs (Table 3). It should become a priority for every TB control programme to ensure that patients (1) are prescribed a suitable regimen and dose, and (2) complete their treatment, to curtail the emergence of more drug-resistant

strains of TB. Better treatment response monitoring tools are crucial to check effectiveness of prescribed therapy.[9,10]

TB Diagnosis

Strong laboratory services are necessary to ensure accuracy of diagnosis and prescription. WHO's *Global Tuberculosis Report 2016* shows low coverage of rapid tests, although more than 50% of the notified TB cases in all EAC states were bacteriologically confirmed (Table 4). A bacteriologically confirmed case is one that has tested positive for TB by either smear microscopy or culture or by rapid molecular tests such as Xpert MTB/RIF and line probe assay.[8] Rapid tests give results in a matter of hours, simultaneously detecting the presence of TB and drug resistance.[11] Low coverage of rapid tests means that TB testing still largely depends on smear microscopy, a method that has been shown to be poor at detecting TB in low-burden patients, particularly children and PLHIV.[12] Coverage of bacterial culture for TB diagnosis and drug sensitivity testing is still low, on average 1 per 5 million population, and thus cannot account for a large proportion of the bacteriologically confirmed cases.[3]

No data on rapid tests was given for Uganda or Tanzania, despite that there are 111 and 74 Xpert MTB/RIF machines, respectively, in these 2 countries.[3] This raises concern over the quality of data collection and reporting, which needs to be addressed. Data quality is crucial to control of TB.[13] Across EAC states, an average of 89% of TB cases had known HIV status, which indicates that the region has a higher testing capacity for HIV than for TB (Table 4).

TB Treatment

The purpose of diagnosis is not accomplished if there is no treatment, and treatment success begins with accessibility. On average, 60% of the EAC has TB treatment coverage. This means that 40% of the population of the EAC has hardly any access to TB treatment. Rwanda posted the highest rate of treatment coverage at 84%, whereas the lowest rate was in Tanzania, at 37% (Table 5). Despite having the least treatment coverage, Tanzania and Burundi achieved 90% treatment success among new TB cases. However, the low treatment coverage in these countries means that many patients may have gone untreated and died. Overall treatment success was lower among previously treated, HIV-associated, and drug-resistant TB cases (Table 5). While recognising the great treatment success achieved among those patients who had access to treatment, the policy ambition should be to expand TB treatment coverage to 100% of the population.

FINANCING OF TB CONTROL PROGRAMMES

All the EAC member states except Burundi had a budget for TB control programme activities in the 2016 financial year. Domestic funding constituted less than 25% in all countries. Unfunded budgets ranged from 0% in Kenya and Rwanda to 55% in Tanzania; although the budget was fully funded in Kenya and Rwanda, most of the funding came from external donors. Funding gaps are common, and EAC states have had yearly budget shortfalls almost every year since 2012, with the unfunded budget sometimes going up to 100% of the total (Table 6).

Going forward, the EAC must strive to increase domestic funding of national TB control programmes to assure citizens of these essential health care services. Bearing in mind the competing demands on the meagre resource envelopes of the EAC states, one realises that domestic funding in the short term can be found in savings made by fostering programme cooperation, such as between HIV and TB control programmes. This could include building well-coordinated

TABLE 4. Proportion of Total Notified TB Cases Tested for TB and HIV, 2015

Country	Total Case Notification 2015	% Confirmed With Rapid Tests	% Confirmed Bacteriologically	% With Known HIV Status
Uganda[a]	43,736	—	71%	91%
Kenya	81,518	10%	59%	82%
Tanzania[a]	62,180	—	53%	92%
Rwanda	5,637	39%	86%	96%
Burundi	6,969	8%	88%	95%
South Sudan	10,250	2%	56%	79%
Average	*35,048*	*15%*	*69%*	*89%*

Note: Data from the World Health Organization's *Global Tuberculosis Report 2016*.
[a] Data for the percentage of cases tested by rapid diagnostic tests in Uganda and Tanzania was missing.

TABLE 5. TB Treatment Coverage by Country and TB Treatment Success by Patient Cohort, 2015

Country	TB Treatment Coverage	Treatment Success Rate (%, *n*)				
		New and Relapsed Cases	Previously Treated Cases	Cases Among PLHIV	MDR/RR-TB Cases	XDR-TB Cases
Uganda	57%	75% (43,628)	67% (2,438)	73% (16,670)	73% (214)	0% (0)
Kenya	76%	87% (89,294)	78% (222)	82% (30,107)	82% (266)	0% (1)
Tanzania	37%	90% (61,573)	81% (1,578)	87% (20,658)	68% (92)	0% (0)
Rwanda	84%	86% (5,846)	80% (94)	—	81% (94)	0% (0)
Burundi	51%	91% (7,309)	82% (83)	86% (901)	89% (38)	0% (0)
South Sudan	54%	71% (8,335)	69% (521)	71% (859)	—	—
Average	*59%*	*83%*	*76%*	*80%*	*76%*	*0%*

Abbreviations: MDR-TB, multidrug-resistant TB; RR-TB, rifampicin-resistant TB; PLHIV, people living with HIV; TB, tuberculosis; XDR-TB, extensively drug-resistant TB.
Note: Data from the World Health Organization's *Global Tuberculosis Report 2016*. In all countries, treatment success rate was lower among previously treated cases than among new cases. Treatment failed for the single XDR-TB case in Kenya.

TABLE 6. Financing of TB Control Programme Activities by EAC Member State, 2015

Country	Budget 2016 (Million US$)	% Domestically Funded	% Externally Funded	% Unfunded 2016	% Unfunded 2012–2015
Uganda	38	4%	78%	19%	20%–80%
Kenya	59	20%	80%	0%	30%–60%
Tanzania	40	5%	40%	55%	60%–100%
Rwanda	15	21%	60%	19%	0%–10%
Burundi	0	—	—	—	20%–100%
South Sudan	12	10%	59%	31%	10%–80%

Note: Data from the World Health Organization's *Global Tuberculosis Report 2016*.

coalitions of stakeholders for coordinated efficient action that results in larger impact. Coordination of local and international players will avoid duplication of interventions and save resources that can be invested elsewhere. Savings can be invested in ensuring a consistent supply of medicines and diagnostics, and in hiring and retaining skilled human resources. As in Rwanda, implementing affordable health insurance premiums could increase access to health care in other member states.[14] Indeed, Rwanda's achievement of 84% TB treatment coverage was the highest in the region. All EAC member states should work towards achieving 15% expenditure on health, as recommended by the African Union. The long-term target should be providing quality health care at the nearest location to the patient and achieving universal health care coverage.

RECOMMENDED ACTIONS TO END TB IN THE EAC

In summary, the following action points, drawn from WHO's *Global Tuberculosis Report 2016*, can enable the EAC to realise the 2035 vision for ending TB:

- Coordinate HIV and TB diagnosis and treatment, which is critical to defeating both diseases. The steps for achieving this coordination are clearly outlined by AVERT's HIV and TB coinfection programmes.[15]

- Invest in building surveillance and data collection capacity to ensure accurate disease burden estimates, which are useful for resource allocation planning.
- Strengthen and expand TB diagnostic and treatment services to cover all citizens. This includes providing the required infrastructure and human resources to deliver the services[16] and investing in postregistration anti-TB-drug clinical trials and affordable diagnostic approaches.
- Implement deliberate socioeconomic empowerment programmes, including health insurance, to increase health care affordability and accessibility.[17,18]
- Commit to increasing domestic funding for health care and reduce overdependency on external donor support.
- Ensure that governments act as team players in harnessing resources by ensuring the coordination and cooperation of local and international stakeholders in health care provision.
- Invest in research that can inform policy. Research into the social determinants of disease and research into new diagnostics and treatments should be equally supported.[19]

The *Global Tuberculosis Report 2016* importantly noted that as far as research is concerned, the diagnostic technology landscape looks quite promising, with new diagnostic platforms, new drugs, and new vaccines in the pipeline. To achieve maximum benefit, the EAC states should, first, not sit and wait to consume finished products but instead actively participate in research to bring these diagnostics, medicines, and vaccines to the clinic. Secondly, EAC states should put in place channels for rapid translation of research into policy and practice. This will happen as long as policy makers go after research evidence from national and international sources to support policies they make.

Thirdly, EAC states should committedly invest in implementing TB policies. As noted regarding TB budget financing, some member states have fallen short of their TB control budget or have not financed it at all. Allocation of resources is what brings a policy to life. Without financing, policies remain 'on the shelf' and are as good as absent. No one benefits from an unimplemented policy.

Acknowledgements: The data presented in this paper is drawn from the World Health Organization's *Global Tuberculosis Report 2016*. The TWENDE consortium is funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) grant No. EDCTP-CSA-2014–283.

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Peer Reviewed

Competing Interests: None declared.

Received: 5 Nov 2016; **Accepted:** 5 Feb 2017

Cite this article as: Sabiiti W, Sabiiti W. Beyond the Numbers: Interpreting the WHO Global Tuberculosis Report 2016 to Inform TB Policy and Practice in the East African Community. East African Health Res J. 2017;1(1):2-7. https://doi.org/10.24248/EAHRJ-D-16-00364

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COMMENTARY

One Health Research in Northern Tanzania – Challenges and Progress

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ABSTRACT

East Africa has one of the world's fastest growing human populations—many of whom are dependent on livestock—as well as some of the world's largest wildlife populations. Humans, livestock, and wildlife often interact closely, intimately linking human, animal, and environmental health. The concept of One Health captures this interconnectedness, including the social structures and beliefs driving interactions between species and their environments. East African policymakers and researchers are recognising and encouraging One Health research, with both groups increasingly playing a leading role in this subject area. One Health research requires interaction between scientists from different disciplines, such as the biological and social sciences and human and veterinary medicine. Different disciplines draw on norms, methodologies, and terminologies that have evolved within their respective institutions and that may be distinct from or in conflict with one another. These differences impact interdisciplinary research, both around theoretical and methodological approaches and during project operationalisation. We present experiential knowledge gained from numerous ongoing projects in northern Tanzania, including those dealing with bacterial zoonoses associated with febrile illness, foodborne disease, and anthrax. We use the examples to illustrate differences between and within social and biological sciences and between industrialised and traditional societies, for example, with regard to consenting procedures or the ethical treatment of animals. We describe challenges encountered in ethical approval processes, consenting procedures, and field and laboratory logistics and offer suggestions for improvement. While considerable investment of time in sensitisation, communication, and collaboration is needed to overcome interdisciplinary challenges inherent in One Health research, this can yield great rewards in paving the way for successful implementation of One Health projects. Furthermore, continued investment in African institutions and scientists will strengthen the role of East Africa as a world leader in One Health research.

INTRODUCTION

Eastern Africa has one of the highest predicted human population growth rates on the planet.[1] It is also home to some of the world's largest wildlife populations, many of which live in close proximity to people and livestock—directly linking people's lives to the health of livestock and the natural environment. The interdependence of human, animal, and environmental health—termed One Health[2]—creates both unique challenges and opportunities that could make East Africa a world leader in the development of One Health research and policy. The instinctive understanding of these interdependencies, together with the maturing of health research institutions and the establishment of One Health research consortia in Africa, provides an opportunity for African scientists and institutions to lead the global One Health agenda. Moreover, in East Africa, One Health is also embraced by policymakers in some countries, such as Tanzania and Kenya, to the extent that they have established One Health departments within their governments.[3,4] These intersectoral units set out not only to establish the interdisciplinary collaborations required for effective responses to emerging zoonotic diseases, but also to recognise the challenges of endemic zoonoses that continue to threaten human and animal health.[5] The growing awareness of endemic zoonoses as an important cause of common human disease

syndromes, including fever,[6–10] emphasises the relevance of One Health as a daily health concern, beyond outbreaks of "newsworthy" diseases such as Ebola, SARS, or highly pathogenic avian influenza (HPAI).

One Health research requires the collaboration of multiple disciplines, including medical, veterinary, and biological scientists alongside quantitative and qualitative social scientists. As dramatic advances were made in medical science and the practice of human medicine in industrialised societies in the 20th century, medicine, veterinary medicine, and social sciences developed as largely separate entities, with separate professional and scientific organisations. The growth of One Health publications has surpassed that of life science publications overall, suggesting an increased uptake of interdisciplinary approaches.[11] Even so, publication silos and differences in attitudes and best practices between and within biological and social sciences continue to exist. These differences impact interdisciplinary research, not just in terms of theoretical and methodological approaches, but also with regard to the operationalisation of the projects through, for example, ethical approvals, consent procedures, laboratory requirements, and authorship of publications.

In this contribution, we share our experiential knowledge gained from implementation and management of One Health research programs in northern Tanzania, concentrating on bacterial zoonoses research—conducted by the University of Glasgow, in collaboration with a wide range of Tanzanian and international partners—and on the development of bespoke zoonoses laboratory space at the Kilimanjaro Clinical Research Institute (KCRI) in Moshi. We begin with a discussion of ethical approval, which needs to be in place before field or laboratory work commences, and present some of the issues that may arise when working across social and biological disciplines. We continue with a description of some of the challenges in developing field-based and laboratory capacity for One Health research, where differences between human and animal health processes and policies come to the fore. Finally, we use case examples from current research projects to demonstrate research opportunities when such challenges are overcome and One Health research can be effectively implemented.

ONE HEALTH RESEARCH ETHICS

Regardless what type of human or animal research is conducted, the safety and protection of research participants should be paramount, and ethical committees and review procedures have a key role in safeguarding them.[12,13] Owing to its interdisciplinary, interinstitutional, and, frequently, international nature, ethical review of One Health projects can pose special challenges, an issue that is also recognised in relation to review of funding applications for One Health research.[14] Different disciplines draw on norms, methodologies, and terminologies that have evolved and become accepted within their respective institutions. As ethics committees are usually embedded within each of these institutions, the review process for interdisciplinary research can be problematic. An ethics committee whose members are familiar with biomedical research may struggle to assess research that is based on social science and vice versa. For example, in the initial review of one of our projects, focused on understanding the dairy value chain in northern Tanzania, members of a biomedical ethics committee were unfamiliar with the use of standard social science research terminology—such as "actor" and "purposive sampling"—which caused an obstacle to approval. The reviewers also expressed a strong preference for written over verbal consent, as is standard in medical research. While written consent is also common practice across the social sciences, it is not always appropriate—such as within communities with high illiteracy rates. When written consent cannot be given, the discipline norm is to record verbal consent. Rigid ethical regulations that insist on a written method of consent could preclude data collection in situations where such procedures would be challenging to apply.[15] A consequence of this could be that research on marginalised groups becomes more difficult or even impossible to carry out, which could lead to unethical outcomes of an ethical review process.[15] Another layer of complexity is added by the fact that many One Health projects involve multiple institutions, often in multiple countries, and often with their own or even multiple consenting procedures, for example human subjects vs. animal research. For some of our bacterial zoonoses projects, ethical review was required by up to 7 committees across 4 countries. Questions about ethical jurisdiction can arise when multiple international and local institutional ethics committees involved in ethical review take different positions.[16]

Human Subjects Research

Informed consent is the cornerstone of ethical scientific research involving people, whether as research subjects or as owners of animal research subjects. The informed-consent process is comprised of 3 key tenets[17]: firstly, the participant must be given full and transparent information about the research and their rights in an easily understandable manner; secondly, the participant must comprehend what is being asked of him or her; and lastly, the participant must freely agree to take part in the research.

The convention for the first tenet—provision of information—is to produce written documents, such as information sheets and consent forms, for participants. In our research, these typically cover the study objectives, potential risks and benefits of participating, research organisations and funding bodies, confidentiality, who has reviewed the study, what will happen to the results, and the researchers' contact details. The forms also include statements encouraging participants to ask questions and informing them that they can withdraw from the research at

any time. In Tanzania, the adult literacy level is 68%,[18] indicating that reliance on written documentation to convey information would likely exclude a considerable proportion of potential study participants. This problem is made worse by the fact that One Health research frequently involves groups—such as women, rural populations, smallholder farmers, or informal traders—who may have literacy rates below the national average. Presenting written information to a person with a low level of literacy may cause embarrassment, something an ethical researcher should strive to prevent rather than to precipitate. In our experience, even literate participants often felt intimidated rather than reassured by our consent documents. For example, our Tanzanian research assistants reported the high level of detail on our information sheets—especially the details of multiple research partners with formal institutional logos—sometimes caused concern about hidden goals of the research. Potential participants refused to believe that so much paperwork could be associated with something as straightforward as taking part in an interview or survey. On multiple occasions, people mistook us for members of a group rumoured to have malicious intent, a problem other researchers also encountered.[19] Additionally, some of the information provided on the forms—such as contact information for UK-based researchers—was not meaningful to participants and thus risked "information overload" for literate and illiterate participants alike. This often resulted in important messages, such as the right to refuse or withdraw participation, being buried in other information. Bhutta drew attention to the drawbacks of written information documents, arguing that they served largely to satisfy researchers' concerns over the legality of the informed consent process rather than the needs of the study participants.[17] Requesting written confirmation of participants' consent, such as a signature, may also pose difficulties when working with individuals or groups with low literacy. In theory, this issue could be solved by taking participants' thumb prints, but in a study of U.S.- and developing country-based researchers, respondents considered oral consent preferable to written consent, both for sharing information and for documenting consent.[20] Likewise, for a survey of informal milk vendors in Kenya, participants chose to give consent verbally (140 of 230, 61%) rather than by signature (39%), and none provided thumbprints.[21]

Considering the second tenet—comprehension—we found it difficult to assess the degree to which consent was genuinely informed even after the agreed informed-consent procedure had been dutifully followed. The consent process tended to take place quickly because the research was taking people away from other activities and because participants often became visibly bored when full details were read out slowly. Moreover, although we attempted to clearly explain the research activities, the gap between the researchers' lived experiences and those of the participants was vast and created communication challenges. Despite high rates of consent, we were not always confident that participants had genuinely understood the research goals, nor had they had time to reflect on whether or not to take part in the study. This is not a new problem in development research. The standard consent process of a single meeting between investigator and volunteer may be insufficient for adequate comprehension of informed consent.[22] Indeed, Sreenivasan argues that full comprehension is rarely achieved in research and should be considered an ethical aspiration rather than a minimum standard,[23] claiming that if full comprehension was a necessary condition of valid consent then much scientific endeavour would be impossible. To protect prospective research participants, their level of understanding can be assessed prior to consenting. Cooper and colleagues used comprehension and engagement scores to test the effectiveness of 3 communication tools—written documents, illustrative photographs, and illustrative cartoons—when providing study information to 22 Tanzanian livestock keepers prior to consent.[21] Cartoons were associated with significantly higher engagement scores, highlighting the usefulness of alternative means of information provision.

Finally, the third tenet—freely agreeing to consent—posed several problems. It proved difficult in some circumstances to identify who should be providing consent. Western ethical models place emphasis on the individual; however, in many African and other low- and middle-income country (LMIC) contexts, additional consent at the family or community level may be necessary.[20,23–25] In northern Tanzania, we sought permission of ward officials or community leaders, such as village chairs, prior to conducting key informant interviews. This was not required by our ethics committees but was recommended as standard practice by researchers with experience working in the area. However, even this level of contingency was not always

Photo 1. Development of bespoke cartoons in consultation with Tanzanian end-users to create culturally appropriate communication tools for One Health research.

sufficient. For one interview we visited a 19-year old informal milk trader at his family's home. We were accompanied by the village chair who had arranged the interview with the trader's prior agreement. Halfway through the interview we were interrupted by the trader's angry father, irate that we were holding an interview on his compound without his express consent. The village chair's presence made no difference and we had clearly violated a social or familial hierarchical norm. Such situations have the potential to compromise the safety and wellbeing of both researchers and participants, thus breaching a fundamental bioethics principle to do no harm.

A further difficulty with the third tenet is ensuring that consent is truly voluntary. Power relations can have coercive effects on consent. The presence of an outsider, the endorsement by a community leader, or the gender of a participant could all serve to socially or politically pressure participants who feel they ought to participate. The social desire to please others—whether conscious or subconscious—is particularly strong if those others are perceived as having power or control.[26] In some instances, women refused to be interviewed if their husbands were not home to consent, although in some cases this may have been a strategy for tacit refusal.[27] Financial incentives can also be coercive. We attempted to avoid this by not offering any remuneration other than the reimbursement of travel expenses. Although we made an exception when reimbursing local officials for the time and costs of arranging research activities, as is the local norm for recruiting these community gatekeepers, this practice could have been construed as reinforcing power imbalances that discriminate against the poor by unintentionally implying a poorer person's time has less value than the time of those privileged with education, wealth, and social status.

Animal Research

Use of animals in research comes with its own ethical challenges, especially where multiple international partners are involved. Animal research performed by researchers at the University of Glasgow is governed by the Animals (Scientific Procedures) Act 1986, implemented under European Union (EU) Directive 2010/63/EU, which sets out measures for the protection of animals used for scientific purposes.[28] Research funded by United Kingdom (UK) funders or conducted by UK universities is required to comply with this Act. However, while the UK Home Office provides detailed guidance on implementation of the Act within the UK, it does not take into consideration implementing work overseas.[28] The EU directive—largely developed with housed laboratory animals in mind—does not necessarily make adequate provision for research on other animals or in other settings.[29] These limitations make it difficult for researchers and local animal welfare ethical review bodies (AWERB) in the UK to interpret the guidance in international contexts. Few African institutions have AWERBS or institutional animal care and use committees (IACUCS) to review animal research within One Health proposals. Instead, issues relating to animal use in African countries are routinely addressed by AWERBs or IACUCs of partner institutions in Western countries. As a result, the policies and practices largely reflect the values and concerns of societies in those countries,[30] which may not necessarily be the same as those in African or other non-Western societies. While reviews of animal care and use in LMICs often emphasise the need for standards to meet those evolving in Western countries,[31] in this commentary we question whether and why decisions made in Western countries are given precedence as they may, in some cases, be inappropriate and ethically unacceptable to non-Western societies in Africa and elsewhere.

Most studies on attitudes toward animal research have been carried out across a narrow range of high-income countries in Europe and North America,[32,33] while little research has been conducted on the attitudes of people in lower-income and agricultural-based societies, especially in the LMICs. Furthermore, such studies tend to focus on a limited number of animal species including non-human primates; companion animals, such as dogs and cats; and laboratory animals used in biomedical research, such as mice and rats. Little attention has been given to species involved in One Health research such as livestock and wildlife. Contact with animals is likely to foster emotional attachment and empathy towards the species involved and affects attitudes towards animal use in research.[34,35] In Western societies, most human–animal contact involves companion animals or equids and the emotional attachment formed with those animals is reflected in the higher level of scrutiny given to the use of these species in research.[28] In those societies people have little direct contact with livestock species, which tends to preclude them from becoming objects of people's positive affections—resulting in attitudes dominated by the utilitarian view of livestock as a source of products.[36] In contrast, in rural and peri-urban communities in African countries, which comprise a large proportion of the population, people live in close contact with livestock. In Maasai pastoral societies, where we conduct research on bacterial zoonoses, many aspects of spirituality and mythology focus on the importance of cattle, which are seen as manifestations of the god, *Ngai*.[37] Additionally, livestock and pastoral livelihoods are crucial to Maasai identity as well as economic, social, and cultural capital.[38] Because the Maasai and other livestock-keeping societies often have complex spiritual and practical relationships with livestock, a strong case can be made for greater understanding of these relationships when making decisions on animal use in research. For example, during recent field trials to evaluate the efficacy of a new vaccine against malignant catarrhal fever (MCF) in Tanzania,[39] residents of the Maasai communities—where the trials were being conducted—expressed considerable misgivings about

exposing cattle to the risk of a potentially fatal disease through contact with wildebeest, even though the cattle in question had been purchased from outside the community specifically for the trial and were unknown to the community. Although the Tanzania study protocol followed UK trial guidelines[40] and was compliant with the UK Animals (Scientific Procedures) Act 1986 for euthanasia of animals with severe clinical disease, euthanasia of healthy animals that posed no disease risk to people or other animals was not carried out at the end of the trial. Euthanasia was considered socially unacceptable in the Maasai communities and the unnecessary loss of valued animals was likely to have caused considerable concern among community members. Even in Western societies, the harms and benefits of culling of healthy animals are being re-evaluated, particularly in the context of One Health, where not just human health but also animal and environmental health are given consideration.[2]

Incorporating the Plurality of Ethical Perspectives

One Health research is challenging because it brings many ethical considerations simultaneously to the fore: it is interdisciplinary and often international, endemic zoonoses disproportionately impact poor and vulnerable populations living in LMICs, and zoonoses research inherently covers both animals and humans. The plurality of processes—with different standards and procedures across committees assessing medical, veterinary or social science research at different organisations in multiple countries—often cause considerable delays to projects due to the varied and sometimes contradictory demands of the different ethical bodies. The multiplicity of standards and ethical procedures and practices is neither suitable nor adequate for this complex area of research, and innovative solutions are urgently required. Although there are legal constraints on modifications of ethics processes, we would like to suggest that the balance between disciplines and between legal and ethical aspects of the process may need to be reconsidered. More specifically, our first suggestion is that One Health applications should be reviewed by a single ethical review committee, rather than separate committees for each discipline. Such committees should be interdisciplinary—including experts in human medicine, animal health, and social sciences. Those committees would become familiar with the language, culture, and standards of the different disciplines—and consider all aspects of an interdisciplinary One Health proposal in a single assessment. While there is international agreement on the need for ethical conduct, consent procedures, and oversight in human subjects research, uniform implementation of Western guidelines for ethical conduct may create conflict between the "spirit" and "letter"—the underlying principles and technical implementation—of such guidelines in other countries or cultures.[12,13,41] Our second suggestion, therefore, is that ethical approvals from the country where the research is to be conducted should be considered as possible basis for ethical approval by the country where the funding originates. In our example, this could mean that the University of Glasgow would consider waiving the need for ethical approval if such approval were already granted by KCRI and the National Institute for Medical Research (NIMR), as the relevant local and national institutions, respectively, in Tanzania. This would prevent "ethical colonialism", whereby ethical standards from one country are imposed on another country without considering the cultural appropriateness of the ethical requirements. Already, waivers for ethical review may be granted by institutions within a single country—for example, across different universities in New Zealand—and such an approach could be adopted more widely, albeit with consideration of applicable legal constraints. Finally, this system would also require development of appropriately trained interdisciplinary ethics committees in LMICs. The development of an ethical and compliance infrastructure should be included in capacity-building initiatives, which, more often than not, focus on physical spaces and equipment or on technical and scientific skills, rather than on institutional, administrative, or ethical-research infrastructure.

