

Research Article

The Incidence of Non-Tuberculous Mycobacteria in Infants in Kenya

Grace Kaguthi ^{1,2}, Videlis Nduba,^{1,2} Wilfred Murithi,¹ and Suzanne Verver^{3,4}

¹Kenya Medical Research Institute-Centre for Respiratory Diseases Research, Nairobi, Kenya

²Amsterdam University Medical Centre, University of Amsterdam, Netherlands

³Department of Public Health, Erasmus Medical Centre, Rotterdam, Netherlands

⁴KNCV Tuberculosis Foundation, The Hague, Netherlands

Correspondence should be addressed to Grace Kaguthi; skiringa@gmail.com

Received 11 February 2019; Accepted 11 June 2019; Published 3 July 2019

Academic Editor: Jean-Paul J. Gonzalez

Copyright © 2019 Grace Kaguthi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

There is inadequate understanding of the epidemiology of Non-Tuberculous Mycobacteria (NTM) among infants in high tuberculosis burden countries. The objective of this study was to document the incidence and diversity of NTM disease or colonisation in sputum specimens from infants with presumptive TB, the risk factors, and clinical characteristics, in a high TB and HIV burden setting in Western Kenya. A cohort of 2900 newborns was followed for 1–2 years to assess TB incidence. TB investigations included collection of induced sputa and gastric aspirates