ONE HEALTH RESEARCH LOGISTICS

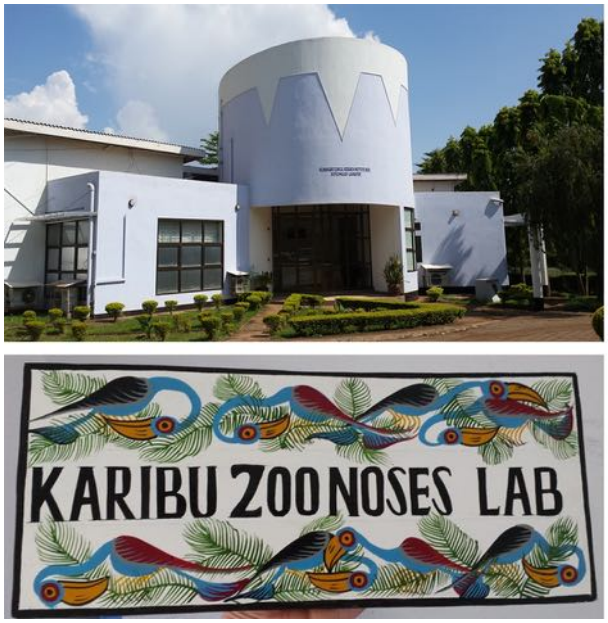
A major aim of zoonoses research is to generate data that informs understanding of the linkages between human and animal infection. To obtain insights into transmission processes between species and populations, epidemiological studies need to capture data on human and animal populations and their connectivity. This requires the design and delivery of multiple linked epidemiological studies and careful consideration of the aims and intended outputs from each component part. Because of the differences in the structure and distribution of species and populations, the sampling units or strategies appropriate for one species or population may not apply to others, especially when contemporaneous collection of samples or data from multiple species is needed. For example, while hospital- or school-based approaches may be the most efficient and cost-effective way to obtain samples from human populations, comparable sampling strategies for animal species are more difficult to find. Where they do exist, such as through veterinary surveillance or sampling at livestock markets or slaughterhouses, human behavioural factors linked to disease reporting or to the sale of particular livestock may make them poorly representative of the true disease situation in animals.[42,43] Household-based sampling strategies where data are gathered from individuals of multiple species present at the same place and time provide a way to gather fine-scale data from linked populations, but these efforts are resource and time intensive, and defining a household may be a challenge in its own right.[44] Careful evaluation of the nature of the linkages between populations, the scales at which they are observed and

sampled, and the trade-offs between optimal study design and feasibility is crucial. The increasing availability of online databases with molecular typing or sequencing data for pathogens may provide a false sense of comparability across studies, as the standardised format of such data masks underlying differences in epidemiological origins and reporting zeal.[45] This may contribute to erroneous interpretations regarding host-adaptation of pathogens or transmission potential between host species,[46] thus emphasising the need for epidemiologically appropriate study designs in One Health research.

Gaining access to appropriate laboratory space for processing of samples from people and animals can be difficult due to differences in regulation, location, and operation of human and animal diagnostic laboratories. For example, quality assurance/quality control check lists, such as those used by the U.S. National Institutes of Health Division of AIDS, may include questions on the presence of animal samples in the laboratory, with the implication that animal samples pose an inherent biohazard that could affect human infectious disease research. This is at odds with the fact that the level of containment needs to reflect the hazard level of the pathogen rather than the host species. For example, *Bacillus anthracis* is a Hazard Group 3 organism regardless of its origin. For some infections, such as HPAI, where false positive results could trigger a pandemic influenza alert, international guidelines explicitly state that human and animal samples should not be processed in the same laboratory.[47] Some would argue that the hazard of cross-reactivity with unrecognised diagnostic targets in samples of animal origin could lead to false-positive results and that prudence dictates separation of laboratories at all times. A counter argument would be that such cross-reactivity can also occur within human samples, meaning that separation of laboratories does not fully resolve this issue.[48] Cross-contamination or cross-reactivity, while highly undesirable, would be less impactful for endemic than epidemic infections. Implementation of good working practices supported by standard operating procedures, risk assessments, and staff-induction protocols should be put in place to minimise cross-contamination risks in microbiological and molecular laboratories, irrespective of the origin of samples. In our experience, sensitisation and training about zoonotic disease research was key to overcoming initial resistance, building consensus, and gaining acceptance of work on projects involving human and animal samples for laboratory accreditation bodies and staff alike.

An unintended consequence of the separation of laboratories and staff processing human and animal samples is lack of communication. For example, our research team was involved in anthrax diagnostics on animal-derived material and was not aware of the availability of molecular diagnostics for anthrax in human samples in an adjacent laboratory until a discussion about diagnostics was sparked by the occurrence of human anthrax in northern Tanzania.[49] Such issues could be resolved by regular communication between staff, staff training and cross-training, and the collocation of human and veterinary diagnostic facilities and experts. To allow for the processing of animal-derived material from our One Health projects in northern Tanzania, a physically separate containment level-2 laboratory was set up within the suite of KCRI laboratories. In this space, serum, milk, vaginal or cloacal swabs, faeces, lymph nodes, kidneys, and meat can be safely processed without the fear of compromising other studies performed at this site. Establishment of this bespoke zoonoses laboratory at KCRI required considerable investment in time, funding, and relationship building as well as a combination of general infrastructure-focused or capacity-building grants and project-specific research grants; its future is dependent on continued income generation. A major benefit of establishing the zoonoses laboratory is that we can now collect and process both human and animal samples without the need to routinely ship material to other laboratories. This leads to more efficient generation of results; improved dissemination of result, for example, to participants, medical or veterinary stakeholders, researchers, and policymakers; enhanced response to findings; and a stronger sense of ownership in the source country. Further, the routine handling and analysis of samples builds and sustains skills and capacity

Photo 2. Kilimanjaro Clinical Research Institute, Moshi, Tanzania (top), and the welcome sign to the bespoke Zoonoses lab set up within KCRI to enable One Health research (bottom).



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within Tanzanian institutions, with potential for onward training and capacity strengthening in other institutions. For example, staff at the KCRI zoonoses laboratory now train technicians and researchers from other institutions, including government veterinary institutions, further enhancing cross-sectoral engagement and capacity.

PROGRESS IN ONE HEALTH PROJECTS

To demonstrate the value of establishing a One Health research platform, we describe some of our projects, which span the spectrum from wildlife and livestock to people and the values and perceptions that drive their behaviour.

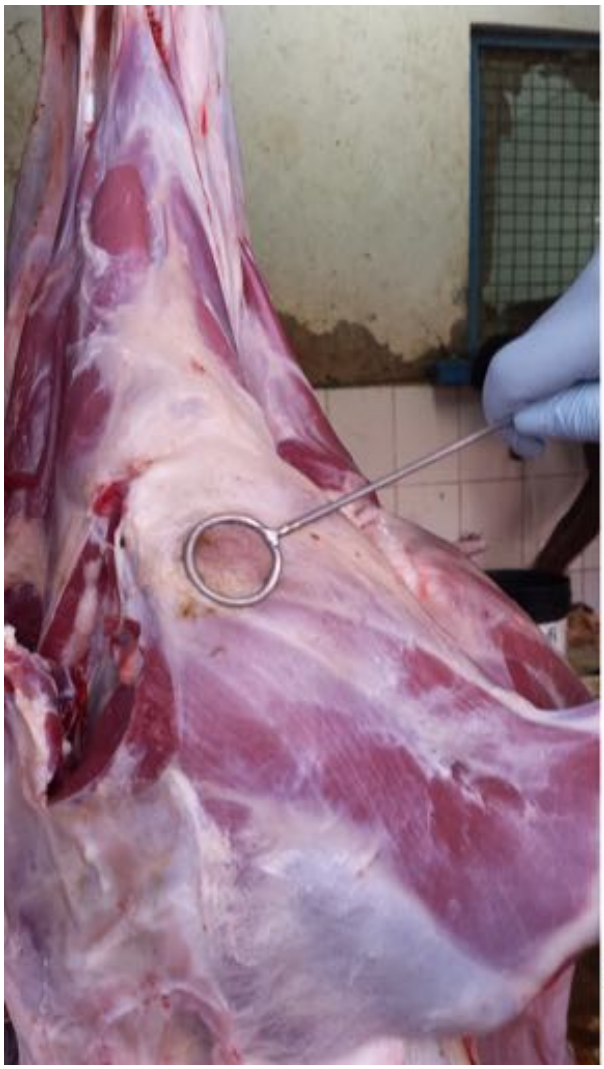
Zoonotic Causes of Febrile Illness

Febrile illness is a common cause of healthcare-seeking behaviour and is often attributed to malaria. Research conducted through the Kilimanjaro Christian Medical Centre-Duke University collaboration, however, showed that bloodstream infections and bacterial zoonoses caused more than half of all febrile illnesses in paediatric and adult patients.[6] Bacterial zoonoses were 20 times more common than malaria as a cause of illness in febrile admissions, yet routine diagnostics for zoonoses are not available and many healthcare providers identify low knowledge and testing capacity as reasons for zoonoses under-diagnosis.[50]

Q fever and brucellosis, among other diseases, were commonly identified in febrile patients and may have a ruminant livestock reservoir. Within the genus *Coxiella*, the causative agent of Q fever, only a single species is known, *C. burnetii*, but different subtypes have been associated with cattle or small ruminants as primary sources for human infection.[51,52] Within the genus *Brucella*, which causes brucellosis, multiple species are recognised: *Brucella abortus* is primarily found in cattle and *B. melitensis* in sheep and goats. This host–association is not absolute, and new *Brucella* species as well as the possibility of adaptation of known *Brucella* species to new host species at the wildlife–livestock interface is increasingly recognised.[53] Thus, a range of questions arises: Which animal species contribute most to human infection and disease? Which pathways of transmission are most important? What are the critical points that can be targeted for interventions? How are health interventions and treatments perceived, understood and valued by livestock keepers? How do or could livestock handling practices, milk and meat supply chains, household preparation, and consumption practices, and livestock vaccination influence disease occurrence?

Serological tests cannot differentiate between infections caused by *B. abortus*, *B. melitensis*, or other *Brucella* species. Because different *Brucella* species have distinct transmission dynamics and control options, it is important to determine the identity of *Brucella* in patients with fevers and in potential reservoir host species. Achieving this requires access to the actual bacteria, which is difficult to obtain due to low levels of bacteremia in patients, transient bacterial shedding by infected animals, and constraints on culture of the organism. Using serological data from field studies and sophisticated modelling approaches, goats were implicated as the most likely source of human infection in northern Tanzania.[54] This study also used simulated data to show that even small amounts of data on bacterial species identity—or comparable subtyping—are sufficient to inform models aimed at quantifying transmission between host species, thus justifying

Photo 3. Staff training in standardized methodology for carcass swabbing to support slaughterhouse-based surveillance of meat borne pathogens such as non-typhoidal *Salmonella* and *Campylobacter* species. Moshi, Tanzania.



considerable investment in obtaining such data.[54] Such efforts are now in progress, using human and animal sampling collection platforms set up in collaboration with human and veterinary medical professionals, and working with molecular bacteriologists in the zoonosis laboratory to prize this valuable information out of drops of urine, blood, and milk.

Foodborne Zoonoses

Zoonoses can be transmitted through the milk supply and value chain as well as the meat supply and value chain. Live animals and animals at slaughter carry numerous bacteria that are harmless, or commensal, when they reside in the animal gut but are potentially dangerous pathogens once they find their way into people. This is particularly true for non-typhoidal *Salmonella* (NTS) and *Campylobacter*, which are—among other signs and symptoms—associated with invasive and often fatal disease and growth shortfalls, respectively.[55,56] In Tanzania, *Salmonella* has been detected on carcasses of pigs and in fresh goat meat,[57,58] while *Campylobacter* has been found in faeces or on carcasses of pigs, cattle, and ducks.[59,60] People may acquire zoonotic *Campylobacter* from beef, particularly when meat preparation and processing is not undertaken properly,[61] but it remains to be determined whether animals or food of animal origin contribute to the burden of human invasive NTS disease.[56,62] Meat for consumption may become contaminated with faecal bacteria from the slaughtered animal, or with human- or animal-derived bacteria found in the environment of slaughter locations, butcheries, and eateries, such as on chopping blocks, butcher knives, or "nyama choma" (ready-to-eat meat). Targeted interventions require an understanding of the relative contribution of those potential sources of contamination and infection. Through a combination of supply- and value-chain analysis, mathematical modelling, and molecular epidemiology studies, we aim to identify the contributions of these different sources to human infections. The detection of NTS and *Campylobacter* in slaughter animals, carcasses, meat, and the environment is a crucial component of this work and is conducted in the zoonoses laboratory at KCRI.

Anthrax

Anthrax is not normally thought of as a foodborne zoonosis, however, many outbreaks in sub-Saharan Africa and elsewhere have been attributed to meat consumption. In 2016, ProMed—a global electronic reporting system for outbreaks of emerging infectious diseases and toxins—reported cases or government warnings related to consumption of cattle in,[63] for example, Kenya, Niger, Nigeria, Tanzania, and Zimbabwe. An outbreak in people in Zambia was linked to wildlife through the consumption of hippopotamus meat.[64] While many Western scientists, regulatory agencies, and funding bodies think of anthrax as a biowarfare agent and impose very strict controls on anthrax research, it is an endemic disease in much of sub-Saharan Africa where it affects wildlife, livestock, and people.[65] The spores of the causative bacterium, *Bacillus anthracis*, survive in the natural environment. Moreover, environmental factors drive interactions between wildlife, livestock, and humans, and between hosts and the environment, all of which impact the risk of disease.[66,67] Communities generally know the risk of eating carcasses from animals that died from anthrax, and some have developed traditional methods to determine whether or not a carcass is safe to eat. When faced with a choice between a high likelihood of malnutrition and a small chance of falling sick, consumption of carcass meat is often considered a risk worth taking, sometimes resulting in fatal outcomes. Similarly, the risk of exposure to MCF from wildebeest may drive herders to take their cattle to alternative grazing areas despite a known risk of anthrax (O. R. Aminu, oral communication, November 2016). To gain a better understanding of the relationships between anthrax in wildlife, livestock, and people, we are working with communities in Ngorongoro Conservation Area (NCA) to map their knowledge of anthrax, its spatial distribution, and its direct and indirect impact on livestock and people. To make possible confirmation of anthrax diagnoses, we provide training to animal health workers in the NCA in safe sample collection methods and the use of personal protective equipment. Samples are shipped to KCRI for culture-free confirmation of the presence of anthrax and for genetic typing. Use of molecular tools will also enable us to identify newly emerging *Bacillus* strains such as *B. cereus* Biovar *anthracis*, which has been associated with anthrax-like disease in wildlife and livestock in numerous countries in West and Central Africa.[68,69]

Photo 4. Cutaneous anthrax in a resident of the Ngorongoro Conservation Area where anthrax is an endemic disease with seasonal peaks in wildlife, livestock, and human case numbers.

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OUTLOOK

While the importance of One Health research is becoming increasingly recognised, it takes time and effort to break down the barriers between disciplines and their ways of working, and to build the interdisciplinary research teams, culture, and infrastructure needed to address One Health questions. Open Access publishing should reduce financial barriers to access to publications from other "silos", but barriers in language and methodology will take longer to come down. In addition, institutional attitudes to ethical review may continue to be dominated by Western legislation and priorities, regardless of the country or culture where the research is conducted. Our experience in Tanzania suggests that when those barriers are overcome, the opportunities for One Health research open up. The importance of One Health research, and the potential for African countries to play a leading role in this arena, is recognised and encouraged by policymakers and by regional and international funding bodies alike. The establishment of the *East African Health Research Journal* provides an opportunity to disseminate and advocate for One Health research and is testament to the confidence this region has in its capabilities. To date, much of One Health research—particularly in the social sciences—has been led or carried out by non-African scientists. A range of initiatives aim to address this imbalance. Examples at the time of writing include the Zoonoses and Emerging Livestock Systems–Associated Studentship (ZELS-AS) program funded by Research Councils UK and the Department for International Development, with PhD studentships and supervision shared between high-, low-, and middle-income countries; the Leverhulme–Royal Society Africa Awards, which fund a PhD studentship and postgraduate training courses for East African scientists; the Program for Enhancing the Health and Productivity of Livestock (PEHPL), funded by the Bill and Melinda Gates Foundation, which supports non-western and western PhD students at the Nelson Mandela African Institution of Science and Technology (NM-AIST) in Arusha, Tanzania; and the Developing Excellence in Leadership, Training and Science (DELTAS) Africa program, which supports the Africa-led development of world-class researchers and scientific leaders in Africa. We hope that current and new generations of scientists will consider the *East African Health Research Journal* as a platform for their publications.

Acknowledgements: Our work on bacterial zoonoses in Tanzania was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/J010367/1], the Biotechnology and Biological Sciences Research Council Zoonoses and Emerging Livestock Systems [grant numbers BB/L017679, BB/L018926, BB/L018845], the Wellcome Trust [grant number 096400/Z/11/Z], an EU Marie Skłodowska-Curie Individual Fellowship [grant number 659223], a Natural Sciences and Engineering Research Council (NSERC) of Canada Postdoctoral Fellowship [grant number PDF-471750-2015], a Leverhulme-Royal Society Africa Award [grant number AA130131], a Medical Research Council Doctoral Training Partnership grant [grant number MR/K500847/1], the Bill and Melinda Gates Foundation Program for Enhancing the Health and Productivity of Livestock and 2 Impact Accelerator Awards through the University of Glasgow Knowledge Exchange Fund.

We would like to thank our many collaborators in Tanzania, including those at the Kilimanjaro Christian Medical Centre; Ministry of Agriculture, Livestock and Fisheries; Ministry of Health, Community, Development, Gender, Elderly and Children; Nelson Mandela African Institution of Science and Technology; National Institute for Medical Research; Ngorongoro Conservation Area Authority; Sokoine University of Agriculture; and the Tanzania Wildlife Research Institute. We would also like to thank our collaborators from Massey University and University of Otago, New Zealand; Institute for Food Research, International Development Institute, Moredun Research Institute, and University of Glasgow, UK; and Duke University and Washington State University, USA.

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Peer Reviewed

Competing Interests: The authors have received funding from the Biotechnology and Biological Sciences Research Council, the Wellcome Trust, the EU Marie Skłodowska-Curie fund, the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Leverhulme-Royal Society Africa Award programme, a Medical Research Council Doctoral Training Partnership, the Bill and Melinda Gates Foundation, and the University of Glasgow Knowledge Exchange Fund.

Received: 21 Nov 2016; **Accepted:** 2 Feb 2017

Cite this article as: Ladbury G, Cleaveland S, Davis A, de Glanville WA, Forde TL, Halliday JE B, et al. One Health Research in Northern Tanzania – Challenges and Progress. East African Health Res J. 2017;1(1):8-18. https://doi.org/10.24248/EAHRJ-D-16-00379

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Tuberculosis Infection: Occurrence and Risk Factors in Presumptive Tuberculosis Patients of the Serengeti Ecosystem in Tanzania

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ABSTRACT

Background: Cross-species tuberculosis (TB) transmission between humans and animals has been reported for quite a long time in sub-Saharan Africa. Because humans and animals coexist in the same ecosystem, exploring their potential for cross-species transmission and the impact the disease may have on the health of humans, animals, and their products is critical.

Objectives: This study aimed to identify risk factors for transmission of TB (*Mycobacterium tuberculosis*) and to assess the potential for zoonotic TB (*Mycobacterium bovis*) transmission in the Serengeti ecosystem where humans and animals are in intense contact. Our aim is to create a base for future implementation of appropriate control strategies to limit infection in both humans and animals.

Methodology: We administered a semi-structured questionnaire to 421 self-reporting patients to gather information on risk factors and TB occurrence. In a parallel study, researchers screened sputum smears using Ziehl–Neelsen staining and confirmed by mycobacterial culture. We then performed descriptive statistics (Pearson's chi-square test) and logistic regression analysis to establish frequencies, association, and quantification of the risk factors associated with TB cases.

Results: Our findings showed 44% (95% confidence interval [CI], 0.40-0.49) of the results were positive from sputum samples collected over a 1-year duration in areas with a high TB burden, particularly the Bunda district, followed by the Serengeti and Ngorongoro districts. Of the culture-positive patients who also had infections other than TB (43/187 patients), 21 (49%) were HIV positive. Contact with livestock products (odds ratio [OR] 6.0; 95% CI, 1.81-19.9), infrequent milk consumption (OR 2.5; 95% CI, 1.42-4.23), cigarette smoking (OR 2.9; 95% CI, 1.19-7.1.2), and alcohol consumption (OR 2.3; 95% CI, 1.22-4.23) were associated with a higher likelihood of TB infection.

Conclusion: There was no evidence of direct cross-species transmission of either *M tuberculosis* or *M bovis* between humans and animals using the study methods. The absence of cross-species TB transmission could be due to limited chances of contact rather than an inability of cross-species disease transmission. In addition, not all people with presumptive TB are infected with TB, and therefore control strategies should emphasise confirming TB status before administering anti-TB drugs.

INTRODUCTION

The incidence and prevalence of human tuberculosis (*Mycobacterium tuberculosis* [TB]) in Tanzania is not adequately determined due to absence of regular screening. This is despite the reported estimate incidence rate of 164 per 100,000 persons per year and a prevalence of 0.4%.[1] In animals, particularly cattle, no national TB surveys have been performed; however, localised studies report the prevalence of bovine TB to range from 0.2% to 13.3%.[2] Reports from some parts of northern Tanzania indicated the presence of TB in humans caused by zoonotic TB (*Mycobacterium bovis*), which is often characterised by TB adenitis features.[3,4] In a separate study, Cleaveland and colleagues[5] reported the potential risk factors for infection by *M bovis* for humans and cattle

in rural Tanzania, and provided possible evidence of a link between infections among humans, cattle, and wildlife where they live in close proximity. Despite reports from the Horn of Africa that *M bovis* makes only a minor contribution to human TB,[6,7] the potential for *M bovis* to infect humans may be significant in some groups, such as pastoral communities, where TB can be acquired through the air (pulmonary TB) and through consumption of contaminated animal products. For example, in 2007 Shitaye and colleagues[8] reported prevalence of TB in cattle ranging from 3.4% (in smallholder production systems) to 50% (in intensive dairy productions) and 3.5% to 5.2% in slaughterhouses in Ethiopia. This level of prevalence is concerning, particularly in areas where humans and animals are in intense contact like in the Serengeti ecosystem. The contribution of *M bovis* to human TB is potentially 37.7% of all human TB cases in Africa.[9] In Tanzania the median contribution of *M bovis* in human TB is 26.1% (range 10.8%-37.7%), further increasing concerns about zoonotic TB with regional variations.

Given the challenges of laboratory TB diagnosis in resource-poor countries,[10] identification of hotspots or at-risk communities could help direct resources, such as laboratory tests, therapy, or intervention programmes, and make them more cost-effective. Directing more resources to communities at risk may be useful in determining the spread of TB in areas where humans and animals coexist, exploring their potential for cross-transmission, and understanding the impact the disease may have on the health of humans, animals, and their products.

We conducted the study by administering a semi-structured questionnaire in health centres in the Serengeti ecosystem to collect information about TB disease status and transmission. We administered the questionnaires in parallel with a different study that tested (using bovine tuberculin skin testing) for the presence of TB in cattle in the Serengeti ecosystem[11] before collecting samples from TB-infected humans and animals. Animal tissues from cattle with presumptive TB and archived tissues with presumptive TB from wildlife were then cultured and mycobacterial DNA characterised to identify circulating TB strains in the ecosystem. These results, published separately, showed no *M tuberculosis* strains in animals indicative of no potential for at least *M tuberculosis* to infect animals.[12] These findings ruled out potential for zoonotic tuberculosis (M bovis) transmission,[13] initially thought to be the case to create a base for future joint implementation of appropriate control strategies to limit infection in both humans and animals.

METHODS

Study Site

The semi-structured interview was carried out between October 2010 and November 2012 in health centres in the Serengeti ecosystem. These centres were Endulen Health Centre (in the Ngorongoro Conservation Area), Bunda district-designated hospitals (serving villages within the district and from neighbouring districts), Mugumu district-designated hospital (in Serengeti District), and Waso district-designated hospital (in Ngorongoro) (Figure 1). The centres were selected because they serve as focal points where TB screening is performed regularly.

TB Testing Methods

As described earlier, sputum smear samples were collected from self-reporting participants (all age groups) with presumptive TB and then tested using Ziehl–Neelsen staining, microscopic examination for acid-fast bacilli, and mycobacterial culture. At the time of sample collection, we administered paper-based semi-structured questionnaires to participants to collect information about TB disease status and transmission in these ecosystems. Our testing methods differed from other groups[2–5] who have conducted TB studies in Tanzania with a focus on either humans or animals. The sampling in this study was at the interface of intense human-animal contact. In such interfaces, the possibility of cross-species transmission might be comparatively high due to a heightened interaction.

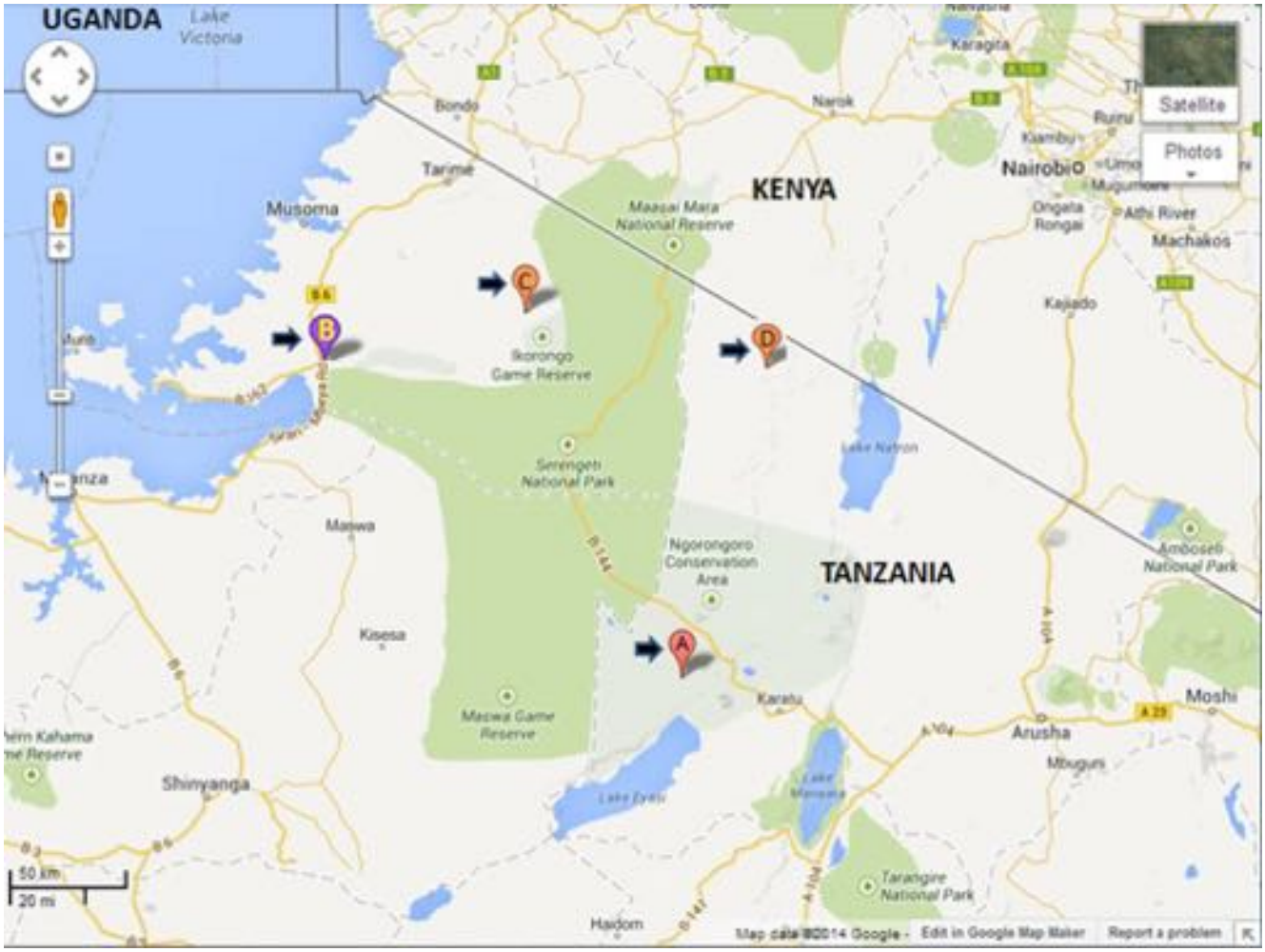
Participant Criteria

Inclusion criteria for participating in the questionnaire consisted of both inpatients and outpatients at health centres within the study sites who had symptoms suggestive of pulmonary TB (eg, prolonged recurrent fever, cough, anorexia, night sweating, weight loss, and general ill health of unknown cause) and who had not yet been on an anti-TB regimen. Patients who were already undergoing TB therapy were not eligible to participate in the study to avoid interference with study analysis outcomes. We understand that without symptomatic inclusion criteria likely to identify cases of *M bovis*, the study is limited; however, we believe that the results from the questionnaire provide an excellent overview regarding the dynamics of the disease in the course of interaction between humans and animals.

Observations About the Study Population

The study population came from diverse home environments, ranging from modern houses to African traditional huts with different thatching and wall construction materials. Iron sheets, thatch grasses, and soil were commonly used as roofing materials, whilst walls were commonly made of cement bricks, mud, and poles with soil and animal dung in combination (traditional housing). These houses were also characterised by the number and size of windows, which affects ventilation and the potential for aerosol TB transmission in case there is a source of infection. We subjectively defined window sizes as small (approximately 30 cm by 30 cm or less), medium (approximately 60 cm by 45 cm), and large (exceeding the above sizes), based on information

FIGURE 1. Map of Health Centres Within the Study Site (Serengeti Ecosystem)



A: Endulen Heath Centre; B: Bunda district-designated hospital; C: Mugumu district-designated hospital; D: Waso district-designated hospital.

provided by respondents and our own experience. The pastoral societies live in clustered houses customarily known as a *boma*, which constitutes a household (Figure 2), with 2 or more houses that have enclosures for protection of animals from thieves and predators. This is particularly the case in Serengeti District and Ngorongoro Conservation Area.

Administration of Questionnaire

At the time of sample collection, we administered a paper-based semi-structured questionnaire to acquire sociodemographic information that was considered useful to understand infection and transmission of TB in this ecosystem. The questionnaire was written in the common language (Swahili) and, where necessary, a translator familiar with the local language was used. The questionnaire was pre-tested in a pilot before the study began. Trained health workers administered the questionnaires and helped participants complete them at health centres during a face-to-face interview. In general, the questionnaire inquired about socioeconomic information, contact with animals and animal products, and household members' health history including TB and HIV/AIDS status.

Ethical Consideration

All patients enrolled in the study gave written informed consent. We obtained ethical clearance from the Muhimbili

FIGURE 2. Different Types of Houses Within the Study Site (Serengeti Ecosystem)

In the top two pictures, the *boma* (clustered houses that constitute a household) represent traditional African huts with different roofing and construction materials, as well as windows (not shown). The clustered poles are an enclosure within the household where cattle are kept close to owners to prevent stock loss. The bottom picture represents a modern house with different kinds of windows.

University of Health and Allied Sciences Research Ethics Committee (Ref.MU/PGS/PhD/R/Vol.1) and the National Institute for Medical Research (Ref. No. NIMR/HQ/R.8a/Vol. IX/1299). Participants received an explanation about the study purpose, what was expected of them, potential risks and benefits, confidentiality, and the voluntary nature of participation. Participants had a chance to ask questions before deciding whether to participate in the study or not. Patients with confirmed TB were offered treatment as stipulated in the Tanzania National Guidelines for Management of TB.[14]

Sample Size Calculation

The sample size for this study was calculated based on the formula $[n=(1.96)^2\ p\ (1-p)/d^2]$ (where p=expected prevalence or proportion and d=precision) as suggested by Thrusfield[15] at a 5% significance level and 5% precision using 0.172% as the estimated prevalence of TB in Tanzania.[1] Based on this calculation, a minimum of 219 subjects was sufficient to be sampled and provide reliable information.

Sample Collection, Storage, and Transport

When someone with presumptive TB presented for clinical evaluation by a medical practitioner at a health centre within the study site, instructions were given to collect 2 sputum samples: 1 collected early in the morning by the patient at home and 1 sputum collected on the spot at the health centre. From these specimens, sputum smears were prepared for microscopy on-site at the health facility, and the remaining sample was transported in cetylpyridinium chloride to the central TB laboratory in Dar es Salaam for mycobacterial culture and drug sensitivity testing.

Sample Processing and Mycobacterial Culture

The Ziehl–Neelsen staining for acid-fast bacilli was performed according to existing standard protocols followed by mycobacterial culture on in-house prepared Löwenstein-Jensen medium containing glycerol (for *M tuberculosis*) or pyruvate (for *M bovis*).[12] Quality control for Ziehl–Neelsen testing was achieved from the point of sample collection, which was done early in the morning at home by the patient and later at the health centre. When collection tubes were delivered to the patients, it was ensured that instructions for sample collection at home were clear and followed up with a demonstration. Ziehl–Neelsen testing was done on-site and later after the samples arrived at the laboratory in Dar es Salaam before culture. Drug susceptibility testing was performed according to standard proportion methods.[16] The concentrations for each respective antibiotic tested were: isoniazid (1 µg/mL), rifampicin (40 µg/mL), ethambutol (2 µg/mL), and streptomycin (5 µg/mL). Species identification was performed by spacer oligonucleotide typing (spoligotyping), whose results have been reported separately.[12,17]

Statistical Analysis

The research team analysed data using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to examine the frequencies and associations between population attributes. Different household characteristics were compared using the Pearson Chi-square test at a significance level of 5% ($P<.05$). Logistic regression analysis was performed to quantify the risk factors associated with TB cases using SAS version 9.0 (SAS Institute Inc. Cary, North Carolina, USA). TB status was treated as a binary outcome variable. We analysed the following explanatory variables to examine their potential association with TB transmission: sex, age, marital status, number of household members, Bacillus Calmette–Guérin (BCG) vaccination history, other infections associated with TB, recent history of TB treatment, cattle keeping, recent contact with livestock and livestock products and nature of contact, milk drinking and frequency, milk boiling, sour milk drinking and frequency of use, source of milk, knowledge about TB, contact with TB patients, house roofing material, house construction material, the number of rooms in a house, the number and size of windows, sleeping in a household with livestock,

cigarette smoking, alcohol consumption, visits to gatherings, visits to local brew centres, and the origin of people they meet during free social time.

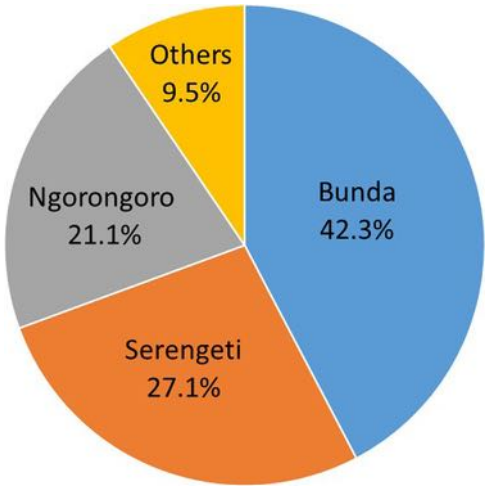
Univariable analysis was run for each explanatory variable and a variable qualified for multivariable analysis when it had a likelihood ratio with a significance level less than 25% ($P<.25$). A multivariable model was built by a backward-stepwise regression, in which case the retention cut-off point was $P<.05$. Assessment of confounders was based on relative or absolute change of 25% or 0.1 respectively in coefficients of other variables. Model goodness of fit was assessed by a Hosmer-Lemeshow test, whereby a P value of more than .05 indicated that the model had a good fit.

RESULTS

General Results

The communities living in the Serengeti ecosystem consist of a heterogeneous mixture of ethnic groups who have migrated from various regions within Tanzania and who pursue a variety of economic activities with a common language, Swahili. A large proportion of presumptive TB patients were found in Bunda (42.3%), compared with Ngorongoro (21.1%), Serengeti (27.1%), and other districts (9.5%) (Figure 3). In the Serengeti and Ngorongoro districts, most presumptive TB patients came from communities involved largely with farming (ie, growing crops and keeping livestock), whereas Bunda consisted of a more heterogeneous population including farmers as well as traders, tailors, and fishermen. Some patients who visited the district-designated hospital in Bunda, however, came from villages outside of Bunda district, such as from Lamadi in the Magu district of Mwanza alongside the Serengeti National Park, and also from Musoma and other nearby districts of Mara. Therefore, data from the Bunda district-designated also represents residents from other nearby districts within Mara and just outside the region. The location of the Bunda district-designated hospital makes it accessible from every corner of the ecosystem.

FIGURE 3. Distribution of Presumptive TB Patients Participating in the Study by District (N=421)



Data Analysis

Sputum samples were obtained from 421 presumptive TB individuals collected over a 1-year period. Gender representation was almost equal. These samples were subjected to mycobacterial culture, of which 187 tested positive for *M tuberculosis*, or 44% (95% CI, 0.40–0.49) of all collected samples. None of the sputum culture growths consisted of *M bovis* or non-tuberculous mycobacteria. Comparison between gender and TB status indicated a significant difference, with males being more frequently culture positive for the disease compared with females (χ^2=5.7, df=1, P=.017) (Figure 4). Antibiotic sensitivity tests showed no phenotypic resistance with a standard minimal inhibitory concentration in Löwenstein-Jensen medium to isoniazid, rifampicin, ethambutol, or streptomycin.

The culture results of TB patients by age are shown in Table 1. Most TB-positive patients were between 20 and 39 years old. The frequency of distribution was skewed toward older ages within this age group, with a statistically significant association between TB culture positivity (TB status) and age (χ^2=14.1, df=4, P=.007). The proportion of TB-positive (45%) and TB-negative (55%) patients did not reflect much difference.

Of all participants analysed for culture results, 291 (71.9%) were married, 89 (22%) were unmarried, 20 (4.9%) were widowed, and 5 (1.2%) were divorced. Of the culture-positive TB patients, married participants accounted for 69.3% of the group followed by unmarried participants (22.9%). Smear-positive and culture-positive participants who had a previous history of BCG vaccination (65, 34.8%) compared with those who had no previous history (114, 61.5%) shows clear evidence that BCG vaccination protected individuals against TB by nearly twofold, indicating that the 2 groups differed significantly in potential susceptibility to TB (χ^2=14.9, df=2, P=.001). Of the participants who tested positive for TB based on smear and culture samples, 61.5% had no BCG vaccination history, 34.8% had a history of vaccination against TB, and 3.7% did not know their childhood BCG vaccination status. Analysis of other infections associated with TB among participants revealed that 21 (49%) had known cases of HIV infection and 22 (51%) had other associated infections or conditions including wounds and skin rashes.

FIGURE 4. Distribution of Presumptive Tuberculosis Patients Participating in the Study by Gender (N=421)

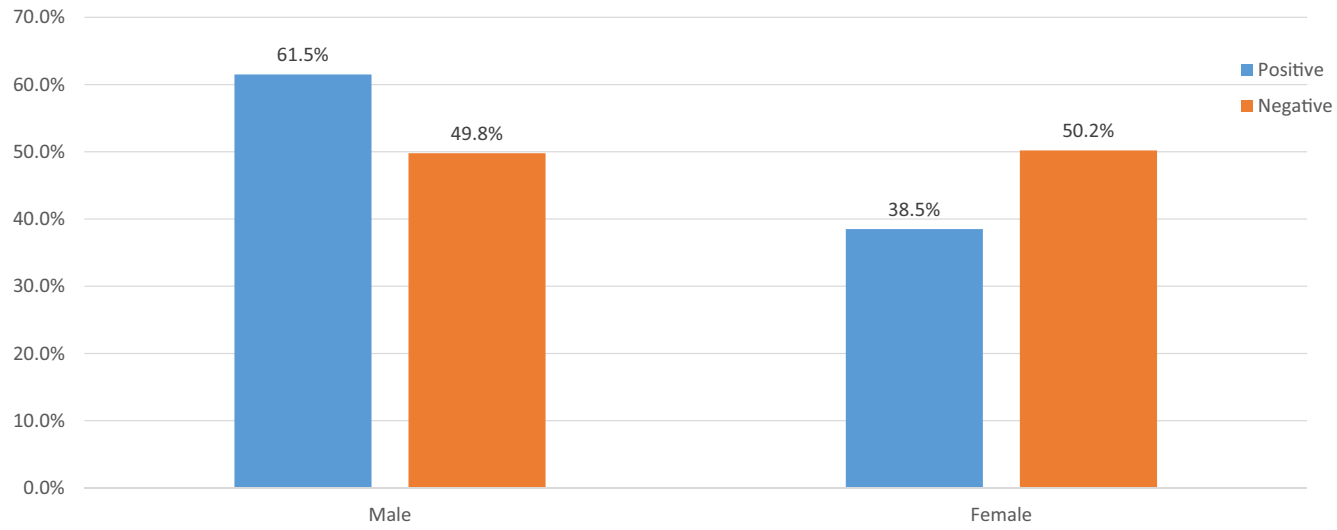


TABLE 1. Tuberculosis Status of Culture-Tested Participants by Age (N=416)

	Tuberculosis Culture		
Age	**Positive (%)**	**Negative (%)**	**Total (%)**
No category	2 (1.1)	1 (0.4)	3 (0.7)
<20 yrs	10 (5.3)	29 (12.7)	39 (9.4)
20–39 yrs	104 (55.6)	91 (39.7)	195 (46.9)
40–59 yrs	51 (27.3)	75 (32.8)	126 (30.3)
60 yrs and above	20 (10.7)	33 (14.4)	53 (12.7)
Total	*187 (45.0)*	*229 (55.0)*	*416 (100.0)*

TB Test Results and Occupation

The Serengeti ecosystem is predominantly inhabited by pastoralists, although many community members engage in other livelihood activities. Pastoralists keep animals and move around the ecosystem in search of better pastures when grazing becomes limited, particularly during the dry season. Our results (Table 2) showed that the majority of TB culture-positive participants (61.5%) were farmers who raised livestock and crops. The proportion of those farmers who kept livestock only (15%) was the second largest group of TB culture-positive participants, followed by the group designated "others" that included street vendors, tailors, plumbers, masonry workers, welders, and bar maids (12.3%). The percentage of the latter group was greater than the percentage of culture-positive students and traders combined (11.2%). Pearson Chi-square group comparisons showed an association between the proportion of TB-positive patients and their occupation (χ^2=10.3, df=4, P=.04), reflecting the impact occupation may have on infection status.

Knowledge About TB and TB Status

Nearly all (92%) of the participants in this study had prior knowledge about how TB infection can be acquired and transmitted from an infection source. As reflected in Table 2, previous contact with a TB patient was not

TABLE 2. Tuberculosis (TB) Status of Culture-Tested Participants by Occupation, Previous Contact With TB Patients, and Window Size (N=416)

Variable	Variable Category	TB Status		
		Positive (%)	Negative (%)	Total (%)
Occupation	Farmers (livestock and crops)	115 (65.1)	111 (85.5)	226 (54.3)
	Farmers (livestock only)	28 (15)	55 (24)	83 (20)
	Students	10 (5.3)	23 (10)	33 (7.9)
	Traders	11 (5.9)	13 (5.7)	24 (5.8)
	Others[a]	23 (12.3)	27 (11.8)	50 (10)
Previous contact with TB patients[b]	No	112 (59.9)	119 (52)	231 (55.5)
	Yes	38 (20.3)	58 (25.3)	96 (23.1)
	Not known	37 (19.8)	52 (22.7)	89 (21.4)
Window size	Small	101 (54.0)	105 (47.1)	206 (50.2)
	Medium	68 (36.4)	106 (47.5)	174 (42.4)
	Large	18 (9.6)	12 (5.4)	30 (7.3)

[a] Includes fishermen, tailors, plumbers, carpenters, and street vendors who were found in small proportions.
[b] Refers to contact with TB patients in the previous 12 months.

significantly associated with TB infection, as only 38 (20.3%) of TB culture-positive participants had known previous contact with TB patients in the last 12 months before testing themselves. In addition, among those participants who had previous contact with TB patients, 16 (42.1%) had contact with patients aged 40 to 59 years.

Culture Results and Housing

Most TB culture-positive participants lived in houses constructed with iron sheets (63.1%), mud walls (62.4%), 3 to 5 rooms (59.1%), and 3 to 5 windows (57.8%). Most participants resided in houses with small windows (54%), and the remainder (36.4% and 9.6%) had medium and large windows, respectively (Table 2). In contrast, most (53%) TB culture-negative participants lived in dwellings with medium to large windows. Thus, window size seems to be associated with TB risk (χ^2=6.5, df=2, P=.04), particularly small windows. The latter group of culture-positive patients who lived in houses with small windows also lived with 2 to 5 other people (163/187, 87.2%). Very few patients who tested positive for TB came from single-person households (3.2%) or more than 6 members per household (9.6%). There was no evidence that sleeping in a household with livestock was associated with testing positive for TB (χ^2=9.8, df=1, P=.002). This is indicated by the results that a higher proportion (88.2%) of TB-positive patients had never slept with livestock in the same household, and only 11.8% habitually slept with livestock in the same household.

Cigarette Smoking, Alcohol Use, and Social Networking

The results also revealed that 85% of the participants who tested positive for TB were non-smokers, providing evidence that cigarette smoking alone might not be a major risk factor for developing disease in this population (χ^2=10, df=1, P=.002), and 85% also did not drink alcohol. In other words, non-smokers were more likely to have TB compared with smokers. Among smokers, however, the majority were TB positive; of all smokers (n=41), 28 (68.3%) had positive TB culture results compared with 13 (31.7%) who were culture negative. This means that if we had tested for TB in smokers only, more smokers could test positive than negative. Similarly, the effect of drinking alcohol was such that not drinking alcohol was associated with being TB negative, whereas drinking alcohol was associated with being TB positive (χ^2=6.9, df=1, P=.01).

Quantification of Risk Factors for TB Occurrence

The data were subjected to multivariable logistic regression to quantify the contribution of different factors to

TABLE 3. Explanatory Variables as Potential TB Risk Factors

Variable	Response	Frequency (n=347)	% TB Case (n=148)	OR	95% CI
Recent contact with livestock (within the past 12 months)	No (0)	160	61.6	1.0	0.25-0.75
	Yes (1)	185	38.4	0.43	
Contact with livestock products	No (0)	20	2.7	1.0	1.81-19.9
	Yes (1)	327	97.3	6.0	
Milk consumption	At least daily (0)	171	33.0	1.0	1.42-4.23
	Not daily (1)	176	67.0	2.5	
Smoke cigarettes	No (0)	315	84.0	1.0	1.19-7.12
	Yes (1)	32	16.0	2.9	
Drink alcohol	No (0)	278	73.0	1.0	1.22-4.23
	Yes (1)	69	27.0	2.3	

Abbreviations: CI, confidence interval; OR, odds ratio; TB, tuberculosis.

TB occurrence. The final logistic regression model had 5 explanatory variables as shown in Table 3. A total of 74 observations were excluded due to missing values. The Hosmer and Lemeshow goodness-of-fit test yielded a *P* value of .72. Contact with livestock products, infrequency of milk consumption, cigarette smoking, and alcohol consumption were the main risk factors for occurrence of TB, whereas recent contact with livestock (within the past 12 months) was found to be protective (Table 3). People in contact with livestock products were 6 times more likely to have TB infection compared with those not in contact. On the other hand, the risk of becoming infected with TB was 2.9 (95% CI, 1.19–7.12) times higher for cigarette smokers and 2.3 (95% CI, 1.22–4.23) times higher for consumers of alcohol compared with those who did not smoke or drink. Habitual milk drinking in one way or the other affected occurrence of TB in the community. Frequency of milk consumption, for example, increased risk of *M tuberculosis* infection by 2.5 times for people who did not consume milk on a daily basis compared with people who drank milk at least once a day (Table 3). There were neither confounders nor significant interactions between explanatory variables in the final model.

DISCUSSION

The participants in this study represent a heterogeneous community of farmers (growing crops and keeping livestock), livestock keepers (who keep only livestock), traders, and other people who engage in formal and non-formal livelihood activities around the Serengeti ecosystem. The prevalence of TB in this area in humans and cattle is 0.2%[1] and 2.4%, respectively.[11] It is highly likely that these groups of people have different levels of exposure to the disease and therefore variability in incidence rates.

Previous studies have indicated the association between age and TB infection with a change in prevalence by age[18–21] because the disease is chronic in nature.[1] This study found more TB culture-positive patients between 20 and 39 years old, potentially the most economically active and productive age group. TB affects mostly young adults and is among the top 3 causes of death for women ages 15 to 44 years, although all age groups are at risk.[1,22] Being in this age range could also be a major risk factor for other infections, particularly in Bunda where contact and interaction between people is very high due to location and access to road infrastructure and connections to other places within the region. Married participants accounted for 69.3% of TB-positive cases, signifying high transmission of TB within this group.

The relationship between HIV and TB in this study can be found in a separate report by Mbugi and colleagues.[23] Interestingly, and probably not surprising, is that HIV accounted for 21 (49%) of other infections active in TB-positive individuals. Previous studies reported more than 30% TB-HIV coinfection,[24] and up to almost 90% TB-HIV coinfection[25] in some parts of Africa. The updated World Health Organization (WHO) report indicates 13% TB-HIV coinfections.[1] More reports address concerns on TB-HIV coinfection,[26–29] despite one study reporting a lack of concrete TB-HIV coinfection in children.[30] In regions where TB and HIV/AIDS are endemic, particularly sub-Saharan Africa, people living with HIV are nearly 20 times more likely to develop TB compared with those who are HIV negative.[24] This is

particularly important as HIV plays a role in compromising the immune response to TB,[28] although the mechanisms by which HIV disrupts TB immune pathology are unclear.[31] Our findings are very similar to findings from Piggott and Karakousis, which reported more than 50% active TB-HIV coinfection.[32] Infection with HIV is known to predispose the host to *M tuberculosis* latent infection and progression to active disease, which increases the risk of latent TB reactivation by 20-fold.[27] The role HIV plays in increasing susceptibility to TB is clear, particularly through reduction in CD4+ T cells count and function.

TB Test Results and Occupation

Study results showed that TB infection was much more prevalent in farmers (Table 2), but this should not be misinterpreted to mean that other groups (students, traders, and others—street vendors, tailors, plumbers, masonry workers, welders, and bar maids) are not at risk. The data suggest that the disease can affect all groups regardless of occupation and age. The study showed that 5.3% of TB-positive cases were students at different stages of school life. This is critical because one would expect the younger generation at school to be healthy. The results of this study imply that special attention should be paid to students to protect them from chronic infections that could jeopardise their school performance and future development. The results of this study do not explain why TB infection was more prevalent among farmers or the association between TB infection and occupation in general. The variability in infection rates by occupation could be attributed to lifestyle and other factors not associated with human-animal interface.

TB Test Results and Keeping Animals

The study findings reflected that TB infection is determined by a combination of factors rather than a single factor. Keeping cattle plays a key role in becoming infected with bovine TB (bTB), especially the type and frequency of contact with infected livestock and their products. Other studies also show evidence of transmission of TB from humans to animals and vice versa, particularly in elephant farms.[33,34] Recent conversations with a Keystone delegate confirm that humans in the United States and Canada have transmitted TB to cattle (unattributed). One report, however, suggests that transmission of TB from cattle to humans is still questionable, as the contribution of *M bovis* to human TB is considered minor.[6] Our study failed to establish the causal relationship because of non-detection of *M tuberculosis* in animals.[12,17] Transmission of bTB to humans is thought to occur through consumption of livestock products, predominantly milk but also meat.[35] Whether the transmission of bTB to humans constitutes a public health concern remains a question. In this study, descriptive analyses indicated no evidence that drinking milk, boiling milk before drinking, or drinking either sour or fresh milk were major risk factors associated with TB infection (an association would have been more likely if patients were infected with *M bovis*). However, results from the logistic regression analysis suggested that a combination of multiple factors may play a role in increasing risk for TB infection (Table 3). For example, frequency of milk consumption revealed a 3.7 times higher likelihood of TB infection for people who did not consume milk on a daily basis compared with people who consumed milk at least once a day. The higher risk in this group might be caused by a confounding factor, for example, people who consume milk less frequently could have a different lifestyle that increases their chances of becoming infected with TB.

Knowledge About TB and TB Status

The majority of patients tested in this study (91.8%) had knowledge about TB infection and factors associated with a positive sputum smear test; 95.7% who tested positive for TB had at least some idea of how TB can be contracted and transmitted. This high level of knowledge implies that other unknown factors are likely playing a role in transmission, for example, environmental contamination through overcrowding in transport vessels. Level of education and frequency of awareness campaigns by credible sources might play a crucial role in reducing TB incidence.

TB is a chronic infection that can be latent over a long period of time, and therefore asymptomatic TB carriers can unknowingly pass it on to uninfected individuals. This is especially threatening if the carrier possesses drug-resistant TB, in particular multidrug-resistant TB or extensively drug-resistant TB. It has been suggested[35] that despite the potentially minor contribution of bTB in human TB, there is a critical need for interventions to control the disease and prevent zoonotic transmission of *M bovis* to human populations consuming dairy products. As reflected in this study, people in contact with livestock products were 5.4 times more likely to become infected with TB compared with those not in contact with livestock (Table 3). The risk of TB transmission is bidirectional (human to animal and animal to human), as indicated by reports on transmission from humans to cattle[36,37] and dogs,[38] which reflect the importance of zoonotic transmission of TB in the Serengeti ecosystem communities.

Education, socioeconomic factors, and living standards may play important roles in reducing transmission among pastoralists. Analysing such factors to determine ways to reduce transmission will be particularly critical to prevent the spread of multidrug-resistant TB in the region.

Culture Test Results and Housing

This study evaluated the construction material of housing and living standards and found that TB culture-positive participants came from houses thatched with iron sheets (63.1%), constructed with mud-made walls (62.4%), and

having 3 to 5 rooms (59.1%) and 3 to 5 windows (57.8%) per house. These findings are contrary to previous findings that support the well-known relationship between poverty, disease, poor indoor ventilation, and transmission of disease. The houses of participants in this study seemed to have space; however, the number of rooms may have led to many people living in one household, thus predisposing them to overcrowding—a risk for disease transmission from infected to healthy individuals. Potential overcrowding would be exacerbated by the fact that a large proportion of TB culture-positive participants lived in houses with small windows (Table 2). The combination of small windows and overcrowding could predispose patients to infection due to the aerosol nature of transmission of the disease. A recent report[39] indicates a higher risk and predisposition to TB transmission in an environment with limited air flow and low rates of ventilation in households. Multiple factors, however, contribute to airborne pathogen transmission from infectious carriers, including cough frequency, respiratory rate, and duration of exposure between source and contact.[40]

The houses lived in by study participants also had at least 2 to 5 members (87.2%) per household, further signalling the potential impact that overcrowding may have on disease transmission. Environment is an important factor in TB transmission[41] and should not be overlooked. Our study also showed that very few TB culture-positive patients were from single-occupancy households (3.2%); this finding aligns with the principles of airborne disease transmission. The risk of contracting a disease increases as the number of occupants per unit room increases. Thus, the increased risk among 163 (87.2%) patients who slept in households that were occupied by 2 to 5 other members is justified.

The evidence for transmission of TB infection from humans to animals is available[42,43] but rarely from animals to humans. We also did not find any association between TB incidence and sleeping in a household with livestock. Most TB-positive participants (88.2%) never slept in a household with animals and only 11.8% originated from a household with animals. The results are predictable, as there were no positive results of *M bovis* in TB-positive participants.

Cigarette Smoking, Alcohol Use, and Social Networking

Our study did not show any association between cigarette smoking or alcohol use and smear-positive TB, indicating that cigarette or alcohol use alone might not be major factors for developing the disease, but a combination of factors might contribute. Visits to gatherings (ie, social networking) had no direct association with culture-positive TB. However, logistic regression analysis revealed that cigarette smokers and consumers of alcohol are at risk of becoming infected with TB (3 and 2 times more likely) compared with non-smokers and non-drinkers alone. The association between TB and smoking has been previously reported as having a strong dose-response relationship, both in terms of quantity and duration of smoking.[44–46] Smoking increases workload to the lungs and is said to potentially decrease the immune response or damage the function of cilia in the airways.[47] It is also reported that cigarettes contain nicotine, which exerts immunosuppressive effects on immune surveillance through functional impairment of the dendritic cell system.[48] By doing so, the ability of immune cells to induce differentiation and expansion of type 1 T immune cells is reduced, thus decreasing the frequency of interferon-γ-producing effector cells for body defence. In such situations, the risk for TB increases due to diminished protection.

With patients in this study coming from families of different economic status, our objective should focus on alleviating these communities from poverty to improve their economic status, which is the key toward solving health problems related to infectious diseases. Tuberculosis is a disease of poverty, and therefore a solution to poverty could help reduce the impact of the disease. Only by successfully containing the disease can we realise the epidemiological and economic projections of averted mortality and economic benefits in sub-Saharan Africa and other high-burden, TB-endemic countries.[49] Education to these communities on the One Health approach in tackling zoonotic infections like TB, combined with a participatory approach from professional and non-professional stakeholders, could play a key role in this context. Joint efforts could have a big impact in finding solutions for cross-cutting infectious diseases in resource-constrained countries, most of them found in Africa. For example, some studies in Africa have called for government and policy makers to work together with other stakeholders to design methods that could control bTB in intensive farming communities.[35] In so doing, any transmission of bTB to humans will be prevented, particularly if contact investigation for early case detection is combined with treating latent TB infection.[50] This study focused on analysing some of the risk factors (eg, milk consumption and occupations involving livestock) to identify potential associations with zoonotic transmission of TB. However, the culture results showed no evidence of zoonotic transmission (*M bovis* growth), disproving the idea that zoonotic transmission was a major risk factor in this population.

CONCLUSION AND RECOMMENDATIONS

There was no evidence of direct cross-species transmission of either *M tuberculosis* or *M bovis* between humans and animals using the study methods. A parallel study that tested wildlife and livestock tissues[12] also showed no evidence of *M tuberculosis* in cattle and wildlife, further limiting the possibility of TB cross-transmission between these species.

The absence of *M bovis* in humans and *M tuberculosis* in animals does not, however, rule out potential cross-species

transmission if the infection happens to be in either of the hosts in these endemic areas. The absence of evidence of cross-transmission could be due to limited chances of contact rather than absence of transmission between these species. Our future plans include tracing households with TB-positive patients and concomitant infection in animals to enable identifying the virulence gene in both *M tuberculosis* and *M bovis* and their probable differences. Precautionary measures are therefore still required in farming communities where animal-human interaction is intense.

Our statistical analysis revealed that recent contact with livestock is a potentially protective factor, similar to frequent milk consumption. We interpret this finding with caution, however, as contact with livestock products such as milk and meat might also be a risk in case of bovine TB transmission in the ecosystem. We also found an association between infrequent milk consumption and TB infection. Although this finding seems counterintuitive and may be attributed to chance, it is important to acknowledge its potential contribution.

This study found a higher rate of TB infection among participants between 20 and 39 years old, potentially the most economically active and productive age group, and also prone to HIV/AIDS. HIV impairs the immune system and can increase the risk of active TB, further contributing to the spread of TB. WHO[51] reports that people infected with HIV are 21 to 34 times more likely to become infected with TB.

This study found that a combination of factors is associated with TB infection—contact with livestock products, infrequent milk consumption, cigarette smoking, and alcohol consumption. These factors may influence the dynamics and impact of the disease in the Serengeti ecosystem.

From this study we can also learn that not all people with presumptive TB are infected with TB, and therefore control strategies should emphasise confirming TB status before administering anti-TB drugs.

Acknowledgements: The Southern African Centre for Infectious Disease Surveillance (SACIDS) is acknowledged for providing a Postdoctoral Research Fellowship to EM and PhD candidacy for BZK. Financial support was received from the Wellcome Trust Grant [WT087546MA]; MUHAS Sida Sarec Small Grant [000/3177]. Professor Mark Rweyemamu, Executive Director of SACIDS, is acknowledged for stimulating the idea and providing advice. The London International Development Centre in the UK and staff are acknowledged for collaboration. We thank the participants for consenting to participate in our study and health authorities in Serengeti, Bunda, and Ngorongoro for granting permission to conduct our study in this Serengeti ecosystem. Laboratory staffs in district-designated hospitals within the ecosystem are acknowledged for their kind assistance. Muhimbili University of Health and Allied Sciences, particularly the School of Medicine and the Department of Biochemistry are acknowledged for providing time for preparation of this work.

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Peer Reviewed

Competing Interests: None declared.

Received: 15 Oct 2016; **Accepted:** 1 Feb 2017

Cite this article as: Mbugi EV, Katale BZ, Lupindu AM, Keyyu JD, Kendall SL, Dockrell HM, et al. Tuberculosis Infection: Occurrence and Risk Factors in Presumptive Tuberculosis Patients of the Serengeti Ecosystem in Tanzania. East African Health Res J. 2017;1(1):19-30. https://doi.org/10.24248/EAHRJ-D-16-00319

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Gridlock from Diagnosis to Treatment of Multidrug-Resistant Tuberculosis (MDR-TB) in Tanzania: Illuminating Potential Factors for Possible Intervention

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ABSTRACT

Settings: Kibong'oto Infectious Diseases Hospital, Kilimanjaro, Tanzania

Objective: Characterise multidrug-resistant tuberculosis (MDR-TB)-treated cases during the scaling up of molecular diagnostics using Xpert MTB/RIF and GenoType MTBDRplus

Design: Retrospective cohort study

Results: A total of 223 MDR-TB patients were referred to the Kibong'oto Infectious Disease Hospital from January 2013 through December 2014. Four cities—Dar es Salaam, Mbeya, Mwanza, and Tanga—contributed 144 (65%) of referrals. Of the total referred patients, HIV coinfection was found in 92 (41%) and 180 (81%) had history of previous TB treatment. Molecular drug susceptibility testing (DST) contributed 201 (91%) of referrals and resulted in a shorter time from diagnosis to start of treatment, 30 days (95% confidence interval [CI], 26–37), compared to conventional phenotypic DST, 212 days (95% CI, 151–272; $P<.001$). Molecular DST found higher proportions of MDR-TB children and people living with HIV without prior treatment, 5 (12%) and 24 (56%), respectively, compared to those with previous treatment for TB, 4 (2%) and 68 (38%), respectively. The median CD4 count correspondingly was 131 cells/μl (IQR, 109–131) and 200 cells/μl (IQR, 94–337) for MDR-TB diagnosed by phenotypic and molecular diagnostics ($P=.70$). Despite the more rapid time to treatment initiation among patients diagnosed by molecular DST, treatment outcomes, including time to sputum culture conversion, did not differ compared to those diagnosed with conventional phenotypic DST. Regardless of the method of diagnosis, MDR-TB/HIV coinfected patients who died had lower CD4 counts (mean 86 ± 87 cells/μl) than survivors (mean 274 ± 224 cells/μl; $P=.02$).

Conclusion: Molecular diagnostics appear to speedup the time to treatment initiation, but may not improve other treatment outcomes.

INTRODUCTION

Multidrug resistance to tuberculosis (MDR-TB) is defined as *Mycobacterium tuberculosis* (MTB) with at least resistance to isoniazid and rifampicin, and is associated with high morbidity and mortality.[1] Diagnosis of MDR-TB in resource-limited settings is immensely challenging, requiring not only identification of MTB but also drug susceptibility testing (DST) to confirm, at minimum, isoniazid and rifampin resistance. While DST can be performed by conventional phenotypic methods such as the 1% agar proportion, it requires mycobacterial culturing of sputum in biosafety facilities and takes upward of 12 weeks from the time the sputum specimen is submitted to produce results. Alternatively, mutations in the drug resistance-conferring regions of the MTB genome have reasonable diagnostic accuracy, compared to phenotypic DST, for several important TB medications, including isoniazid and rifampin. In the best-case scenario, Xpert MTB/RIF or GenoType MTBDRplus can deliver a result from an uncultured sputum specimen within a 2-day period, often as early as within the same

clinic visit. The near immediacy of this faster testing approach has led to commercialised assays and a rather unprecedented global roll out.[2]

We previously highlighted several unique aspects of the first cohort of patients treated for MDR-TB in Tanzania, 2009–2011, the majority of whom were diagnosed using conventional phenotypic DST.[3] Importantly, the average time from specimen collection to the start of MDR-TB treatment for that cohort was 9 months. At that time, the proportion of patients with MDR-TB with HIV coinfection was only 14%, while the overall country prevalence of HIV in TB notification was 40%. We hypothesised that patients with MDR-TB treated in the first cohort were those who were relatively healthy enough to survive the prolonged diagnostic and referral process, a tip of the iceberg, and that many others with MDR-TB either died before referral or the conventional phenotypic method of DST created a barrier of cost, logistical complexity, and lack of availability that led clinicians to send for this testing only when a patient had failed multiple rounds of TB treatment. Supporting these assumptions were data indicating that 86% of cases had been treated for TB at least twice previously, and those with HIV coinfection were relatively immune reconstituted, with the majority on antiretroviral therapy (ART).[3] We further hypothesised that an increase in the availability of rapid molecular DST would identify sicker patients before they died—including more of those with HIV and advanced immunosuppression—and proportionally more with primary MDR-TB or no prior TB treatment.

The scale up of molecular diagnostic testing using XpertMTB/RIF and GenoType MTB/DRplus assays began as early as 2011 in Tanzania. A preliminary study found significantly reduced time from specimen submission to MDR-TB treatment to an average of 2 months;[4] however, the duration was still long compared to the test characteristics and projections from more widespread roll out. This study was limited in that it did not examine the impact of molecular DST on MDR-TB treatment outcomes. Additionally, findings from a large prospective evaluation of Xpert MTB/RIF testing in South Africa showed the testing method did not result in a decrease in mortality or increase in the retention of patients in TB treatment.[5]

In 2014, the World Health Organization (WHO) officially recommended DST using XpertMTB/RIF for all people living with HIV (PLHIV) with presumptive TB regardless of treatment history, thus providing additional advantage for early screening of MDR-TB.[6] Although Tanzania continues to scale up molecular DST for MDR-TB diagnosis in accordance to global recommendations,[7] it remains to be seen if this paradigm shift more regionally translates to a change in MDR-TB clinical presentation or treatment outcomes.

To examine this issue, from 2013 to 2014, we had the unique opportunity to characterise consecutive patients with MDR-TB admitted at Kibong'oto Infectious Disease Hospital (KIDH)—the only national center coordinating all MDR-TB treatment—during the country's transition from conventional phenotypic DST to the faster, more specific molecular DST in order to ascertain the effect of molecular diagnostics on these important clinical parameters.

METHODOLOGY

Participants

From 1 January 2013 through 31 December 2014, the study team recruited patients with MDR-TB referred from all regions of the country to Kibong'oto Infectious Disease Hospital (KIDH) for MDR-TB treatment. Patients with previously treated MDR-TB and readmitted as failures or relapse or lost to follow-up were excluded. Those suspected of MDR-TB were screened in the domicile regions using molecular diagnostics (Xpert MTB/RIF or GenoType MTBDRplus),[8] or conventional drug-resistance surveillance performed programmatically at the Central TB Reference Laboratory (CTRL) in Dar es Salaam.

Once diagnosed by a positive test for rifampin resistance, MDR-TB patients were transported to and treated at KIDH with a standardised regimen comprised of at least 4 new anti-TB drugs and pyrazinamide during the inpatient intensive phase.[9] The four new drugs included 1 injectable agent (kanamycin or capreomycin), 1 flouroquinolone (levofloxacin), and 2 group-4 drugs (ethionamide and cycloserine). Medications were given based on weight and administered under direct observation daily for a period of 8 months. For patients experiencing drug intolerance of any of the group-4 drugs, para-aminosalicylic acid was substituted. Novel or repurposed anti-TB agents, such as bedaquiline, delamanid, clofazimine, and linezolid, were not available at that time.

Prior to initiation of second-line anti-TB drugs, baseline measurements and levels were taken such as height, weight, complete blood count, liver and renal function tests, sputum mycobacterial culture, and HIV serodiagnostics; if the latter was positive, a CD4 count was also tested per hospital routine. Treatment responses were monitored monthly and the patient was considered as culture converted if 2 consecutive negative sputum cultures were collected at least 1 month apart. Thereafter, clinically improved patients were discharged for an additional 12 months of continuation treatment that excluded the injectable agent.

Design

This retrospective cohort study reviewed consecutive medical charts of MDR-TB patients admitted to KIDH during the study period. Demographic and clinical data were extracted from charts. Treatment outcome variables were comprised of the time from sputum culture conversion to negative (intermediate outcome) and the final treatment response was categorised as either favorable or unfavorable. The predictors of treatment outcome included demographic features, the method of susceptibility testing, time from diagnosis to

referral, HIV status, CD4 count, the number of previous TB treatment episodes, and nutrition status.

Definition and Measures

Patients who had never been exposed to TB drugs or had taken anti-TB drugs for less than a month were classified as primary MDR-TB. Patients with a history of previous TB treatment—with exposure of category I or II treatments for at least 1 month or more—and a recognised World Health Organization (WHO) treatment outcome of cured, treatment complete, treatment failure, relapse, or lost to follow-up, were classified as secondary or acquired MDR-TB.[9] PLHIV patients were those with a known HIV diagnosis and on antiretroviral therapy (ART) prior to referral; all other patients were tested for HIV at KIDH at the time of MDR-TB treatment initiation regardless of their prior testing history. MDR-TB treatment outcomes were determined at the conclusion of both intensive and continuation phases of therapy. Favorable outcomes included treatment completed as recommended without evidence of failure with or without consecutive culture taken at least 30 days apart and reported culture negative during the continuation phase. Unfavorable outcomes included death by any cause; lost to follow-up, if treatment was interrupted for a greater than 8 weeks; treatment failure, if treatment was terminated or there was a need for a permanent regimen change of at least 2 anti-TB drugs because of lack of culture conversion by the end of intensive phase; or bacteriological reversion to culture positive in the continuation phase.[10] The body mass index (BMI) was defined as the ratio of weight (kg)/height^2 (m^2) at baseline. Low BMI (<18.5) was further classified into 3 categories: BMI of (17.00–18.49), (16.00–16.99), and (<16) were considered as mild, moderate, and severe malnutrition, respectively, according to WHO criteria.[11]

Data Quality Assurance and Statistical Analysis

Data were double entered from source documents in Microsoft Excel (Version 14.2.3), then transferred to SPSS (Version 20) for analysis. Descriptive results were conveyed as simple proportion with a percentage, as a mean with 95% confidence interval (CI) or standard deviation (SD), or as a median with interquartile range (IQR), when applicable. Proportions were compared using a chi-square test, while means were compared using an independent t-test and medians using the Mann Whitney U test for non-parametric data. Determinants of final treatment outcome were examined using logistic regression with HIV status; CD4 count, in cases coinfected with HIV; age; gender; history of previous TB treatment; and nutrition status as predictors. All statistical tests were two-tailed with a P value of <.05 considered as significant.

Ethical Approval

The Kilimanjaro Christian Medical University College Research Ethics and Review Committee (CRERC), the KIDH management, and the University of Virginia approved this study.

RESULTS

Demographics and Clinical Characteristics

A total of 223 patients with MDR-TB were referred to KIDH from January 2013 to December 2014. Four (17%) regions that include 4 large cities—Dar es Salaam, Mbeya, Mwanza, and Tanga—contributed 144 (65%) of MDR-TB referrals. Although the mean age was 38 ± 15 years, children under 12 years were only 9 (4%). Men constituted 145 (65%) of referrals and 92 (41%) had HIV coinfection with median CD4 count of 200 cells/μl (IQR). History of previous TB treatment was found in 180 (81%) of the study population, 121 (76%) of whom had at least 2 prior treatment episodes. The MDR-TB diagnosis in 204 (91%) was through the molecular diagnostic tools, the vast majority 184 (83%) by Xpert MTB/RIF (Table 1).

Effect of the DST Methods on the Presenting MDR-TB Features

For patients diagnosed with the conventional phenotypic DST, the mean time from diagnosis to MDR-TB treatment initiation was 212 days (95% CI, 151–272), compared to those diagnosed with the molecular DST method that had a mean time of 31 days (95% CI, 26–37; P<.001) (Table 2). Interestingly, patients diagnosed with Xpert MTB/RIF had a shorter duration to treatment initiation 27 days (IQR, 20–30) than those diagnosed by GenoType MTBDRplus (70 days, IQR, 40–100). All 19 patients diagnosed with conventional phenotypic DST had a previous history of TB treatment (100%), compared to 161 (79%) of those diagnosed by molecular DST (P=.03). Furthermore, 89 (44%) of PLHIV were diagnosed by molecular DST compared to only 3 (16%) diagnosed by phenotypic DST (P= .02).

Given the increased proportion of primary MDR-TB patients diagnosed with molecular DST compared to historical norms using only phenotypic DST, we further examined the clinical characteristics of primary and previously treated MDR-TB patients. A higher proportion (12%) of children (<12 years) were classified as primary MDR-TB compared to those with prior treatment (2%) (P=.02). Additionally, PLHIV comprised a higher proportion in primary MDR-TB cases, 24 (56%), than in previously treated cases, 68 (38%) (P=.03), although CD4 counts did not vary between those groups (Table 2). Despite primary MDR-TB having a non-significantly shorter time from sputum collection to start of MDR-TB treatment—likely due to their mode of diagnosis—history of previous TB treatment did not comparatively prolong time to

TABLE 1. Distributions of Demographic and Clinical Characteristics of Patients with MDR-TB Referred for Treatment (N=223).

Characteristics	Subcategories	Number
Age, years, mean (SD)	NA	38 (15)
Paediatric, No. (%)	Under 5 years	4 (1.8)
	5–12 years	5 (2.2)
Sex, No. (%)	Male	145 (65)
	Female	78 (35)
HIV status, No. (%)	Negative	129 (58)
	Positive	92 (41)
	Unknown	2 (1)
CD4 count for PLHIV, median (25–75 IQR)	NA	200 (99, 333)
Region of referral/domicile, No. (%)	Dar es Salaam	99 (44)
	Mwanza	23 (10)
	Tanga	11 (5)
	Mbeya	11 (5)
	Others[a]	79 (37)
History of PTB treatment, No. (%)	Yes	180 (81)
	No	43 (19)
Number of PTB episodes, No. (%)	1	59 (33)
	2	84 (47)
	3	27 (15)
	4 or more	10 (5)
Method of drug susceptibility test, No. (%)	Conventional	19 (9)
	Molecular	204 (91)
Type of molecular DST, No. (%)	GenoType MTB/RIF	184
	GenoType MTBDRplus	19
Duration from specimen submission to treatment, days, mean (95% CI)	Conventional	210 (150, 270)
	GenoType MTB/RIF	27 (20, 30)
	GenoType MTBDRplus	70 (40, 100)
Time to culture conversion, No. (%)	3 months or less	166 (72)
	More than 3 months	27 (12)
	Unknown	37 (16)

Continued

TABLE 1. Continued

Characteristics	Subcategories	Number
End of MDR-TB treatment outcomes, No. (%)	Favorable	179 (80)
	Unfavorable	44 (20)
Unfavorable treatment outcomes, No. (%)	Died	34 (15)
	Defaulted	6 (3)
	Reverted	3 (1)
	Unknown	1 (<1)

Abbreviations: CI, confidence interval; DST, drug susceptibility testing; IQR, interquartile range; MDR-TB, multidrug-resistant tuberculosis; PLHIV, people living with HIV; SD, standard deviation; TB, tuberculosis.
[a] Others refer to Iringa, Morogoro, Kilimanjaro, Tabora, Tanga, Mara, Dodoma, Pwani, Kagera, Geita, Ruvuma, Singida, Manyara, Arusha, Mtwara, Rukwa, Lindi, Shinyanga, Zanzibar, and Kigoma.

TABLE 2. Comparison of the Clinical Characteristics of Patients with Patients with MDR-TB Referred After Diagnosis with Molecular and Conventional Methods

Characteristics	Phenotypic DST (N=19)	Molecular DST (N=204)	*P* Value
Age, years, mean (SD)	34 (13)	38 (15)	.29
Sex, male, No. (%)	10 (53)	135 (66)	.30
Paediatric, No. (%)	0 (0)	9 (100)	.99
HIV status, No. (%)	3 (16)	89 (44)	.02
CD4 count for PLHIV, median (IQR)	131 (109, 131)	200 (94, 337)	.70
History of TB treatment, No. (%)	19 (100)	161 (79)	.03
Episodes of TB treatment, median (IQR)	2 (2, 3)	2 (1, 2)	.22
Duration from diagnosis to treatment, days, mean (95% CI)	212 (151, 272)	30 (26, 37)	<.001
Time to culture conversion after month 3, No. (%)	4 (25)	23 (13)	.19
Unfavorable treatment outcomes, No. (%)	5 (26)	39 (19)	.54

Abbreviations: CI, confidence interval; DST, drug susceptibility testing; IQR, interquartile range; MDR-TB, multidrug-resistant tuberculosis; PLHIV, people living with HIV; SD, standard deviation; TB, tuberculosis.

culture conversion (*P*=.11) or lead to a greater proportion of unfavorable treatment outcomes (*P*=.6) in other patients (Table 3).

Comparison of Clinical-Demographic Factors with Final Treatment Outcomes

Twenty-seven patients (12%) had delayed culture conversion (>3 months); however, for 37 (16%) of the patients time to culture conversion could not be estimated. In follow-up, 44 (20%) had unfavorable treatment outcomes, which included death in 33 (77%) and culture reversion to positive in 3 (7%) (Table 1). The latter yielded 1 (2%) extensively drug-resistant (XDR)-TB case. Of the patients who died, 15 (44%) were within 2 months of MDR-TB treatment initiation. Logistic regression analysis of the association of final treatment outcomes with potential covariates such as age, gender, HIV, CD4 count, and history of previous TB

TABLE 3. Comparison of Clinical–Demographic Characteristics of Patients with MDR-TB With and Without History of Previous TB Treatment

Characteristics	Primary MDR-TB (N=43)	Acquired or Secondary MDR-TB (N=180)	*P* Value
Age, years, mean (SD)	36 (15)	38 (14)	.38
Sex, male, No. (%)	22 (51)	123 (68)	.49
Paediatric, No. (%)	5 (12)	4 (2)	.02
HIV status, No. (%)	24 (56)	68 (38)	.03
CD4 count for PLHIV, median (IQR)	161 (80, 308)	209 (99, 367)	.27
Duration from diagnosis to treatment, days, mean (95% CI)	27 (19, 34)	53 (41, 64)	.51
Time to culture conversion after month 3, No. (%)	2 (5)	24 (16)	.11
Unfavorable treatment outcomes, No. (%)	7 (16)	37 (21)	.60

Abbreviations: CI, confidence interval; DST, drug susceptibility testing; IQR, interquartile range; MDR-TB, multidrug-resistant tuberculosis; PLHIV, people living with HIV; SD, standard deviation; TB, tuberculosis.
2 MDR-TB patients had unknown HIV status.

treatment was performed; only CD4 count had statistical significance in predicting unfavorable treatment outcomes (*P*=.02). The mean CD4 count of MDR-TB patients who died was 86 ± 87 cells/μl compared to those with favorable outcome that had a mean CD4 count of 274 ± 224 cells/μl (Table 4).

DISCUSSION

Following the roll out of molecular diagnostics for MDR-TB in Tanzania, we expectedly found more cases of MDR-TB with HIV coinfection, paediatric MDR-TB, and primary MDR-TB referred for treatment initiation. However, we were surprised to discover little difference in treatment outcomes between patients diagnosed by molecular or phenotypic methods. This observation could be for a few reasons. While the molecular tests may speed treatment initiation, the original hypothesis claiming molecular tests would reduce early mortality from MDR-TB may have been overstated. Our findings support those of a large cohort in South Africa where the roll out of Xpert MTB/RIF did not improve TB treatment outcomes.[12] Instead, outcomes may depend on other factors such as enrollment in and adherence to HIV ART, use of second-line anti-TB DST, and other host factors, including pharmacokinetic variability. We still found delays of nearly 1 month on average from diagnosis with a molecular test to treatment initiation; however, if those delays could be further reduced then a treatment outcome benefit may be observed. Also, of those with prior TB treatment, comparatively few were diagnosed by the conventional phenotypic DST, and thus may have other uncharacterised reasons for survival.

While it still possible that molecular diagnostics may prevent mortality, this benefit will be difficult to quantify in the Tanzanian setting without a cluster randomised trial, which may now be difficult to study given the widespread attempt to roll out Xpert MTB/RIF. Interestingly, compared to the GenoType MTBDRplus used in our settings, Xpert MTB/RIF reduced delay of treatment initiation by 2.5 fold. The estimated time for GenoType MTBDRplus was 70 days, which is slightly worse than 55 days reported in South Africa,[13] while the delay for Xpert MTB/RIF was 27 days, compared to 26 in similar settings and 4 days in other well-resourced settings.[12,14] These differences may be explained by the hospital laboratories that use GenoType MTBDRplus compared to Xpert MTB/RIF or other factors not tested, including specimen transport time and the means of relaying results back to patients and providers.[15] Also, the GenoType MTBDRplus suffers additional delay of testing sputum specimen on smear results prior to test.[16] Regardless of the test type, the official policy of the Tanzania National TB and Leprosy Programme accepts health system delays of no more than 14 days, yet this research suggests that considerably longer delays still exist. We are currently undertaking a nationwide study to investigate patient- and provider-related and health system-associated factors to understand the magnitude of these delays on disease transmission and to inform the design of strategies for intervention.

In Tanzania, we have observed a decline in the well-known risk factor for MDR-TB—the proportion of patients with MDR-TB categorised as having multiple episodes of

TABLE 4. Comparison of Patients Who Completed MDR-TB Treatment to Those Who Died

Characteristics	Classification	Favorable Outcomes (N=179)	Died (N=34)	*P* Value
Age, years, mean (SD)	N/A	37 (13)	41 (20)	.6
Duration from diagnosis to treatment, mean (95% CI)	N/A	48 (37, 59)	42 (19, 65)	.4
Sex, No. (%)	Male	113 (64)	26 (76)	.4
	Female	65 (36)	9 (24)	
HIV status, No. (%)	Positive	75 (84)	13 (15)	.3
	Negative	102 (88)	21 (16)	
CD4 count for PLHIV, mean (SD)	N/A	274 (224)	86 (87)	.02
History PTB treatment, No. (%)	Yes	142 (79)	30 (17)	.27
	No	35 (83)	5 (12)	
Episodes of TB treatment episodes, mean (SD)	N/A	2 (1)	2 (1)	.23
Nutrition status, No. (%)	Normal	62 (90)	4 (6)	.28
	Mild malnutrition	19 (83)	3 (13)	
	Moderate malnutrition	9 (64)	4 (29)	
	Severe malnutrition	26 (79)	5 (15)	

Abbreviations: N/A, Not applicable, CI, confidence interval; MDR-TB, multidrug-resistant tuberculosis; PLHIV, people living with HIV; SD, standard deviation; TB, tuberculosis.

retreatment for drug-susceptible TB—which was initially as high as 86% and is now down to 67%.[3,4] This statistic can be used as a crude marker for how widespread DST for MDR-TB has matured throughout the country. Indeed, in 2014 it was estimated that only 9,506 (40%) of new and 882 (34%) retreatment cases were screened for MDR-TB in 2014. Regardless of methodology, phenotypic DST or one of the molecular methods, these proportions in new and retreatment cases seem woefully low. Continued evidence points to the fact that even in the setting of adequate adherence, patients with prior TB treatment failure are at risk for acquiring drug resistance largely due to suboptimal serum drug exposure originating from the individual's pharmacokinetic variability.[17] While a rare event, this phenomenon occurs more often among those presenting with prior TB treatment failure. This hypothesis is further supported by several studies in Tanzania that found very low serum drug levels to first-line anti-TB drugs in a noteworthy proportion of patients.[18–20] To prevent the emergence of MDR-TB or more complex forms of resistance, therapeutic drug monitoring (TDM) for optimal treatment could be beneficial.[21] We support expert recommendations of applying TDM in resource-limited settings to individualise dosing to ensure adequate pharmacokinetic/pharmacodynamic (PK/PD), speed the time to culture conversion, decrease relapse, and prevent the development of resistance.[22,23] Barriers of cost and expertise may be overcome by using more field-appropriate methods, such as the use of capillary dried blood spots that do not require cold storage. When compared to the health system effort necessary to implement a molecular diagnostics strategy for MDR-TB diagnosis, TDM may be a life-saving complementary strategy.[24–26]

Lastly, this cohort has a remarkable high number of new MDR-TB in children and PLHIV compared to those previously treated for TB. A recent systematic review confirmed that HIV infection is an independent risk factor for MDR-TB, emphasising several risk factors including defects in infection prevention control, especially in hospital settings.[27] Therefore, routine screening of PLHIV to identify and test patients presenting with at least one of the signs and symptoms of MDR-TB—such as current cough, fever, weight loss, or night sweats—should eventually impact morbidity and mortality in this group.[6] To achieve the STOP TB Partnership global plan of 90(90)90—to diagnose least of 90% of all the people with TB, reach 90% of key populations (the most vulnerable or underserved), and achieve 90% treatment success—then

diligent approaches are needed for MDR-TB identification in currently underserved populations, such as children.[28] Estimates have shown that every year there is an enormous detection gap for children with not only TB but also MDR-TB.[29] Several challenges surround diagnosis of MDR-TB in children; the paucibacillary nature of TB and the inability of paediatric patients to produce an adequate sputum specimen complicate diagnostic processes. Further operational research that combines available diagnostic strategies, such as active specimen collection in children using sputum induction or gastric aspiration, and tests a wide variety of diagnostics in different settings and age groups may begin to address this diagnostic gap.

Despite the inclusion of all patients treated for MDR-TB in Tanzania during the study period, there were limitations to the analyses, as the hospital-based observational approach included only those who were successfully referred to KIDH for MDR-TB treatment and excluded patients unable to reach the MDR-TB facility due to early death and loss to follow-up during the diagnosis process.

In summary, we have observed a clear shift in the use of molecular diagnostics for referral to MDR-TB treatment in Tanzania, but that shift has not been associated with obvious improvements in MDR-TB treatment outcomes. Molecular diagnostics appear, however, to have contributed to an earlier detection, diagnosis, and treatment of MDR-TB in children and PLHIV. However, despite changes in diagnostic practices, delays persist and should be considerably reduced. As such, we propose MDR-TB diagnostic strategies include a community education component that explains the factors contributing to diagnostic delay for both patients and health systems. Alternative strategies to prevent MDR-TB may also be necessary; and, once MDR-TB is diagnosed, further actions to address MDR-TB treatment failure—such as the use of quantitative second-line DST and individualised regimens—may ultimately lead to the full potential gains envisioned for the roll out of molecular MDR-TB diagnostics.

Acknowledgements: The authors would like to acknowledge the Malaria Capacity Development Consortium (MCDC), which supported A. L. in his Master of Public Health training. S. G. M. is supported by project ID B40121, which received financial support from TDR—the Special Programme for Research and Training in Tropical Diseases, co-sponsored by UNICEF, the United Nations Development Programme (UNDP), the World Bank, and WHO—which enabled the final drafting of the paper.

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Peer Reviewed

Competing Interests: None declared.

Received: 21 Oct 2016; **Accepted:** 1 Feb 2017

Cite this article as: Liyoyo AA, Heysell SK, Kisonga RM, Lyimo JJ, Mleoh LJ, Mutayoba BK, et al. Gridlock from Diagnosis to Treatment of Multidrug-Resistant Tuberculosis (MDR-TB) in Tanzania: Illuminating Potential Factors for Possible Intervention. East African Health Res J. 2017;1(1):31-39. https://doi.org/10.24248/EAHRJ-D-16-00330

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Multidrug-Resistant Bacterial Isolates Recovered from Herbal Medicinal Products Sold in Nairobi, Kenya

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ABSTRACT

Background: Medicinal herbs have been reported to be contaminated with microorganisms indigenous to the environment. These microbes become a threat when they harbour drug-resistant traits.

Objective: The aim of this study was to evaluate phenotypic and genotypic drug-resistant traits of bacteria isolated from herbal medicinal products in Nairobi, Kenya.

Methods: We employed an exploratory as well as laboratory-based experimental design. Herbal products were purchased from markets and transported to Kenya Medical Research Institute laboratories for processing and analysis. Microbial contamination and antibiotic susceptibility were determined following standard methods. Antibiotic-resistant genes were determined using polymerase chain reaction. Data were coded and analysed accordingly.

Results: We collected 138 samples of herbal products in the form of liquids, powders, capsules, creams/lotions, and syrups. In total, 117 samples (84.8%) were contaminated with bacteria and 61 (44.2%) were contaminated with fungi. *Bacillus*, *Klebsiella*, *Proteus*, *Staphylococcus*, *Streptomyces*, *Escherichia*, *Enterobacter*, *Serratia*, *Yersinia*, *Morganella*, *Citrobacter*, *Erwinia*, and *Shigella* were the bacterial genera identified. Most of the isolated bacteria were generally sensitive to the panel of antibiotics tested, although a few (35 [36.5%]) were resistant; more than half of these were resistant to more than 1 of the antibiotic agents we tested.

Discussion: We found an association between phenotypic and genotypic drug resistance among the drug-resistant bacteria. This study makes it evident that herbal medicinal products sold in Nairobi are contaminated with drug-resistant bacteria.

Conclusions: The results show that herbal medicinal products are a potential source of dissemination of multidrug-resistant bacteria. There is an urgent need for specific education programmes, policies, and regulations that address herbal products' safety to prevent the possibility of these pathogens being involved in deadly invasive infections.

INTRODUCTION

Antibiotic-resistant bacteria have been a source of an ever increasing therapeutic challenge.[1] Continued mismanagement of antibiotics and the resulting selective pressure have contributed to the emergence of multidrug-resistant bacteria; this has been regarded as an inevitable genetic response to antimicrobial therapy.[2] Drug-resistant infectious microbes have become an important public health concern that warrants organisations in public and private sectors worldwide working together.[3,4] Aside from the public health threat, the search for newer microbial-sensitive treatments to overcome resistant microbes is usually very expensive and contributes to the higher costs of health care, which is attributed to longer hospital stays.[4]

Microbial resistance to antimicrobial agents is usually mediated through gene coding for resistance. The resistant genes are either chromosomal (intrinsic) or plasmid encoded (extrinsic). Plasmids are self-replicating extra chromosomal DNA molecules found in Gram-negative and Gram-positive bacteria as well as in some fungi (yeast and moulds).[5]

Determination of antibiotic-resistant genes through the use of polymerase chain reaction (PCR) techniques provides insights on genetic information relating to resistance to 1 or more antibiotics. The genetic information may also reflect the extent or amount of the multidrug resistance.[6] Bacteria which are resistant to antibiotic agents may develop anywhere, especially in a confined environment previously contaminated with drug-sensitive bacteria. One such environment

can be in herbal medicinal products (HMPs), and HMPs have been previously implicated as a pool for such contamination.[7,8]

The use of HMPs as a form of complementary medicine is becoming increasingly popular in both developing and developed countries.[7] About 70% to 80% of the world's population, particularly in the developing world, has been shown to depend on herbal drug regimens for their primary health care.[9] As the pros and cons of HMPs are pondered, it is important to monitor and ascertain their pureness, as HMPs contaminated with microbes, especially drug-resistant microbes, may pose important health, medical, and economic implications.[7]

Monitoring of HMPs will help to identify microbial contamination, provide information on the rate of antimicrobial resistance, and devise mechanisms to slow down the rate of emergence of drug-resistant strains from HMPs.[10] In the present study, we evaluated selected HMPs from Nairobi, Kenya, for the presence of contaminating microorganisms. These microorganisms were later subjected to susceptibility studies to establish their resistance profiles. The DNA for phenotypic-resistant isolates were extracted and used to determine genotypic resistance using specific primers coding for antibiotic-resistant genes.

MATERIALS AND METHODS

Study Site and Design

The study was undertaken in Nairobi, the capital and largest city in Kenya. Nairobi has several herbal clinics, especially in densely populated areas. However, HMPs are also sold in health food stores, pharmacies/chemists, supermarkets, local retailers, and hawkers, among other outlets. This study employed an exploratory as well as laboratory-based experimental design.

Sample Collection

We collected HMPs from different herbal vendors across Nairobi County. The study sample included 138 different HMPs in different preparations, which included liquids, powders, capsules, creams/lotions, and syrups.

Herbal Products Displayed on Shelves. A, Herbal products sold in herbal chemist or health food stores. B, Herbal products sold in herbal clinic.

Formulations of Herbal Products. A, Capsules. B, Liquid (concoctions). C, Syrups and concoctions. D, Powdered roots and stem barks.

Isolation and Identification of Contaminating Bacteria

Each HMP was serially diluted and plated in triplicate on selective, differential, and general purpose media for bacteria growth. HMPs were incubated at 37°C for 12 to 18 hours. The ensuing colonies were further purified, isolated, and characterised using standard methods.[11]

Susceptibility Testing of the Bacterial Isolates

Briefly, the following antimicrobial discs were placed onto Mueller-Hinton agar plates seeded with the bacteria strains: piperacillin, ciprofloxacin, norfloxacin, cefotaxime, gentamicin, sulphamethoxazole/trimethoprim, chloramphenicol, and ceftazidime. The plates were incubated overnight for 12 to 18 hours, and any microorganism that showed resistance[12] to any of the antibiotics was isolated for further resistance DNA isolation studies. After isolation, the bacteria were stocked and stored in a negative 40°C deep freezer.[13]

DNA Extraction, PCR, and Gel Electrophoresis

The bacteria were retrieved from the freezer, thawed, and cultured in brain heart infusion broth at 37°C overnight. Total DNA were extracted from 5 mL of a broth culture grown overnight. After incubation, bacterial cells were harvested by centrifugation at 3,000 rpm (radius 7.20 cm) for 10 minutes; the cell pellets were suspended in phosphate-buffered saline with 100 µg of lysostaphin per millilitre and incubated at 37°C for 30 minutes. The phenol/chloroform extraction method was used for nucleic acid extraction, and the DNA was precipitated in 1 mL of 70% ethanol. The DNA precipitate was dissolved in 50 µl of TE buffer (10 mM Tris-

Cl, 1 mM EDTA; pH 8.0) and stored at negative 20°C until processing.[13]

The PCR amplification was performed in a 25 µl reaction mixture (2.5 mL of 10× reaction buffer without MgCl2; 200 µM of each deoxynucleoside triphosphate, 2 mM MgCl2; 2.5 pmol of each primer and approximately 2–4 µl of template DNA) and brought up to a 25 µl final volume with sterile DNA/RNA-free distilled water. To reduce the formation of nonspecific extension products, a 'hot-start' protocol was adapted. The PCR reactions were hot-started for 5 minutes at 95°C and placed on ice, and 2 µl of Taq polymerase was added. Reaction mixtures were subjected to 30 PCR cycles (95°C for 2 minutes, then 1 minute at 54°C, and 1 minute at 72°C). A final elongation step of 7 minutes at 72°C was applied in a thermal cycler.[13]

Among the drug-resistant isolates, the following genes were investigated:

- The *aacA-aphD* gene coding for gentamicin resistance, with 227 base pairs. The primers are *aacA-aphD*: F-TAA TCC AAG AGC AAT AAG GGC and *aacA-aphD*: R-GCC ACA CTA TCA TAA CCA CTA.
- The bla_{CMY} gene, which has 205 base pairs and is responsible for fourth-generation cephalosporin (cefepime, ceftazidime) resistance. Its primers are bla_{CMY}: F-GAC AGC CTC TTT CTC CAC and bla_{CMY}: R-TGG AAC GAA GGC TAC GTA.
- The bla_{CTX-M} single gene coding for cefotaxime and piperacillin resistance, which has 499 base pairs. Its primers are CTX-M1: F3-GAC GAT GTC ACT GGC TGA GC and CTX-M1: R2-AGC CGC CGA CGC TAA TAC A.
- The *gyrA* is a single gene that codes for norfloxacin and ciprofloxacin resistance and has 574 base pairs. Its primers are *gyrA*: F1-ATG TCA GAC AAT CAA CAA CAA GC and *gyrA*: R3-ACA TTC TTG CTT CTG TAT AAC GC.
- The *SulA* gene that codes for sulphamethoxazole/trimethoprim resistance and has 360 base pairs. Its primers are *SulA*: F-AC TGC CAC AAG CCG TAA and *SulA*: R-GTC CGC CTC AGC AAT ATC.[14]

The DNA products were loaded on an agarose gel, and gel electrophoresis was performed to separate the mixture of DNA pellets. The DNA bands (products) were visualised using ultraviolet transmission light and were photographed alongside the controls and molecular weight markers.[13] We then identified the DNA bands in reference to the controls to determine the associated genes. This information was used to correlate the phenotypic and genotypic characteristics of the targeted drug-resistant bacteria.

RESULTS

We collected and analysed 138 samples of HMPs. These samples included 106 powders (76.8%), 18 liquids (13.0%), 8 syrups (5.8%), 4 creams/lotions (2.9%), and 2 capsules (1.4%). Seventy-four of the samples (53.6%) came from street vendors/hawkers, 34 (24.6%) from herbal clinics, 19 (13.8%) from supermarkets/shops, 7 (5.1%) from manufacturers/wholesalers, and 2 each (1.4%) from chemists and health food stores.

Bacteria isolated from the collected HMPs were grouped into 13 genera: *Bacillus*, *Klebsiella*, *Proteus*, *Staphylococcus*, *Streptomyces*, *Escherichia*, *Enterobacter*, *Serratia*, *Yersinia*, *Morganella*, *Citrobacter*, *Erwinia*, and *Shigella*. The genera and species of the bacteria isolated from HMPs are shown in the Table.

For this study, we tested 96 (100.0%) isolates of bacteria for susceptibility to the commonly used antibiotics. Most of the isolated bacteria (61 [63.5%]) were generally sensitive to the panel of antibiotics. Thirty-one (32.3%) bacterial isolates were resistant to ceftazidime; 33 (34.4%) were resistant to cefotaxime; 2 (2.1%) were resistant to gentamicin; 5 (5.2%) were resistant to chloramphenicol; 1 (1.0%) was resistant to piperacillin; and 2 (2.1%) each were resistant to norfloxacin and ciprofloxacin, respectively.

The isolated bacteria were resistant to either 1 or more than 1 antibiotic. The following isolates were resistant to only 1 antibiotic: *Morganella morganii* (MM2), resistant to chloramphenicol; *Enterobacter cloacae* (EC3), resistant to ceftazidime; and *Proteus penneri* (PP20), resistant to cefotaxime. Four isolates were resistant to 3 antibiotics: *Citrobacter diversus* (CD1), resistant to gentamicin, cefotaxime, and norfloxacin; *M morganii* (MM1), resistant to sulphamethoxazole/trimethoprim, chloramphenicol, and cefotaxime; *Enterobacter aerogenes* (EA2), resistant to ceftazidime, cefotaxime, and piperacillin; and *Klebsiella pneumoniae* (KP2), resistant to sulphamethoxazole/trimethoprim, ceftazidime, and cefotaxime.

Two isolates exhibited resistance to 4 antibiotics: *C diversus* (CD2), resistant to sulphamethoxazole/trimethoprim, chloramphenicol, norfloxacin, and ciprofloxacin; and *E cloacae* (EC5), resistant to sulphamethoxazole/trimethoprim, ceftazidime, cefotaxime, and norfloxacin. Twenty-six isolates were resistant to 2 antibiotics. Most of these isolates (22 [62.9%]) were resistant to both ceftazidime and cefotaxime.

Bacterial DNA extraction was performed and was later amplified independently with reverse and forward primers in a single reaction in order to determine antibiotic-resistant genes among the phenotypic-resistant isolates. The bacteria were *C diversus* (CD1 and CD2), *E aerogenes* (EA2), *E cloacae* (EC2, 3, 4, 5, 8, 9, and 10), *Erwinia chrysanthemi* (ERC1), *K pneumoniae* (KP2), *M morganii* (MM1 and 2), *P penneri* (PP4, 8, 9, 10, 11, 12, 13, 17, 18, 20, 21, and 22), *Serratia marcescens* (SM1 and 2), *Serratia rubidaea* (SR4, 6, and 7), and *Yersinia enterocolitica* (YE3, 6, 7, and 8).

Except for 2 isolates, all the bacteria found to be resistant to the drugs were found to contain drug-resistant genes. There was an association between the phenotypic and

TABLE. Genus and Specific Epithets of the Isolated Bacteria

Genus	Organism(s)	Gram Reaction	Frequency (%)
Citrobacter	*C diversus*	Gram −	3 (100.0)
Enterobacter	*E aerogens, E cloacae*	Gram −	22 (100.0)
Streptomyces	*S* spp.	Gram +	74 (100.0)
Bacillus	*B anthracoides, B* spp.	Gram +	64 (100.0)
Erwinia	*E chrysanthemi*	Gram −	1 (100.0)
Escherichia	*E coli*	Gram −	7 (100.0)
Morganella	*M morganii*	Gram −	2 (100.0)
Klebsiella	*K pneumoniae*	Gram −	4 (100.0)
Proteus	*P penneri*	Gram −	25 (100.0)
Serratia	*S fonticola, S marcescens, S rubidaea*	Gram −	14 (100.0)
Shigella	*S sonnei*	Gram −	1 (100.0)
Staphylococcus	*S aureus*	Gram +	5 (100.0)
Yersinia	*Y enterocolitica*	Gram −	11 (100.0)
Total			*233 (100.0)*

Abbreviations: Gram −, gram negative; Gram +, gram positive.

genotypic drug resistance among the drug-resistant isolates. All the isolates that were phenotypic resistant to cefotaxime and ceftazidime contained the $bla_{CTX\text{-}M}$ gene and the bla_{CMY} gene.

Figure 1 shows DNA fragments for isolates that were resistant to cefotaxime; the $bla_{CTX\text{-}M}$ gene has 499 base pairs. Figure 2 shows DNA fragments for isolates that had genes coding for ceftazidime resistance; the bla_{CMY} gene has 205 base pairs.

DISCUSSION

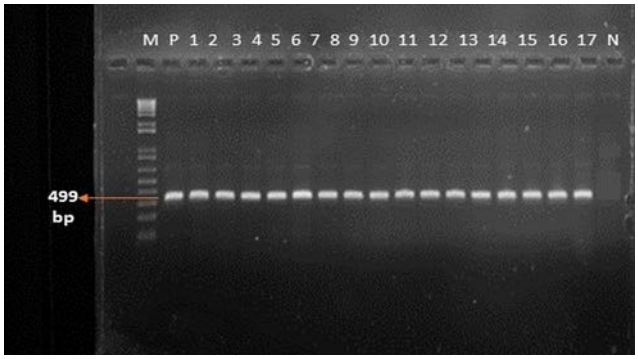
HMPs designed for chemotherapeutic and pharmacological benefits should be effective against the targeted medical condition. Several factors could compromise this goal, including contamination with pathogenic and nonpathogenic microorganisms.[15] Apart from possible microbial degradation of the active constituents contained in the HMPs, the presence of contaminating microorganisms could constitute a source of infection and a serious health risk to consumers, who were probably already overwhelmed by the serious medical conditions for which the HMPs were initially indicated.[16] Drug-resistant traits in products that are consumed can lead to serious health conditions which do not respond to antibiotic agents.

In this study, soil bacteria formed the bulk of the isolates found. These bacteria were *Streptomyces* species (74 [53.6%]) and *Bacillus anthracoides* (64 [46.4%]), which indicate environmental contamination. According to a study done by Grierson,[17] *B anthracoides* is pathogenic to guinea pigs and mice under experimental conditions, and it would appear to occupy a position between the virulent *Bacillus anthracis* and the nonpathogenic members of the group of aerobic sporing bacilli (e.g., *Bacillus subtilis*, *Bacillus mesentericus*). When people who are sick are exposed to *B anthracoides* through consumption of contaminated HMPs, this could pose a serious health risk.

Escherichia coli were isolated in liquid HMP formulations, which include concoctions, decoctions, and infusions. Generally all the liquids were dissolved in water; hence the water for dissolution might have contained *E coli*. *E coli* is an indication of faecal contamination and is associated with gastroenteritis.

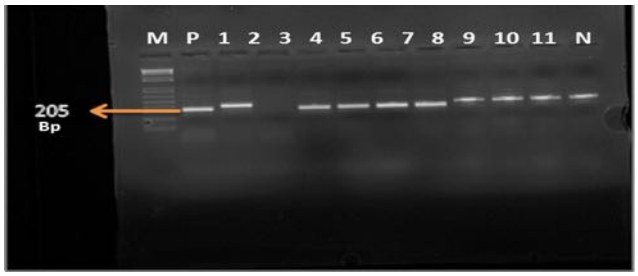
The most important bacteria we isolated in terms of potential human diseases were *K pneumoniae*, *Staphylococcus aureus*, *Proteus* species, *Shigella sonnei*, and *E coli* (among other coliforms). These results concur with a similar study by Frazier and Westhoff,[18] who isolated bacteria of clinical importance such as *Bacillus* species, *Salmonella* species, and *E coli* from herbal products, although the current study did

FIGURE 1. Bacterial Isolates with *bla*$_{CTX-M}$ Gene Coding for Cefotaxime Resistance with 499 Base Pairs



Lane M is a molecular marker with different bands denoting different molecular weights: each band stands for 150 base pairs. Lane P is the positive control; Lane N is the negative control; and Lanes 1–17 show bacterial isolates containing the *bla*$_{CTX-M}$ gene. The *bla*$_{CTX-M}$ gene has 499 base pairs.

FIGURE 2. Bacterial Isolates with *bla*$_{CMY}$ Gene Coding for Ceftazidime Resistance with 205 Base Pairs



Lane M is a molecular marker with different bands denoting different molecular weights: each band stands for 50 base pairs. Lane P is the positive control; Lane N is the negative control; and Lanes 1–11 show bacterial isolates containing the *bla*$_{CMY}$ gene. The *bla*$_{CMY}$ gene has 205 base pairs.

not find any species of *Salmonella*. Shukla and colleagues,[19] in a similar study, reported a high recovery rate of these suspected infectious bacteria from indigenous orally consumed herbal medications. Danladi and colleagues[20] found similar results in their study on herbal preparations. However, these other studies did not determine drug susceptibility of the isolated bacteria.

The majority of the bacterial isolates found in this study (61 [63.5%]) were sensitive to the antibiotics tested. These results concur with a study done by Alwakeel on microbial contaminants of herbal medicine, where he found that most (75%) of the bacteria isolated were sensitive to the antibiotics,[21] but did not determine the presence of drug-resistant genes.

Testing bacterial pathogens for their responses to chemotherapeutic agents is common practice in clinical and food microbiology.[22] In our study, 36.5% of the isolated bacteria were resistant to the panel of antibiotics we tested. Other studies have observed a higher level of resistance (46.2% to 51.7%) to the commonly used antibiotics.[22,23] Angulo and colleagues[24] alleged that the ability of bacteria to evolve mechanisms to resist attack by antimicrobials was recognised soon after the widespread deployment of the first antibiotics. DeWaal and colleagues[25] have also suggested that resistance is an inevitable consequence of antibiotic use; the more antibiotics are used, the more bacteria will develop resistance to them.

All the bacteria we found to be drug-resistant, except for 2 (5.7%) isolates, were found to contain resistant genes. The antibiotic-resistant bacterial isolates were resistant to either 1 or more than 1 of the antibiotics tested and were also found to contain drug-resistant genes. There was a direct association between phenotypic resistance and genotypic resistance.

Antibiotics are used to treat bacterial infections. They may be used as a short- or long-term treatment, depending on whether the problem is acute or chronic. A study by Ash and colleagues[26] found that bacteria with intrinsic resistance to antibiotics are found in nature. Such organisms may acquire additional resistant genes from other bacteria introduced into soil or water, and the resident bacteria may be the reservoir or source of the widespread drug-resistant organisms found in many environments. In bacteria, antimicrobial resistance is facilitated by the ability to quickly adapt to new environments and to replicate very quickly. From this comes the aptitude to mutate the DNA acquired from other drug-resistant bacteria.[4]

The acquisition of resistance to drugs may be due to chromosomal mutations or mobile genetic elements like plasmids that are often capable of transfer from one strain of organism to another, even across the species barrier. Plasmid transfer within and across species is further enhanced through the activities of transposons, which are mobile genetic elements that can confer resistance determinants.[5] The ability of transposons to integrate into either conjugative plasmids or into an organism's chromosomes enhances the transferability of a given determinant of resistance.[25]

This process is a natural phenomenon exacerbated by the abuse, overuse, and misuse of antimicrobials in the treatment of human illness and in animal husbandry, aquaculture, and agriculture.[2] When drug-resistant organisms are

present in medicaments, such as HMPs, they could behave as opportunist pathogens and initiate an infection, particularly in immune-compromised patients. They can also lead to transfer of antibiotic-resistant traits to hitherto drug-sensitive microorganisms that cohabit within the consumers of the contaminated products.

Given the increasing rate of development of resistant bacteria strains, the main challenge is to slow the rate at which resistance develops and spreads. To do this, physicians, pharmacists, researchers, and consumers alike need to be more aware of the selective pressures that drive these bacteria to decrease their susceptibility.[2] These selective pressures include the abuse, overuse, and misuse of antimicrobials in therapy; improper manufacturing and mishandling of HMPs;[2,5] and numerous other socioeconomic factors that govern the development of multidrug-resistant bacteria strains.[26] In such circumstances, a collective and concerted effort towards preventing the development of resistant bacteria strains through rational antimicrobial use policy, right practices, and intensive research leading to novel and alternative drug therapies would help check the emergence of multidrug-resistant bacteria strains.

CONCLUSION AND RECOMMENDATIONS

The results of the present work show that HMPs were contaminated with both pathogenic and nonpathogenic bacteria. Of concern was the multidrug resistance found among the isolated bacteria, since only 3 isolates were resistant to only 1 drug, while 32 isolates were resistant to more than 1 antibiotic. All the drug-resistant bacteria harboured drug-resistant genes. The high rate of strains with multidrug resistance that were isolated from these herbal preparations may indicate widespread antibiotic resistance among microorganisms from different sources. It is therefore important that quality assurance is built into the whole process of manufacturing HMPs. Thus, there is a need for constant monitoring and control of the microbial standards of herbal medicines available on the market. Further studies should sequence bacteria that are found to have genotypic resistance in order to determine their relatedness. Mobile-resistant genes should be determined, because bacteria that share the same environment can transfer mobile genes to antibiotic-sensitive bacteria through plasmids and transposons.

Acknowledgements: We are very grateful to the National Commission for Science, Technology and Innovation (NACOSTI) for funding this work. We would also like to sincerely acknowledge the heads of the Kenya Medical Research Institute and the University of Nairobi for providing an enabling environment for this work to be done.

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Peer Reviewed

Competing Interests: None declared.

Received: 9 Jan 2017; **Accepted:** 10 Feb 2017

Cite this article as: Korir R, Anzala O, Jaoko W, Bii C, Keter L. Multidrug-Resistant Bacterial Isolates Recovered from Herbal Medicinal Products Sold in Nairobi, Kenya. East African Health Res J. 2017;1(1):40-46. https://doi.org/10.24248/EAHRJ-D-17-00027

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Frequency of Uropathogens and Antimicrobial Susceptibility in Childhood Urinary Tract Infection at Kamenge University Hospital, Bujumbura, Burundi

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ABSTRACT

Background: Increasing resistance to antimicrobials is a worldwide problem. The aim of our study was to determine the pathogens and antimicrobial susceptibility of bacteria causing urinary tract infection (UTI) in children.

Methods: This is a prospective cohort study conducted over a 10-month period with 101 children hospitalised at Kamenge University Hospital for acute UTI. The infections were confirmed by Kass urinalysis criteria, and culture and susceptibility antibiotic tests were performed for isolated microbial agents.

Results: Frequency of UTI in the overall population of children hospitalised at Kamange University Hospital was 8.4%. Of the 101 children with UTIs, 87 (86.1%) were under the age of 24 months. Diagnosis of pyelonephritis (82%) was the most common, followed by cystitis (18%). *Escherichia coli* (82%) was the most frequent pathogen causing UTI. We found *E coli* and *Klebsiella pneumonia* to be resistant to aminopenicillins (100%), cotrimoxazole (98.2%, 100%), Augmentin (amoxicillin/clavulanic acid) (70.5%, 80%), cefotaxime (45.8%, 28.6%), cefuroxime (36.8 to 45.5%, 50%), fluoroquinolones (33.3 to 53.6%, 28.6 to 50%), gentamicin (27.5%, 20%), and nitrofurantoin (9.3%, 50%).

Conclusion: *E coli* is the main causal agent of UTI in childhood with a high resistance to antibiotics. Appropriate antibiotics for empiric therapy should be based on local circulating bacterial strains and resistance profiles.

INTRODUCTION

Antimicrobial resistance to common bacterial infections has become an increasingly challenging worldwide problem, as many currently available drugs have been found to have little or no effect on new resistant bacteria.[1] Unfortunately, in many regions of the world, this issue is compounded by inadequate surveillance methods and a lack of accurate and reliable data, particularly in sub-Saharan Africa.[1] Information concerning the antimicrobial susceptibility for bacteria causing urinary tract infections (UTIs) in children is more limited. UTI is one of the most common infection in children, and as many as 2% of children are diagnosed with at least 1 episode by the age of 10 years.[2] Current research shows a range of frequencies of UTI infection in children: Adonis-Koffy et al[3] reported the frequency of 0.77% in Abidjan, Côte d'Ivoire; Bourskraoui et al[4] noted the frequency of 1.33% in Marrakech, Morocco; and Taques et al[5] found a high prevalence between 5 and 10% in France. The prevalence of UTI in children is variable, based largely on sex and age. Within the first 12 months of life, the risk of UTI has been estimated at 6% of girls and 3% of boys. Between 12 and 24 months, the difference between the sexes increases with incidence estimated at 8% of girls and 2% of boys.[2] Each year, 150 million UTIs are reported worldwide with estimated cost of more than 6 billion US dollars.[6,7] Clinical diagnosis of UTI is not easy during childhood. The signs and symptoms in infants and young children are variable and nonspecific of urinary tract, which can delay diagnosis and treatment.[8] Empirical and appropriate antimicrobial therapy should lead to rapid recovery and avoidance of complications. Delayed or incorrect antibiotic treatment may result in recurrence, particularly in children with renal abnormalities who risk developing progressive

renal damage with hypertension and chronic renal failure, which can affect growth and quality of life of the child in long run.[9,10] The antibiotic choice for empiric therapy should be based on local circulating bacterial strains and resistance profiles. In Burundi, antibiotic resistance has become a growing concern, particularly in children. Our research focused on the pathogens and antimicrobial susceptibility of bacteria causing UTIs in children hospitalised in paediatric service at Kamenge University Hospital, Bujumbura, Burundi.

METHODS

Design and Setting

We conducted a prospective cohort study of children hospitalised for UTIs with a positive urine culture in the paediatric service at Kamenge University Hospital from 1 January to 1 November 2013. A UTI was defined by the Kass criteria as leukocyturia ≥ 10^4 cells/ml and bacteriuria ≥ 10^5 cells/ml with the presence of a single bacterial pathogen.[11]

Study Population

The study population began with the 1,200 children who had been hospitalised in the paediatric service at Kamenge University Hospital during the study period. Children aged 0 to 16 years with signs and symptoms leading to suspect acute UTI—including temperature ≥ 38°C, chills, frequency of urination, dysuria, urgency to urinate, suprapubic and/or flank tenderness, and fever with unknown source—with clinical evidence of sepsis were included in the study. We excluded children who had symptoms or signs implicating another infection source in order to rule out asymptomatic bacteriuria accompanied with other infections. Patients who had been on antibiotics for at least 3 days were also excluded. A total of 156 children provided samples for urinalysis. UTIs were confirmed for 101 children corresponding to our sample.

Urine Collection and Transport

Urine samples were collected from younger children and infants using sterile urine bags, and from children older than 24 months using midstream clean-catch method. For the younger population, urine bags were initially placed by trained nurses, using standard perineal cleansing procedures. Parents were trained how to place another, according to the same cleansing procedures, if the urine bags were rejected or separated from the skin, which could result in leakage or stool contamination. Midstream clean-catch specimens were obtained by trained parents using a sterile urine container, taking care that the perineum did not touch the inside of the container. Within 30 minutes of collection, specimens were sent in ice bags for analysis to the laboratory of Kamenge University Hospital. Specimens were processed promptly or refrigerated at 4°C for a maximum of 12 hours before being analysed.

Laboratory Methods

Microscopic and macroscopic tests assessed the color and turbidity of urine and the number of leukocytes and flora on Malassez cell, respectively. Samples containing ≥ 10^4/ml of leukocytes were cultured on a selective medium Eosin Methylene Blue (EMB) Agar (Titan Media, India) and on a non-selective medium cysteine lactose electrolyte deficient (CLED) (Titan Media, India) with an incubation period of 18 to 24 hours at 37°C. Cultures of a single organism with a count of ≥ 10^5 colony-forming units/ml were considered to represent infection and were then identified by Gram-stain to distinguish the bacilli from the cocci. The biochemical characteristics of Gram-positive bacilli were determined on a gallery including Kligler Iron Agar (glucose, H_2S, lactose, gas) (Titan Media, India), Mannitol mobility test medium, Simmons Citrate Agar (Fortress Diagnostics, UK) and Urea Indole Medium (HiMedia Laboratories, India). The characteristics of Gram-positive cocci were determined by culture on mannitol salt agar, or Chapman medium, and D coccossel agar. Routine diagnostic antimicrobial susceptibility results were determined using the agar disk-diffusion method, in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations.[12] Colonies from EMB were inoculated on sterile Mueller Hinton Agar (HiMedia Laboratories, India) using antibiotic discs and then incubated at 37°C for 18 to 24 hours. The antibiotics tested during this process were ceftazidime (30 µg), cefuroxime (30 µg), furadantine (300 mg), cefotaxime (30 µg), ciprofloxacin (10 µg), cloxacillin (4 µg), tetracycline (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), Augmentin (amoxicillin 20 µg and clavulanic acid 10 µg), amoxicillin (25 µg), cotrimoxazole (1.25/3.75 µg), and gentamicin (10 µg). Following the incubation period, results were determined by measuring the diameter of the inhibition of zone. Zones of inhibition greater than 10 mm were considered sensitive, 5 to 10 mm moderately sensitive, and no zone of inhibition resistant.[13]

Data Analysis

For this analysis, bacteria with intermediate resistant were not considered as resistant. Data were analysed using EpiInfo (Version 3.5.3). The proportions were compared using both Fisher's exact test and chi-square test to examine the difference in antimicrobial susceptibilities, a P value of <0.05 was used as a cut-off point for statistical significance.

Ethics

The urine samples were taken only after researchers explained the purpose of the study to every parent of a child, and verbal and free consent was given.

RESULTS

During the study period, 1,200 children were hospitalised in paediatric service at Kamenge University Hospital, of whom

TABLE 1. Sample Distribution by Age and Sex (N=101)

Sex	No. ≤ 24 Months	No. > 24 Months	Total
Male	47	3	50
Female	40	11	51
Total	87	14	101

700 (302 boys and 398 girls) were aged ≤ 24 months and 500 (240 boys and 260 girls) were aged more than 24 months. After applying the exclusion criteria and conducting a series of tests, 101 children (8.4%)—50 boys and 51 girls—diagnosed with UTIs were included in the study. The study population was comprised of 87 children (86.1%) aged ≤ 24 months—47 (46.5%) boys and 40 (13.9%) girls—and 14 children (13.9%) aged more than 24 months—3 (3%) boys and 11 (10.9%) girls (Table 1). A peak frequency of UTI was observed between the ages of 7 and 9 months, which gradually decreased and remained stable after the age of 30 months. The UTIs were defined by the site of predominant alteration, resulting in 83 cases of pyelonephritis (82%) and 18 cases of cystitis (18%).

Organisms

We noted that 96 (95%) of isolates were enterobacteriaceae, of which 82% were *E coli,* 10% were *Klebsiella pneumoniae,* and 3% were *Proteus mirabilis*. Other bacteria isolated were *Staphylococcus aureus* (3%), *Enterococcus faecalis* (1%), and *Pseudomonas aeruginosa* (1%) (Table 2).

TABLE 2. Frequency of Bacteria Responsible for Urinary Tract Infections

Bacteria	Frequency	Percent
Escherichia coli	83	82
Klebsiella pneumoniae	10	10
Proteus mirabilis	3	3
Staphylococcus aureus	3	3
Enterococcus faecalis	1	1
Pseudomonas aeruginosa	1	1
Total	101	100

Antimicrobial Susceptibility Patterns

The antimicrobial susceptibilities of the most common organisms, *E coli* and *K pneumoniae*, are shown in Table 3. *E coli* isolates were resistant to cotrimoxazole in 56 of 57 (98.2%) of cases followed by Augmentin in 55 of 78 (70.5%), cefotaxime in 27 of 59 (45.8%), cefuroxime in 20 of 44 (45.5%), ciprofloxacin in 25 of 75 (33.3%), gentamicin in 14 of 51 (27.5%), and nitrofurantoin in 4 of 43 (9.3%). *K pneumoniae* isolates presented high resistant to cotrimoxazole in 10 of 10 (100%) cases followed by Augmentin in 8 of 10 (80%), cefuroxime in 2 of 4 (50%), ciprofloxacin in 2 of 6 (33.3%), cefotaxime in 2 of 7 (28.6%), gentamicin in 1 of 5 (20%), and nitrofurantoin in 1 of 2 (50%). For all antibiotics tested, no observed statically significant differences were found in resistance prevalence between *E coli* and *K pneumoniae* (Table 3).

DISCUSSION

This study has shown the prevalence of isolation and antibiotic resistance pattern of uropathogenic bacteria in paediatric service at Kamenge University Hospital. Our results were compared with those of other studies of UTIs in hospitalised children. According to our findings, frequency of UTI among hospitalised children at Kamenge University Hospital was 8.4%. In France, Taques et al[5] also found a high prevalence, between 5 to 10%, among the children in their study. Interestingly, Adonis-Koffy et al[3] reported the frequency of 0.8% in children care for in the paediatric service of the Centre Hospitalier et Universitaire de Yopougon in Abidjan, Côte d'Ivoire, and Bourskraoui et al[4] noted the frequency of 1.3% in children cared for in the paediatric services of the Teaching Hospital Mohammed VI in Marrakech, Morocco. The low prevalence of the 2 African studies has been explained by faulty diagnosis, and is therefore probably underestimated.[3,4] In contrast, the high prevalence of UTIs in our study can be attributed to easily accessible diagnostics and free care for children under 5.

According to our research, UTIs are more prevalent in boys before the age of 2 years and in girls after the age of 2 years. In our sample, of the 87 children (86.1%) aged ≤ 24 months, 47 (54%) were boys. Of the 14 remaining children older than 2 years, 11 (78.5%) were girls. These results are similar to those of previous studies. In the study of Ferjani et al, 80% of children were infants.[14] In 2010, Bouskraoui et al found that infants accounted for 70.9% of UTIs of which 55% were boys; in children older than 2 years, UTIs in girls predominated preschool (68%) and school age (76%) populations.[4] In 105 cases in Burundi, Maloba found that 60% were infants aged less than 18 months of which 66.2% were boys; of those who were aged 18 months or older, 40% were girls.[15] The predominance of UTIs in male infants can be attributed to the increased incidence of malformative uropathies and

TABLE 3. Antibiotic Resistance of *Escherichia Coli* and *Klebsiella Pneumoniae*

	E Coli		*K Pneumoniae*		
Tested Drugs	**No. of Tests Made**	**Resistance No. (%)**	**No. of Tests Made**	**Resistance No. (%)**	***P* Value**
Augmentin	78	55 (70.5)	10	8 (80)	.80
Cotrimoxazole	57	56 (98.2)	10	10 (100)	1.00
Cefuroxime	44	20 (45.5)	4	2 (50)	1.00
Cefotaxime	59	27 (45.8)	7	2 (28.6)	.71
Ciprofloxacin	75	25 (33.3)	6	2 (33.3)	1.00
Gentamicin	51	14 (27.5)	5	1 (20)	1.00
Nitrofurantoin	43	4 (9.3)	2	1 (50)	.27

vesicoureteral reflux during this timeframe. Before age of 2 years, the infant preputial sac is still not free of the glans, which can lead to a stagnation of urine around the meatus causing a favorable bed for the local growth of bacteria and inflammation that cause phimosis, if the emptying of the bladder is incomplete, contamination of male meatus is exaggerated by the preputial sac. However, with age the foreskin retracts more easily and exposure to infection is reduced. Accordingly, the risk of infection could be reduced up to 10 times by circumcision or by daily retraction of foreskin before 6 months.[16–18] At preschool age, the higher female prevalence reflects potential hygiene challenges related to a urogenital tract with a short urethra and the location of the urethral and vaginal meatus so close to the anal meatus. In our study, the main cause of UTIs in children was enterobacteriaea (95%): *E coli* (82%), *K pneumoniae* (10%), and *P mirabilis* (3%). Other bacteria were found at a lesser level: *S aureus* (3%), *P aeruginosa* (1%), and *E faecalis* (1%). *E coli* was the most frequent bacteria isolated, as found in other studies.[3,4,14,15,18–21] The predominance of *E coli* can be attributed to its ability to attach to the urinary tract endothelium.

Depending on the sensitivity of the tests, the 2 primary isolated bacteria—*E coli* and *K pneumoniae*—had variable rates of resistance to all tested antibiotics. For aminopenicillins (Augmentin, ampicillin, and amoxicillin), resistance varied from 70.5 to 100% for *E coli* and 80 to 100% for *K pneumoniae*. High resistance was also found by other authors: Adonis-Koffy et al showed the *E coli* resistance ranged from 68% to 100%,[3] Bourskroui et al demonstrated a range of 58 to 68%,[4] and Ferjani et al discovered a range of 65 to 78%.[14] In 2002, researchers at Kamenge University Hospital found the antibiotic resistance of *E coli* was 50% for ampicillin and 47% for Augmentin.[15] Furthermore, the klebsielles are naturally resistant to aminopenicillin. Due to these high levels of resistance, aminopenicillins are no longer the drug of choice for the treatment of UTIs. Cotrimoxazole resistance was also high in our study—98.2% for *E coli* and 100% for *K pneumoniae*—while in a previous study at Kamenge University Hospital the range for all bacteria was between 82 and 100%.[15] However, in certain African studies, cotrimoxazole was found more or less effective for certain serotypes of *E coli*.[4,14] In contrast, second- and third-generation cephalosporins showed resistance to *E coli* (45%) and to *K pneumoniae* (28 to 50%), while other pathogens were 100% sensitive to these antibiotics. Our findings differ from studies where cephalosporins are still proven to have an efficacy ranging from 95% to 100%.[3,4,14] It has been 10 years since there was no resistance to cephalosporins in Burundi.[15] Similarly, in France, cephalosporin resistance is $< 1\%$.[22] The increasing resistance to cephalosporins in our study could be due to the emergence of new resistant strains related to incorrect prescribing, by association with other antibiotics; purchasing drugs without a prescription; or poor antibiotic adherence, by taking insufficient doses to address the infection. We found that gentamicin, an aminoglycoside regularly associated with other antibiotics for empiric therapy, was resistant to *E coli* (27.5%), *K pneumoniae* (20%), and *S aureus* (50%) at the lower levels, and at 100% for other bacteria. In other studies, gentamicin resistance was less than 10%.[3,4,14] The rate of resistance to ciprofloxacin was 33.3% for *E coli*, 28.6% for *K pneumoniae*, and ranged from 33.3% to 100% for other bacteria, except *P aeruginosa* for which the sensitivity was 100%. Other reported findings show that quinolone resistance to uropathogenic organisms ranged from 0 to 10%.[3,4,14,15] In our study, nitrofurans (nitrofurantoin or Furadentine) were efficient with less than 10% of resistance to *E coli.* Our observation supports previous studies indicating that nitrofurantoin exhibited less than 10% of resistance rates in the most countries investigated.[3,4,15,20,23–25] This may have been due to the fact that this antibiotic has

not been widely used for treating UTI cases. Therefore, nitrofurantoin may be a good option for the empirical treatment of UTI in Burundi. However, it should not be used to treat UTI in febrile infants because it is excreted in the urine and does not achieve therapeutic concentrations in the bloodstream.[26]

For more than 10 years, we have observed the progressive rates of antibiotic resistant in Burundi. In our study, the higher resistance rates to all antibiotics tested, with the exception of nitrofurantoin, may be explained by high and uncontrolled usage of these antimicrobial agents, since these antibiotics are also prescribed for other infections.

Methods for testing antimicrobial susceptibility and discovering novel antimicrobial agents have been extensively used. In our study, we used the agar disk-diffusion method often used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. However, this method cannot accurately test all fastidious bacteria, due to their complex nutritional requirements. Antibiograms are overall profiles of antimicrobial susceptibility testing results, which categorise bacteria as susceptible, intermediate, or resistant. It is a typing tool based on the resistance phenotype of the microbial strain tested. The test outcomes guide clinicians in the appropriate selection of initial empiric treatments. However, this method is limited in that it cannot distinguish between bactericidal and bacteriostatic effects and is not as appropriate to determine the minimum inhibitory concentration (MIC) as the antimicrobial gradient method (Etest). The latter method is used to determination the MIC of antibiotics, antifungals, and antimycobacterials,[27] and is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The Etest can confirm or detect a specific resistance phenotype like extended spectrum beta-lactamases (ESBL) producers.[28] This technique has shown a good correlation between the MIC values determined by Etest and those obtained by broth dilution or agar dilution methods.[29,30] The Etest is simple to implement and can be performed to investigate the antimicrobial interaction between 2 drugs,[31] which is why it is routinely requested by clinicians. However, if many drugs need to be tested, the Etest process becomes costly. In contrast, the agar disk-diffusion test offers simplicity, low cost, the ability to test a large number of microorganisms and antimicrobial agents, and the ease to interpret results provided, giving it many advantages over other methods. Due to the good correlation between the in vitro data and the in vivo evolution this method demonstrated the great interest in patients who suffer from bacterial infection of an antiobiotherapy based on the antibiogram of the causative agent.[32,33]

This study has limitations. Our study focused on a range of bacteria known for causing UTIs in children, validating *E coli* as the primary strain of concern. However, it would be important to further examine the subtypes of *E coli* to show which is resistant to available antibiotics. Likewise, additional research would be useful to identify ESBL producers since they indicate conferred resistance to antibiotics and are associated with poor outcomes.

CONCLUSION

UTI is a common disorder in a paediatric hospitals. During the period studied, the prevalence of UTIs in children at Kamenge University Hospital was 8.4%. Enterobacteriaceae were determined to be the main uropathogenic bacteria, with *E coli* being the main causative agent in over 80% of cases. Since 2002, Kamenge University Hospital research has determined that bacterial agents have shown absolute resistance to widely prescribed antibiotics such as cotrimoxazole and aminopenicillins, and increasing resistance to cephalosporins, aminoglycosides, and most fluoroquinolones, except nitrofurantoin. This work highlights the critical importance of pathogen and resistance surveillance over time.

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Peer Reviewed

Competing Interest: None declared.

Received: 24 Oct 2016; **Accepted:** 5 Feb 2017

Cite this article as: Nyandwi J, Manirakiza S, Ndirahisha E, Baptiste Ngomirakiza J, Nisubire D, Nduwayo E, et al. Frequency of Uropathogens and Antimicrobial Susceptibility in Childhood Urinary Tract Infection at Kamenge University Hospital, Bujumbura, Burundi. East African Health Res J. 2017;1(1):47-52. https://doi.org/10.24248/EAHRJ-D-16-00331

ORIGINAL ARTICLE

Prevalence, Aetiology, and Antimicrobial Susceptibility Patterns of Urinary Tract Infection Amongst Children Admitted at Kilimanjaro Christian Medical Centre, Moshi, Tanzania

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ABSTRACT

Background: Urinary tract infections (UTIs) in the paediatric population are well recognised as a cause of acute morbidity and chronic medical conditions, such as hypertension and renal insufficiency later in adulthood. Although antimicrobial treatment of UTIs is simple, the disease is still largely misdiagnosed and mismanaged. Moreover, increasing resistance to conventional antimicrobials is eroding the success of empiric therapy.

Objective: To determine prevalence, aetiological agents, and antimicrobial sensitivity patterns of UTIs amongst children admitted at Kilimanjaro Christian Medical Centre (KCMC).

Methodology: A cross-sectional, hospital-based study was conducted at the KCMC Department of Paediatrics and Child Health between December 2013 and April 2014. All children ages 2 months to 14 years who were admitted in the paediatric ward during the study period and fulfilled study criteria were enrolled. Data were collected by structured questionnaires. A urine dipstick test was done to detect the presence of nitrites and leucocytes, and to perform microscopic analysis of leucocytes and bacteria. All positive cases with the urine dipstick were cultured to determine bacterial species and antimicrobial susceptibility. Urine culture is considered the gold standard to confirm UTI.

Results: A total of 343 children enrolled in the study. Of these, 208 (60.6%) were male and 135 (39.4%) were female. The urine dipstick test was positive for leucocyte esterase and nitrate in 87 (25.4%) and 33 (9.6%), respectively, and urine microscopy showed leucocytes and bacteria by microscope in 38 (11.1%) and 24 (7.0%) samples, respectively. UTI was confirmed by culture in 11.4% (39/343) of the samples. Female children and children less than 24 months old had a higher prevalence of UTI (17% and 15.8%, respectively). Female sex (odds ratio [OR] 2.46, 95% confidence interval [CI], 1.25–4.86), presence of leucocytes esterase (OR 32.20, 95% CI, 12.03–86.19), and nitrate in urine dipstick (OR 5.87, 95% CI, 3.44–3.65) were associated with UTI. Leucocyte esterase, nitrite, microscopic leucocyte, and bacteria were positive in 34 (87.2%), 24 (61.5%), 30 (78.9%), and 23 (59%) samples, respectively, using culture as a gold standard. Antimicrobial sensitivity of nitrites, leucocyte esterase, microscopic leucocyte, and bacteria was 38.1%, 87.2%, 97.4%, and 59.0%, respectively, and specificity was 94.1%, 82.6%, 82.2%, and 99.7%. The most common bacterial species isolated were *Escherichia coli* 46.2% (18/39) and *Klebsiella pneumoniae* 30.8% (12/39); both exhibited low susceptibility to ampicillin, co-trimoxazole, and clindamycin, but they were susceptible to ciprofloxacin, nalidixic acid, and ceftazidime.

Conclusions: UTIs are common conditions affecting children admitted at KCMC. The prevalence is higher in infants and children younger than 24 months. *E coli* and *K pneumoniae* were the most common isolated organisms with low susceptibility in commonly used antibiotics. Antimicrobials, such as ciprofloxacin, ceftriaxone, and gentamicin, are more likely to be successful for empirical treatment of UTIs.

INTRODUCTION

Urinary tract infections (UTIs) are amongst the most common infections in children, occurring in as many as 5% of girls and 1%–2% of boys.[1] It has been estimated that globally, symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments, and 100,000 hospitalisations annually.[2] UTIs occur in 10% of all febrile children, 13.6% of febrile infants, and 7% of febrile newborns.[3] UTIs have great clinical significance due to their acute complications, such as mortality in infants, as well as chronic complications, such as hypertension and chronic renal failure following chronic pyelonephritis. Pyelonephritis accounts for 7%–30% of end-stage renal failure in some countries.[4] The early phase of tissue invasion by microorganisms is the critical determinant in the pathogenesis of kidney lesions following a UTI; therefore, early diagnosis with prompt and effective antimicrobial treatment for acute renal infection can prevent or significantly inhibit the development of renal damage.[1]

The prevalence of UTIs is roughly 9% in developed countries and 45% in developing countries of children seeking medical attention for any cause.[4,5] The few studies in Tanzania have shown a prevalence range from 16.7% to 39.7%.[6–8] A variety of bacteria are thought to cause UTIs, in particular *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.[9,10] This wide range of causative agents suggests that many UTIs may be opportunistic infections.

Although UTIs generally respond well to antimicrobial treatment, on a global basis there is increasing resistance amongst organisms that cause UTIs. There is a need to gain local knowledge about the types of pathogens responsible for UTIs and know their resistance patterns to help clinicians choose effective empiric treatment.[11] Moreover, the community needs to know whether we are exhausting treatment options and whether more effort is required with prevention strategies. Our immediate aim was to determine which bacteria commonly cause UTIs in our locality and to gain knowledge on their antimicrobial susceptibilities.

MATERIAL AND METHODS

Study Area

The study was conducted at Kilimanjaro Christian Medical Centre (KCMC) at the Department of paediatric and Child Health. KCMC is a referral hospital for over 15 million people in the Northern Zone of Tanzania (Kilimanjaro, Tanga, Arusha, and Manyara). It has daily attendance of 500 outpatients, nearly 500 in-patients with 640 official bed capacity, 1,000 staff, and hundreds of visitors daily. As a consultant hospital, it receives children from other health facilities both within the catchment area and from other regions of Tanzania. The paediatric bed capacity is over 100 beds.

Study Design

This was a cross-sectional, hospital-based study conducted from December 2013 to April 2014.

Study Population

The study included children from 2 months to 14 years of age admitted to the general paediatric ward during the study period with or without symptoms of UTI. Children under 2 months of age and children whose parents and guardians refused to give consent were excluded from the study.

The prevalence of UTI, uropathogens, and antimicrobial sensitivity patterns were considered to be dependent variables whereby age, sex, antibiotics use, nutritional status, and duration of treatment were all independent variables.

Sample Size and Sampling Technique

A required minimum sample size for this study was calculated using a standard formula for calculating sample size, $N=[Z^2P(1-P)]/E^2$. Taking the prevalence of UTI as 16.3%, as reported by Fredric and colleagues in 2013 at Muhimbili,[9] the critical value Z=1.96, equivalent to a confidence interval (CI) level of 95% with a minimum tolerable error of 5%. The minimum sample size was estimated to be 210. Convenient sample technique was considered. Children were assessed during admission, and parents or caretakers received an explanation about the study and signed informed consent forms if the child fulfilled inclusion criteria for enrolment and they agreed to participate. Children whose parents or caretakers were not ready to give consent received routine services.

Data Collection

All children who fulfilled the inclusion criteria during admission and whose parents or caretakers signed the informed consent were included in the study. A standardised, structured questionnaire was used to gather demographic and clinical characteristics of children and a laboratory data sheet was used to collect laboratory information, which included urine dipstick and urine microscopic results, pathogen species, and antimicrobial susceptibility. Urine was collected by both clean-catch midstream for older children and urethral catheterisation for young children (<2 years). Two urine specimens were collected in 2 sterile urine containers. One specimen underwent spot testing using the dipstick method to determine the nitrite and leucocyte levels in the urine. For specimens that had any positive results on the urine dipstick (leucocyte esterase and nitrite), the second specimen was sent to the laboratory for microbial identification, culture, and susceptibility testing.

Laboratory Methods

Urine samples were processed within 1 hour of collection, and if any delay was anticipated, the specimen was stored at 4–8°C and analysed within 24 hours. Urine microscopy was

performed to identify the presence of white blood cells and bacteria. Culture processing was performed by inoculation of urine into a cysteine-lactose electrolyte-deficient medium using a standard 1µL loop. Culture of each urine specimen prior to centrifugation was done quantitatively on 5% blood agar and MacConkey agar plates that were incubated aerobically at 37°C for 24 hours. Colonies were counted, mixed-growth cultures were interpreted as negative culture, and only the growth of single-uropathogen colonies of $\geq 10^5$ CFU/ml was interpreted as a positive culture. Bacterial colonies on solid agar were then identified based on characteristic morphology and Gram stain appearance. All Gram-negative bacteria were identified using API 20E strips (bioMérieux Inc., Marcy-l'Étoile, France). Urine culture bacterial growth was used as a gold standard for confirmation of UTI.

Drug Susceptibility Testing

Antimicrobial susceptibility testing was performed with selected commonly used antibiotics in our settings. These included ampicillin (10 µg), co-trimoxazole (30 µg), gentamicin (10 µg), amoxicillin-clavulanate (20/10 µg), nitrofurantoin, ciprofloxacin (5 µg), chloramphenicol (10 µg), nalidixic acid (10 µg), clindamycin (20 µg), and cephalosporins such as ceftriaxone (30 µg), cefazolin, and ceftazidime (30 µg). The testing was done by the Kirby-Bauer disc-diffusion method using Mueller-Hinton agar that was incubated for 18 to 24 hours at 37°C. Antimicrobial susceptibility was reported as resistant, intermediate, and sensitive according to Clinical Laboratory Standards Institute guidelines.[12]

Statistical Analysis

Data were coded and entered into a computer using SPSS version 20 (SPSS Inc., Chicago, IL, USA) and summarised by frequency, median (interquartile range), and percentage. Odds ratios (OR) with a 95% confidence interval were used for reporting the association, with $P<.05$ considered significant. Furthermore, we compared the diagnostic tests with a gold standard method, and specificity, sensitivity, and positive and negative predictive values were estimated.

Ethical Consideration

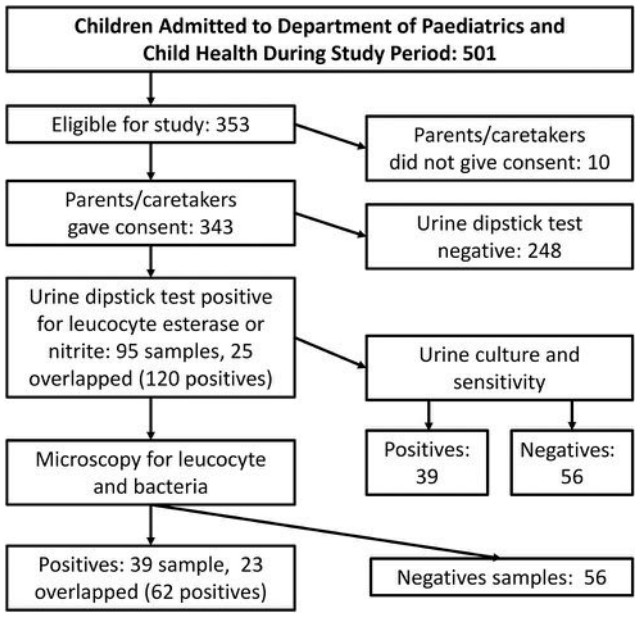
Ethical approval was secured from Kilimanjaro Christian Medical University College Research Ethics Committee. Children whose parents or caretakers who signed informed consent forms were included in the study. Those who did not give consent received routine service and care.

RESULTS

Demographic Characteristics of Study Participants

In total, 501 children were admitted to the Department of paediatric and Child Health during the study period; 148 children were below 2 months of age, and 10 did not give consent. Thus, 343 eligible children enrolled in the study as shown in Figure 1.

FIGURE 1. Number of Participants Enrolled in Study and Test Results



The median age at enrolment was 29 months (range: 2 months to 14 years). Male participants constituted a higher percentage (60.6%) compared to female participants. Table 1 shows the age and sex distribution of the study participants.

Prevalence of UTIs

Of the 343 children enrolled in this study, the urine dipstick test was positive for leucocyte esterase and nitrate in 87 (25.4%) and 33 (9.6%) samples, respectively, and urine microscopy showed leucocytes and bacteria in 38 (11.1%) and 24 (7%) samples, respectively.

Urine culture was used as the confirmatory test for UTI; therefore, the prevalence of UTI was 11.4% (n=39). Children younger than 24 months had a higher prevalence of UTI (15.8%) compared to children ages 24–59 months and over 60 months (9.2% and 8.7%, respectively), and female children had a higher prevalence of UTI (17%) compared with their male counterparts (7.7%). High prevalence of UTI was observed in children with fever for about 2–10 days (17.7%), diarrhoea (16.7%), poor weight gain (16.7%), abdominal pain (16.2%), pain during urination (15.4%), and fever (14%) at baseline. Overall, 135 (39.4%)

TABLE 1. Age and sex distribution of the participants (N=343)

Characteristics	n (%)
Age groups (years)	
≤1	152 (44.3)
2–4	87 (25.4)
5–7	39 (11.4)
8–10	29 (8.5)
11–13	36 (10.5)
Median (IQR)	42 (29–54)
Sex	
Male	208 (60.6)
Female	135 (39.4)

Abbreviation: IQR, interquartile range.

children were on antimicrobial treatment prior to enrolment, with observed lower prevalence of UTI compared with the group not exposed to antibiotics (8.1% vs. 13.5%) (Table 2). Only 86 (63.7%) knew the type of antibiotic used, with 47 (56%) being on ampicillin or amoxicillin alone, 12 (14.3%) on ampicillin in combination with gentamicin, and 10 (11.9%) on cephalosporin, with only (3.6%) exposed to co-trimoxazole.

Amongst the baseline characteristics, female sex (OR 2.46, 95% CI, 1.25–4.86), and presence of leucocytes esterase (OR 32.0, 95% CI, 12.03–86.18) or nitrate in urine (OR 5.87, 95% CI, 3.44–3.65) were the only factors associated with UTI. Although not statistically significant, young age had nearly twice increased odds of having UTI (OR 1.79, 95% CI, 0.79–4.05).

Comparing Urine Dipstick and Microscopic Examination With Urine Culture

Of the children with UTI confirmed by urine culture (n=39), urine dipstick showed positive results for leucocyte esterase and nitrite in 34 (87.2%) and 15 (38.5%) samples, respectively, and 15.4% were positive for both leucocyte esterase and nitrite; urine microscopy showed positive results for leucocyte and bacteria in 30 (76.9%) and 23 (59%) samples, respectively. Antimicrobial sensitivity of nitrites, leucocyte esterase, microscopic leucocyte, and bacteria was 35.1%, 87.2%, 97.4%, and 59.0%, respectively, and specificity was 94.1%, 82.6%, 82.2%, and 99.7% (Table 3).

Isolated Microorganisms and Their Antimicrobial Susceptibly Patterns

A total of 9 pathogenic bacteria were isolated from 39 positive cultures. *E coli* was the most common isolated pathogen in 18 (46.2%) children, followed by *K pneumoniae* in 12 (30.8%) children, and only 1 (2.6%) child had Gram-positive bacteria *S aureus* (Figure 2).

Isolated pathogens were tested for their antimicrobial susceptibility patterns with a total of 12 antibiotics. *E coli* isolates (n=18) were least susceptible to clindamycin (22.2%), co-trimoxazole (33.3%), and ampicillin (38.9%). *K pneumoniae* isolates (n=12) were highly susceptible to ciprofloxacin (75%), nalidixic acid (75%), and ceftazidime (66.7%). Table 4 shows the antimicrobial susceptibility patterns of the isolated organisms.

DISCUSSION

This study on UTIs was done to determine the prevalence, aetiological agent, and antimicrobial susceptibility in children with UTIs admitted in a tertiary care hospital in northern Tanzania. Since we aimed to look at the overall prevalence in the paediatric ward, we did not restrict the study to patients with fever, UTI symptoms, or not on antibiotics; and we used urine culture as a gold standard for diagnosis of UTI. The prevalence of UTIs in this population was 11.4%, a finding that is comparable to previous studies done in both developed and developing countries. [1,13,14] Our findings are similar to a study done at Muhimbili National Hospital by Fredrick and colleagues, where a UTI prevalence of 16.8% was found amongst febrile children.[8] The prevalence of UTI in our study population was lower, however, than the 20.3% reported by Msaki and colleagues in Tanzania[7] and a study done in Bugando Medical Centre that reported a prevalence of 39.7%.[8] The higher prevalence in Msaki's study might be because their study was conducted in a primary health facility and included children with fever only, whereas our study was conducted at a tertiary health facility and included all children who were symptomatic and asymptomatic. Of the 135 (39.4%) children in our study who were on antibiotics prior to admission, the majority were on ampicillin or amoxicillin (56%), ampicillin and gentamicin (14.3%), and cephalosporin (11.9%). Previous exposure to antibiotics might be the cause of the lower susceptibility to ampicillin.

In this study, positive leucocyte esterase and nitrite were the only risk factors of UTI (OR 32.2 and 5.87, respectively). UTI may present with nonspecific symptoms in children; however, abdominal pain, flank pain, vomiting, diarrhoea, duration of fever, antibiotics use, painful urination, and poor weight gain were not associated with UTI. Similar findings have been observed in Nigeria, in keeping with nonspecific symptoms of UTI in children.[13] Children enrolled were of different ages, with the majority being younger, which

TABLE 2. Prevalence of UTI Among Study Population by Characteristic (N=343)

Characteristics	Total	UTI No. (%)	OR (95% CI)	*P* Value
Age groups (months)				
<24	152	24 (15.8)	1.79 (0.79–4.05)	0.165
24–59	87	8 (9.2)	1.07 (0.40–2.90)	0.896
≥60	104	9 (8.7)	1.0	
Sex				
Male	208	16 (7.7)	1.0	
Female	135	23 (17.0)	2.46 (1.25–4.86)	0.008
Abdominal pain				
Yes	37	6 (16.2)	1.5 (0.69–3.44)	0.299
No	304	32 (10.5)	1.0	
Flank pain				
Yes[a]	5	1 (20.0)	1.78 (0.30–10.53)	0.455
No	338	38 (11.2)	1.0	
Vomiting				
Yes	108	13 (12.0)	1.09 (0.58–2.03)	0.792
No	235	26 (11.1)	1.0	
Diarrhoea				
Yes	54	9 (16.7)	1.59 (0.80–3.17)	0.188
No	287	30 (10.5)	1.0	
Fever				
Yes	179	25 (14.0)	1.64 (0.88–3.03)	0.114
No	164	14 (8.5)	1.0	
Duration of fever (n=179)				
0–2 days	51	9 (17.7)	1.49 (0.60–3.6)	0.823
3–7 days	119	15 (12.6)	1.0	0.897
7+ days	7	1 (14.3)	1.16 (0.13–10.28)	
Antimicrobial use				
Yes	135	11 (8.1)	1.75 (0.84–3.65)	0.13
No	208	28 (13.5)	1.0	
Painful urination				
Yes[a]	13	2 (15.4)	1.37 (0.0.37–5.89)	0.642
No	330	37 (11.2)	1.0	

Continued

TABLE 2 Continued

Characteristics	Total	UTI No. (%)	OR (95% CI)	*P* Value
Poor weight gain				
Yes	42	7 (16.7)	1.57 (0.74–3.32)	0.248
No	301	32 (10.6)	1.0	
Leucocyte esterase				
Positive	87	34 (39.1)	32.0 (12.03–86.18)	<0.001
Negative	256	5 (2.0)	1.0	
Nitrite				
Positive	33	15 (45.5)	5.87 (3.44–3.65)	<0.001
Negative	310	24 (7.7)	1.0	

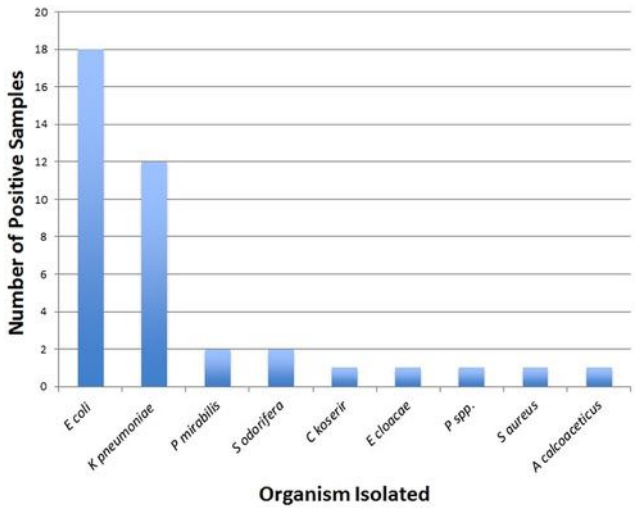
Abbreviations: OR, odds ratio; UTI, urinary tract infection.
[a] The Fisher exact test was used, as some cells have fewer than 5 events.

TABLE 3. Comparison of Urine Dipstick and Urine Microscopic Results With Urine Culture

	Urine Culture			
Tests	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
Urine dipstick				
Nitrite	45.5	92.3	38.5	94.1
Leucocyte esterase	39.1	98	87.2	82.6
Microscopy				
Leucocyte	41.3	99.6	97.4	82.2
Bacteria	95.8	95	59	99.7

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

FIGURE 2. Number of Positive Samples and Isolated Organisms (n=39)



may underscore the presence of some symptoms such as abdominal pain and painful micturition. Vomiting alone is unlikely to be a sign of UTI, as the observed risk was only 9%. However, it should not be ignored as one of the signs of UTI.

Female children had a higher prevalence of UTI compared with males, with a female-to-male ratio of 2:1. This result is consistent with previous studies in Gaza, Tanzania, and India with female-to-male ratios of 4:1, 1.7:1, and 1.5:1, respectively.[7,9,10] A possible explanation for this difference is that females have anatomical and physical features (eg, short urethra) that predispose them to ascending infection. Therefore, the significant twofold increased risk of UTI in female children shows that one should highly suspect UTI in female children presenting with specific or nonspecific symptoms. Dipstick tests (for nitrites and leucocyte esterase)

TABLE 4. Antimicrobial Susceptibility Patterns of the Isolated Organisms (N=39)

	Isolated Organism No. (%)								
Antibiotics	***E coli***	***A c***	***C k***	***E c***	***K p***	***P m***	***P s***	***S o***	***S a***
Ampicillin	7 (38.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)	1 (50.0)	1 (100.0)
Co-trimoxazole	6 (33.3)	1 (100.0)	1 (100.0)	0 (0.0)	3 (25.0)	1 (50.0)	0 (0.0)	1 (50.0)	0 (0.0)
Gentamicin	14 (77.8)	1 (100.0)	1 (100.0)	0 (0.0)	7 (58.3)	1 (50.0)	0 (0.0)	1 (50.0)	1 (100.0)
Chloramphenicol	15 (83.3)	0 (0.0)	1 (100.0)	1 (100.0)	9 (58.3)	1 (50.0)	0 (0.0)	2 (100.0)	1 (100.0)
Clindamycin	4 (22.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Amoxicillin-clavulanate	13 (72.2)	1 (100.0)	1 (100.0)	0 (0.0)	7 (58.3)	1 (50.0)	0 (0.0)	1 (50.0)	1 (100.0)
Nitrofurantoin	15 (83.3)	0 (0.0)	1 (100.0)	1 (100.0)	7 (58.3)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)
Ciprofloxacin	16 (88.9)	1 (100.0)	1 (100.0)	1 (100.0)	9 (75.0)	1 (50.0)	0 (0.0)	2 (100.0)	1 (100.0)
Ceftriaxone	15 (83.3)	0 (0.0)	1 (100.0)	1 (100.0)	6 (50.0)	2 (100.0)	0 (0.0)	2 (100.0)	1 (100.0)
Nalidixic acid	12 (66.7)	1 (100.0)	1 (100.0)	1 (100.0)	9 (75.0)	2 (100.0)	0 (0.0)	2 (100.0)	1 (100.0)
Cefazolin	15 (83.3)	0 (0.0)	1 (100.0)	1 (100.0)	5 (41.7)	1 (50.0)	0 (0.0)	2 (100.0)	0 (0.0)
Ceftazidime	14 (77.8)	1 (100.0)	1 (100.0)	0 (100.0)	8 (66.7)	2 (100.0)	1 (100.0)	2 (100.0)	1 (100.0)
Total	**18**	**1**	**1**	**1**	**12**	**2**	**1**	**2**	**1**

Abbreviations: *A c, Acinetobacter calcoaceticus; C k, Citrobacter koseri; E c, Enterobacter cloacae; K p, Klebsiella pneumoniae; P m, Proteus mirabilis; P s, Pseudomonas spp; S o, Serratia odorifera; S a, Staphylococci aureus.*

and microscopic examination (for leucocyte and bacteria) in urine can be used as screening tools for UTI since they have shown a high specificity and negative predictive value; however, the observed low antimicrobial sensitivity for nitrites and leucocytes indicate a need for a confirmatory test like urine culture, as used in this study. Furthermore, positive urine dipstick results for nitrites and leucocytes esterase, as observed in other studies, should prompt clinicians to consider a diagnosis of UTI in children,[8] especially when culture testing is not available.

Infants and children younger than 2 years old tend to have high chances of acquiring UTIs. In this study, children under the age of 2 had a higher prevalence of UTIs, similar to other studies in India, Nigeria, and Tanzania, which showed that children under 24 months had a higher prevalence, ranging from 15.3% to 53.6%.[8,15,16] This age group is at risk because of their immature immune systems and a high chance of colonic pathogens colonising the urinary tract system. Wearing nappies, as well as the possibility of having urinary tract malformation such as vesicle ureteric reflux, makes this age group susceptible to recurrent UTI.

UTIs can be caused by virtually any pathogen colonising the periurethral region, such as bacteria, viruses, and fungi. This study focused on bacterial causes of UTIs. We found a predominance of Gram-negative bacteria, with *E coli* and *K pneumoniae* being the most common pathogens causing UTI in children (prevalence of 46.2% and 30.8%, respectively). These findings are consistent with previous studies in India and Bangladesh, where *E coli* and *K pneumoniae* were predominant uropathogens.[5,16,17] The only Gram-positive bacterium in this study was *S aureus*, reported in 1 urine sample, which might be due to contamination; however, *S aureus* in urine has been reported from other studies.[8,18] In view of this finding, Gram-negative bacteria should be considered in children with UTI when selecting antibiotics for empiric treatments.

In this study, antimicrobial susceptibility to commonly used antibiotics for empiric treatment of UTI (aminoglycosides, ampicillin, penicillin, cephalosporin, co-trimoxazole, and quinolones) was low, particularly amongst the main bacterial isolates. Susceptibility was moderate to high for gentamicin, ciprofloxacin, ceftriaxone, ceftazidime, and nalidixic acid. However, we did identify amoxicillin-clavulanate and chloramphenicol as likely to be effective, with clindamycin having the lowest susceptibility in all organisms isolated. Similar sensitivity patterns have been observed in the United Kingdom by Bean and colleagues who found low susceptibility to ampicillin and co-trimoxazole, whereas susceptibility to nitrofurantoin, gentamicin, and ceftriaxone was high.[19]

These findings are also in agreement with studies done in Asia, the Middle East, other parts of Africa, and in previous work in Tanzania.[5,8,20,21,22] Thus, with continue empiric treatment, UTI with resistance to antibiotics will become a global problem.

As a referral hospital, children admitted to our hospital are already being exposed to antibiotics that are overprescribed and widely available without prescription. Such practice is likely driving the increase in antibiotic resistance and lowers the chance of antibiotic choice. Exposure to antibiotics may play a role in lower culture-positive results in children exposed to antibiotics, as seen in this study, thus masking the situation. Therefore, clinicians and other health-care providers in lower-level health facilities should consider prescribing empiric antibiotics as per recommended national treatment guidelines, after performing simple screening tests like urine dipstick or microscopic examination, if available. Thus, public health awareness and education efforts may help the general population appreciate that antibiotics should be purchased only with a prescription after proper diagnosis is made. Health-care practitioners should prescribe antibiotics according to national guidelines; however, when unavailable, prescriptions for empiric antibiotics should be given only after excluding all other causes (eg, malaria and viral infection).

Study Limitations

This study included all children with or without fever regardless of antibiotic exposure, which may underestimate the prevalence and aetiology of UTI. Moreover, caregivers of 36.3% of children previously exposed to antibiotics had no information about what kinds of antibiotics were used; it is therefore possible that this study underestimated the levels of antibiotic resistance.

CONCLUSIONS AND RECOMMENDATION

UTIs are amongst the most common conditions affecting children admitted at KCMC, with a higher prevalence amongst infants and children younger than 24 months and females. The most common aetiological bacterial pathogens were *E coli* (46.2%) and *K pneumoniae* (30.8%), both of which showed low susceptibility to ampicillin, cotrimoxazole, and clindamycin. Antimicrobials, such as ciprofloxacin, ceftriaxone, and gentamicin are more likely to be successful for empiric treatment of UTIs. However, the susceptibility of the pathogens is still not high. Because susceptibility is not static, monitoring of antibiotics for UTI needs to be done continuously and guided with urine culture and antimicrobial sensitivity whenever possible.

Acknowledgements: We acknowledged the HRSA Award T84HA21123 for supporting this project through the Medical Education Partnership Initiative (MEPI). We are also grateful to all resident and nurses at KCMC Department of Paediatric and Child Health for assistance in data collection; the authors thank Mr Isaack Afwamba of KCMC-Duke Collaboration, KCRI biotechnology laboratory for laboratory sample processing.

Authors' Contribution: JGG conducted study design, clinical sample collection, data analysis, and developed the initial manuscript. RN supervised initial proposal, data analysis, and manuscript writing. Both MSA and ASM participated in study setting, design, data entry as well manuscript review. Both TT and IK participated in proposal development and data collection. BTM worked on data analysis, interpretation of results, and approved final manuscript writing. LJM participated in reviewing the entire project from proposal writing to final manuscript. All authors have read and approved manuscript.

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Peer Reviewed

Competing Interests: None declared.

Received: 28 Oct 2016; **Accepted:** 5 Feb 2017

Cite this article as: Gidabayda JG, Philemon R, Abdallah MS, Saajan AM, Temu T, Kunjumu I, et al. Prevalence Aetiology, and Susceptibility Patterns of Urinary Tract Infection Amongst Children Admitted at Kilimanjaro Christian Medical Centre Antimicrobial Moshi Tanzania. East African Health Res J. 2017;1(1):53-61. https://doi.org/10.24248/EAHRJ-D-16-00341

EAHRC
EAST AFRICAN HEALTH RESEARCH COMMISSION
Research for Health and Prosperity

ORIGINAL ARTICLE

Food-Handling Practices and Environmental Factors Associated With Food Contamination Among Street Food Vendors in Nairobi County, Kenya: A Cross-Sectional Study

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ABSTRACT

Background: Lack of adequate sanitation and refuse disposal facilities are among the factors found to contribute to food contamination among street food vendors. Most vending facilities are near crowded places, such as bus terminals or markets to attract consumers, and the few basic amenities, such as toilets, are inadequate. The objective of the study was to determine which sanitation practices were associated with food contamination in Githurai and Gikomba markets in Nairobi County.

Methodology: Using a cross-sectional study design, we systematically randomly sampled 149 street food vendors and used questionnaires to interview them and make observations.

Results: A significant negative association was observed between access to a toilet facility and food contamination ($P<.001$), with a decreased risk of occurrence of food contamination observed where vendors had access to a toilet facility (OR 0.095; 95% confidence interval [CI], 0.039–0.227). Accessibility of running water around the toilet facility was negatively associated with food contamination ($P<.001$), with vendors who reported access to running water having a lower occurrence of food contamination (15.9%) compared with those who had no access to running water (30%). Presence of pests/rodents was significantly associated with food contamination ($P<.001$), with vendors who reported presence of pests/rodents having a 5.9-fold risk (OR 5.921; 95% CI, 2.831–12.383) of contaminated food. Access to fresh running water while preparing food, hand washing before handling food, and use of an apron were the food-handling practices that were negatively associated with food contamination ($P<.005$). Use of a head cover, hand washing after handling raw food, and the way food was served and stored had no statistically significant association with food contamination ($P>.05$).

Conclusions: Access to a toilet facility and availability of running water within the toilet facility decreased the likelihood of food contamination. The presence of pests/rodents had a positive association with food contamination. There is a need for more basic amenities, especially toilets and water facilities, within these markets, as well as sensitisation on pest control.

INTRODUCTION

Street-vended foods are defined as those foods prepared on the street and ready to eat, or prepared at home and consumed on the street without further preparation.[1] Foodborne diseases are common in developing countries, including Kenya, because of the prevailing poor food-handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers.[2] The street food trade is a growing sector in many developing countries today, and the expansion is linked with urbanisation and the need of urban populations for both employment and food. The safety of street foods is a major consideration, which deserves and has received attention. Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors for outbreak of foodborne diseases in United States.[3]

Studies conducted in different parts of Ethiopia also showed the poor sanitary conditions of catering establishments and the presence of pathogenic organisms like *Campylobacter*, *Salmonella*, *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*.[4–8] Factors implicated in causing microbial contamination include poor food preparation and handling practices, inadequate storage facilities, a lack of personal hygiene among

vendors, and a lack of adequate sanitation and refuse disposal facilities.[9,10]

MATERIALS AND METHODS

Study Area

The study area was Gikomba and Githurai markets within Nairobi County, Kenya. Two study areas were involved to expand the sample and to avoid vendors influencing each other in their responses, since vendors are closely positioned in both of the 2 markets.

Gikomba is a market located about 800 metres from the town centre in Kamukunji Constituency. Today there are more than 4,000 traders in Gikomba. Gikomba is famous for secondhand clothes sellers, but there are other products sold, including food. It is a very busy market where there are various businesses and activities such as human carriers and hand carts (*mikokoteni*) who ferry goods across the market. The surrounding communities include mainly low-income earners who largely depend on low-cost street-vended foods.

Githurai market is located in the eastern part of Nairobi, about 12 km from the city centre in Kasarani Constituency. This area has a population of over 300,000, and Githurai is a busy market famous for the sale of secondhand goods as well as food. The Githurai market is very congested, and movement within it is severely hampered as traders try to display their goods for passersby to buy. The majority of people in the surrounding communities are low-income earners who depend on the market to buy cheaply priced goods and street-vended foods. Studying food safety in these 2 areas is important as contamination in these areas may imply a possible foodborne disease outbreak that may affect a large population of those who reside in Nairobi or who visit the markets.

Study Design

The study used a descriptive cross-sectional study design to establish the bacteriological safety of street-vended foods and assess the food-handling practices and environmental factors associated with food contamination at consumption point.

Study Population

The study population comprised street food handlers who were selling ready-to-eat foods in Gikomba and Githurai markets. We interviewed the selected food handlers and then purchased a food sample from them for microbial analysis. The inclusion criteria for the study were street food vendors 18 years and older who gave consent. Those who were under 18 years old and who did not give consent were excluded from the study.

Sample Size Determination

The total sample size was determined by the formula of Fisher and colleagues, where n = the desired sample size for a target population greater than 10,000; Z = normal standard deviation corresponding to 95% confidence interval (CI), that is 1.96; P = proportion of the population estimated to have desired characteristics; q=1−P; and d = degrees of accuracy desired (0.05). Hence, this study used a *P* value of 20% as used in a similar study in Ethiopia.[11]

The sample size was therefore calculated as follows:

$n = [Z^2(1-\beta)pq]/d^2$

Description:
n = required sample size
Z = confidence level at 95% (standard value of 1.96)
P = estimated prevalence (.20)
d = level of precision at 5% (0.05)
n = 245.86

However, the population under study was less than 10,000: a preliminary study done at the 2 areas revealed the population of interest was a total of 380 street food vendors. Hence the Cochran 2000 formula[12] was further used to calculate the actual sample size.

Sample size therefore was as follows:

$n_f = n/1 + n/N$

[where N = population size,
n = sample size if N is infinite (N > 10,000),and
nf = sample size if N is finite (N < 10,000)]
= 245.86/1 + 245.86/380
= 149

Then, sharing the sample proportionate to size:
Gikomba = 197/380 * 149 = 77, and
Githurai = 183/380 * 149 = 72.
Sampling interval (K) = N_1/n_1
= 380/149
= 2.55 ≈ 2.0

Sampling

We used random sampling to sample the first street food vendor who was specifically preparing and selling the foods on-site within the 2 study areas, after which systematic random sampling, using a sampling interval of 2 as calculated above, was used to sample the rest of the food vendors. We then bought and aseptically collected food samples from the same street food vendors for the purpose of microbial analysis.

Data Collection

We first distributed an informed consent form to the street food vendors to obtain consent. We then administered a structured questionnaire to gather relevant information from the street food vendors. The questionnaire included

questions about food-handling practices (such as washing of hands before and after handling food, use of soap during washing of hands, washing of utensils used for food preparation, storage of foodstuffs, training on food handling, frequency of medical examination) and environmental factors (such as availability of toilet facilities, accessibility and availability of clean water near the toilet facilities, and disposal of solid waste).

We then bought food samples, which were collected aseptically in sterile universal bottles, transported to the National Public Health Laboratories under low temperature in an ice cooler box, and stored at 4°C until testing. All the samples were analysed within 24 hours of sampling. We used standard methods for enumeration, isolation, and identification of bacteria.

Data Management and Analysis

Data from the study was first coded. Double entry was then done using Microsoft Access for comparison purposes. Errors were minimised by cleaning and rechecking all the entries with the original data forms. Data analysis was done using SPSS software (Version 20) where descriptive statistics like mean, frequencies, and percentages were used to describe the data and presentation was done through tables, pie charts, and graphs. Chi-square was used to establish the relationship between categorical independent variables (such as food-handling and environmental factors) and the dependent variable (food contamination). Correlation technique was used to establish the association between variables under study. Multivariate analysis was performed to calculate the adjusted odds ratio for the independent association between food contamination and the predictive variables.

Ethical Considerations

Approval to conduct this study was obtained from the Scientific Steering Committee at the Kenya Medical Research Institute and the Scientific Ethical Review Committee for scientific and ethical approvals respectively. Study participants were assured that there would be no risks involved in responding to the questions and that they were free to respond or not.

The respondents were informed that the direct benefit of being involved in this study was that the information gathered would reveal some of the environmental challenges they face for the relevant parties to take action. They were also informed that the feedback from this study would assist them in understanding some of the hygienic practices they personally need to improve to prevent food contamination.

RESULTS

Several environmental factors were significantly associated with food contamination. A negative association ($P<.001$) was observed between access to a toilet facility and food contamination, whereby a decreased risk of occurrence of food contamination (OR 0.095; 95% CI, 0.039–0.227) was observed if one had access to a toilet facility (Table 1). Vendors who had access to a toilet had a lower occurrence of food contamination (22.1%) compared with those who had no access to a toilet facility (75%) (Table 2). The vendors were further probed on the type of toilet facility they had access to, and 46.3% of the vendors had access to a modern toilet, which can be described as a pour/flush toilet, while 29.5% had access to a latrine, which is basically a deep pit that is dug for use as a toilet facility.

This variable was assessed in relation to food contamination, and a significant association was observed

TABLE 1. Bivariate Analysis of Environmental Factors in Relation to Occurrence of Food Contamination

Environmental Factors	Chi-square	df	*P* Value	OR	Lower CI	Upper CI
Access to a toilet facility	33.598	1	<.001*	0.095	0.039	0.227
Type of toilet facility	37.270	2	<.001*	...	NA	NA
Running water within or outside the toilet facility	36.046	2	<.001*	...	NA	NA
Waste disposal	1.369	2	.504	...	NA	NA
Presence of pests/rodents	24.176	1	<.001*	5.921	2.831	12.383
Type of pests/rodents	35.489	2	<.001*	...	NA	NA
Environmental contaminants	2.514	1	.113	0.363	0.099	1.327

Abbreviations: CI, confidence interval; df, degrees of freedom; OR, odds ratio. Ellipses indicate the OR could not be calculated due to multiple responses to the variable.
* Variables significant at the 5% level.

TABLE 2. Occurrence of Food Contamination in Relation to Environmental Factors

Environmental Factors	Food Contamination		
	Yes No. (%)	No No. (%)	Total No. (%)
Access to a toilet facility			
Yes	25 (22.1)	88 (77.9)	113 (100)
No	27 (75.0)	9 (25.0)	36 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Type of toilet facility			
Latrine	5 (11.4)	39 (88.6)	44 (100)
Modern	20 (29.0)	49 (71.0)	69 (100)
N/A	27 (75.0)	9 (25.0)	36 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Running water within or outside the toilet facility			
Yes	10 (15.9)	53 (84.1)	63 (100)
No	15 (30.0)	35 (89.5)	50 (100)
N/A	27 (75.0)	9 (25.0)	36 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Waste disposal			
Open area	31 (33.3)	62 (66.7)	93 (100)
Municipal container	19 (40.4)	28 (59.6)	47 (100)
Other	2 (22.2)	7 (77.8)	9 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Presence of pests/rodents			
Yes	33 (60.0)	22 (40.0)	55 (100)
No	19 (20.2)	75 (79.8)	94 (100)
Total	52 (34.9)	75 (79.8)	149 (100)
Type of pests/rodents			
Rats	18 (46.2)	21 (53.8)	39 (100)
Rats & moles	15 (93.8)	1 (6.2)	16 (100)
N/A	19 (20.2)	75 (79.8)	94 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Environment free of contaminants			
Yes	3 (17.6)	14 (82.4)	17 (100)
No	49 (37.1)	83 (62.9)	132 (100)
Total	52 (34.9)	97 (65.1)	149 (100)

TABLE 3. Bivariate Analysis of Food-Handling Practices in Relation to Occurrence of Food Contamination

Food-Handling Practices	Chi-square	df	*P* Value	OR	Lower CI	Upper CI
Access to fresh running water for hand washing while preparing food	8.711	1	.003*	0.355	0.177	0.713
Hand washing before handling food items	82.768	1	<.001*	0.018	0.006	0.051
Hand washing after handling raw food items	0.265	1	.607	1.288	0.490	3.383
Method of hand washing	60.657	3	<.001*	. . .	NA	NA
Acquisition of skills	1.317	3	.725	. . .	NA	NA
Medical check-up	4.216	3	.239	. . .	NA	NA
Use of an apron	21.296	1	<.001*	0.190	0.091	0.394
Use of a head cover	2.994	1	.084	0.531	0.258	1.093
Storage of leftover food	4.431	2	.109	. . .	NA	NA
Serving of food	0.755	3	.860	. . .	NA	NA

Abbreviations: CI, confidence interval; df, degrees of freedom; OR, odds ratio. Ellipses indicate the OR could not be calculated due to multiple responses to the variable.
* Variables significant at the 5% level.

($x^2_{2,\ 0.05}$ = 37.270, P<.001). Those who had access to a modern toilet had a higher occurrence of food contamination (29%) compared with those who had access to a latrine (11.4%) (Table 2). Accessibility of running water around the toilet facility was thus negatively associated with food contamination ($x^2_{2,\ 0.05}$ = 36.046, P<.001), whereby a higher occurrence of food contamination was observed among those who did not have running water (30%) compared with those who had running water (15.9%) (Table 2).

Open area dumping was the most common method (62.4%) used by vendors to dispose waste. The relationship between waste disposal and food contamination was, however, not statistically significant ($x^2_{2,\ 0.05}$ = 1.369, P=.504) (Table 1). Food contamination was highest among vendors who used municipal containers for waste disposal (40.4%). Presence of pests/rodents was significantly (P<.001) associated with food contamination, with vendors who reported presence of pests/rodents having a 5.9-fold risk (OR 5.921; 95% CI, 2.831–12.383) of having contaminated food (Table 1). The type of pests or rodents on-site was observed to have a significant association with food contamination ($x^2_{2,\ 0.05}$ = 35.489, P<.001), with vendors who reported presence of both rats and moles having the highest occurrence of food contamination (93.8%) compared with those who reported rats only (46.2%) (Table 2).

Raw sewage lines, dust, flies, and vehicle fumes were some of the potential environmental contaminants reported by the vendors. The relationship between environmental contaminants and food contamination was not statistically significant (OR 0.363; 95% CI, 0.099–1.327; P=.113). However, those reporting the presence of such contaminants around their vending site had a higher occurrence of food contamination (37.1%) compared with those who did not (17.6%) (Table 2).

Some food-handling practices were significantly associated with food contamination. Access to fresh running water for hand washing while preparing food was negatively associated with food contamination (P<.005), whereby a decreased risk of having contaminated food was observed if one had access to fresh running water (OR 0.355; 95% CI, 0.177–0.713) (Table 3). The occurrence of food contamination was lower among those who had access to fresh running water for hand washing (24.1%) compared with those who did not (47.1%) (Table 4). Hand washing before handling food items was also negatively associated with food contamination (P<.005), whereby there was a decreased risk of contaminated food if vendors practised hand washing before handling food items (OR 0.018; 95% CI, 0.006–0.051) (Table 3). There was a lower occurrence of food contamination among those who washed hands before handling food items (10.8%) compared with those who did not (87.2%) (Table 4). Hand washing after handling raw food items was practised by only 13.4% (20) of the vendors. This variable was however not significantly associated with food contamination (OR 1.288; 95% CI, 0.490–3.383; P=.607) (Table 3).

TABLE 4. Occurrence of Food Contamination in Relation to Food-Handling Practices

Food-Handling Practices	Food Contamination		
	Yes No. (%)	No No. (%)	Total No. (%)
Access to fresh running water for hand washing while preparing food			
Yes	19 (24.1)	60 (75.9)	79 (100)
No	33 (47.1)	37 (52.9)	70 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Hand washing before handling food items			
Yes	11 (10.8)	91 (89.2)	102 (100)
No	41 (87.2)	6 (12.8)	47 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Hand washing after handling raw food items			
Yes	8 (40.0)	12 (60.0)	20 (100)
No	44 (34.1)	85 (65.9)	129 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Medical check-ups			
< 3 months apart	2 (20.0)	8 (80.0)	10 (100)
Every 3 months	6 (26.1)	17 (73.9)	23 (100)
> 3 months apart	17 (47.2)	19 (52.8)	36 (100)
Never	27 (33.8)	53 (66.2)	80 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Use of an apron			
Yes	16 (19.0)	68 (81.0)	84 (100)
No	36 (55.4)	29 (44.6)	65 (100)
Total	52 (34.9%)	97 (65.1)	149 (100)
Use of a head cover			
Yes	15 (26.3)	42 (73.7)	57 (100)
No	37 (40.2)	55 (59.8)	92 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Storage of leftover food			
Fridge	5 (18.5)	22 (81.5)	27 (100)
Cupboard	36 (40.4)	53 (59.6)	89 (100)
Other	11 (33.3)	22 (66.7)	33 (100)
Total	52 (34.9)	97 (65.1)	149 (100)
Serving of food			
Plastic bag	34 (33.7)	67 (66.3)	101 (100)
Both newspaper and plastic	5 (35.7)	9 (64.3)	14 (100)
Plastic or plate	4 (42.9)	9 (69.2)	13 (100)
Other	9 (30.8)	12 (57.1)	21 (100)
Total	52 (34.9)	97 (65.1)	149 (100)

The method of hand washing was also assessed in relation to food contamination. The highest occurrence of food contamination (85%) was among vendors who did not wash their hands at all, either before handling food items or after handling raw food items. This variable was significantly associated with food contamination ($x^2_{3,\ 0.05}$ = 60.657, $P<.001$) (Table 3). In terms of acquisition of knowledge on food preparation, a majority of vendors (63.8%) had acquired food preparation skills through observation, and only 3.4% had been formally trained. On examining this variable in relation to food contamination, there was no significant association ($x^2_{3,\ 0.05}$ = 1.371, P=.725) (Table 3).

Although the relationship between having a medical examination (check-up) and food contamination was not significant ($x^2_{3,\ 0.05}$ = 4.216, P=.239) (Table 3), the occurrence of food contamination was highest among vendors who had regular check-ups more than 3 months apart (47.2%) and least among those who had their medical check-ups less than 3 months apart (20%) (Table 4).

Use of protective clothing while vending food was also assessed in relation to food contamination. There was a significant association between use of an apron and food contamination ($P<.001$), whereby a decreased risk was observed if one wore an apron (OR 0.190; 95% CI, 0.091–0.394) (Table 3). Although use of a cap to cover hair was not significantly associated with food contamination (OR 0.531; 95% CI, 0.258–1.093; P=.084), there was a higher occurrence of food contamination among the respondents who did not use a cap (40.2%) compared with those who did use a cap while vending food (26.3%) (Table 4).

Storage of leftover food was also assessed in relation to food contamination. It was observed that a majority of vendors (59.7%) stored leftover food in a cupboard, and only 18.1% stored it in a refrigerator. Although this variable was not significantly associated with food contamination ($x^2_{2,\ 0.05}$ = 4.431, P=.109), there was a higher occurrence of food contamination among respondents who stored leftover food in a cupboard (40.4%) compared with those who either stored the food in a refrigerator (18.5%) or those who did 'other' and either consumed leftovers with family, gave it to friends, or had no leftover food (33.3%) (Table 4). In terms of serving food, a majority (67.8%) used a plastic bag since they sold takeaway food. There was no significant association between the way food was served and food contamination ($x^2_{3,\ 0.05}$ = 0.755, P=.860). However, a higher occurrence of food contamination (42.9%) was observed among vendors who used a plastic bag or plates, and the least occurrence of food contamination (30.8%) was seen among those in the 'other' category, who mainly used labelled paper bags distributed by manufacturers for serving sausages (Table 4).

DISCUSSION

According to Baluka and colleagues, environmental hygiene is important for food safety and necessary to support safe food handling and hygiene by employees.[13] The present study explored the relationship between various environmental factors and food contamination, and a significant ($P<.05$) association was observed for some of them. A negative association was observed between access to a toilet facility and food contamination ($P<.001$), whereby a decreased risk of occurrence of food contamination was observed if one had access to a toilet facility (OR 0.095; 95% CI, 0.03–0.227). Similarly, Idowu and Rowland[14] reported that vending sites usually lack basic facilities such as toilets and hand washing facilities, since nearness to customers is the primary target of street food vendors. These conditions enhance the incidence of foodborne illnesses and transmission of diseases.

Sludge formed by wastewater from a burst sewer.

The main type of toilet facility observed in the 2 study areas was the modern toilet (46.3%). The greatest challenge that was observed with this type of toilet facility was the inadequate sewerage system. In 1 study area, wastewater flowed along the street as a result of a burst sewer. The vendors along the street continued selling food, oblivious to the hazard posed by the burst sewer. This provided a favorable environment for flies and other types of vectors of transmission of pathogens. This may have contributed to the higher occurrence of food contamination (29%) among vendors who had access to a modern toilet.

Accessibility of running water around the toilet facility for hand washing was negatively associated with food contamination in this study ($x^2_{2,\ 0.05}$ = 36.046 $P<.001$). A higher occurrence of food contamination was observed among vendors who had no access to running water (30%) compared with those who had access to running water (15.9%). This implies that water is a basic necessity that ensures better personal hygiene, which in return serves to reduce the potential for food contamination. Other studies have observed that food that has been properly prepared can become

Waste disposed on the ground by a vendor.

contaminated when handled by people with unwashed hands and that poor access to hand washing water can be a source of bacterial contaminants for food.[15,16]

A majority (62.4%) of the vendors in this study practised open area dumping to dispose of waste. The open area dumping sites were, however, located at a distance away from most vending sites, and hence the vendors stored their waste in plastic bags within the site, then disposed of it later. Although the relationship between waste disposal and food contamination was not statistically significant ($P>.05$), vendors who used municipal containers had the highest occurrence of food contamination (40.4%), probably because these containers were filled beyond capacity and attracted flies that could serve as vehicles of transmission of food contaminants. These vendors would then resort to discarding waste on the ground. These findings agreed with observations made in a study in Uganda where focus group discussions revealed that the municipal council containers were not regularly emptied, therefore in most cases, they were also overflowing. This created a dirty environment that compromised sanitation, became a habitat for rodents and a breeding point for flies, and promoted the growth of microorganisms.[17]

This study found that the relationship between environmental contaminants and food contamination was not statistically significant ($P>.05$). Nevertheless, vendors reporting the presence of contaminants such as flies, dust, insects, rodents, and sludge around the vending site had a higher occurrence of food contamination (37.1%) compared with those who thought that the environment had none of these contaminants (17.6%). According to Muyanja, dust carries many microbes that may be pathogenic if left to settle onto prepared foods.[17] The presence of rats and moles, however, was significantly associated with food contamination ($P<.05$). Vendors who reported the presence of these rodents had a higher occurrence of food contamination (60%) compared with those who did not (20.2%). The rodents may have found an environment that was conducive for breeding as a result of the poor methods of waste disposal and may have served as agents of transmission of contaminants onto prepared foods.

This study observed that access to fresh running water where food was being prepared was negatively associated with food contamination ($P<.005$), whereby a decreased risk of having contaminated food was observed if there was access to fresh running water (OR 0.355; 95% CI, 0.177–0.713). A study in Malawi similarly observed that poor access to fresh running water provides harbour to faecal bacteria that can serve as a source of bacterial contaminants in food.[15] Hand washing before handling food items was also negatively associated with food contamination ($P<.005$), whereby there was a decreased risk of having contaminated food if one practised hand washing before handling food items (OR 0.018; 95% CI, 0.006–0.051). This suggests that observing personal hygiene can help in reduction of food contamination. The findings of this study agree with a study in Ethiopia that observed that vendors with poor personal hygiene had a 4-fold risk of having contaminated food as compared with those who had good personal hygiene.[18]

Contrary to what has been found in the available literature, food contamination was higher among vendors who said they washed their hands after handling raw food items (40%) compared with those who said they did not (34.1%). This finding may have been a result of use of recycled water for hand washing. The vendors cleaned the raw food items using water placed in a container, then used the same water to clean the knives used for cutting and also to wash their hands. This presented a potential risk of cross-contamination as a result of using recycled water for hand-washing. On the other hand, those who did not wash their hands after handling raw food items were mainly vendors who sold takeaway food items such as boiled eggs and sausages that were served with raw vegetables. The raw vegetables were mainly tomatoes and onions which they had already prepared at home. In the event that it was necessary for them to prepare the vegetables on-site, they claimed that they used a plastic bag to wrap their hands while serving to prevent direct contact with prepared foods. Through observation, it was noted that this practice was used by most of these vendors.

The method of hand washing was significantly associated with food contamination ($x^2_{3,\ 0.05} = 60.657$, $P<.001$). The highest occurrence (85%) was among vendors who neither washed their hands before handling food items nor after handling raw food items. The vendors who washed their

hands using soap and running water had no occurrence (0%) of food contamination. Occurrence of food contamination was 17.2% among vendors who used plain water placed in a container and 16.7% among those who used soap and water placed in a container. This may imply that proper hand washing skills reduces the potential for occurrence of food contamination.

These findings are consistent with findings of Todd and colleagues, who reported that several foodborne disease outbreaks were a result of poor handling practices, such as cross-contamination between raw and cooked products and poor personal hygiene of food handlers, such as failure to wash hands.[19]

We found a significant negative association between use of an apron and food contamination ($P<.05$). Vendors who wore an apron had a lower occurrence of food contamination (19%) compared with those who did not (55.4%). On the other hand, although use of a head cover was not significantly associated with food contamination ($P>.05$), there was a higher occurrence of food contamination among the vendors who did not use a head cover (40.2%) compared with those who did (26.3%). Similarly, in a study in Togo, failure to wear aprons and caps was observed to be the likely causative factor for contamination of food samples.[20] These findings suggest that use of protective clothing is necessary to reduce the likelihood of food contamination.

In this study, storage of leftover food was not significantly associated with food contamination ($P>.05$). However, the study explored only the method of storage and not the duration of storage. This could be the reason why our findings differ from those of a study in Ethiopia that observed that storage of leftover street-vended foods for more than a day was a risk factor for contamination ($P<.05$).[18] Findings from this study showed no significant association between the way food was served and food contamination ($x^2_{3,\ 0.05} = 0.755$, $P=.860$). However, a higher occurrence of food contamination was observed among vendors who used a plastic bag or plates (42.9%), and the least amount of contamination was found among vendors who used labelled paper bags normally distributed by manufacturers (30.8%).

The labelled paper bags were mostly used for the sale of sausages or 'smokies', which is a type of sausage. Newspapers were used for wrapping food only after it was first wrapped inside a plastic bag. The type of foods that were mainly served in this manner were fried chips and fish. The plastic bags, however, were poorly stored, as most vendors kept them in the open, which posed a risk of contamination from the environment. Some vendors also blew air into the plastic bags before serving the food, and others would cut the bags into small pieces so as to avoid 'misuse'. The small pieces of plastic bags would then be used to serve foods such as boiled eggs, with some vendors charging a higher price if a consumer needed to have the food completely wrapped. These kinds of practices may have led to the higher occurrence of food contamination among vendors who used plastic bags or plates to serve food, since the plates were, on the other hand, cleaned using recycled water. These findings were consistent with observations made in a study in Haiti where bags and plates were identified to be some of the possible sources of food contamination.[21] According to Barro and colleagues, plastic bags are usually contaminated by the food handlers, as pathogens may invade the interior surfaces of the bags during packaging of the food due to poor handling practices.[22]

Some environmental factors and food-handling practices did not show any significant relationship with food contamination in this study. This may imply that there was no risk of food contamination if direct contact was not made with food. Similar findings were observed in a study in Accra that found that environmental hygiene and the vendor's appearance did not show any significant relationship with the levels of contamination.[23]

CONCLUSION

Access to a toilet facility and availability of running water within the toilet facility were the environmental factors found likely to decrease the risk of food contamination. On the other hand, the presence of pests and rodents around the vending sites was likely to increase the risk of food contamination. Access to fresh running water while preparing food, hand washing before handling food items, and use of protective clothing (apron) were the food-handling practices likely to decrease the risk of food contamination. There is a need for market vendors to be provided with more basic amenities as well as with sensitisation on pest control to reduce the potential for food contamination.

Acknowledgements: I would like to acknowledge my supervisors Professor Zipporah Ng'ang'a and Dr. Peter Wanzala for their great assistance in writing this paper. I acknowledge Ms. Mikali Waswa for her great role in the laboratory work. I also sincerely appreciate the support from the management of the National Public Health Laboratory, Kenya, especially Ms. Jedidah Kahura and the other laboratory technicians within the laboratory.

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Peer Reviewed

Competing Interests: None declared.

Received: 23 Nov 2016; **Accepted:** 9 Feb 2017

Cite this article as: Nyambura Kariuki E, Waithera Ng'ang'a Z, Wanzala P. Food-Handling Practices and Environmental Factors Associated With Food Contamination Among Street Food Vendors in Nairobi County, Kenya: A Cross-Sectional Study. East African Health Res J. 2017;1(1):62-71. https://doi.org/10.24248/EAHRJ-D-16-00382

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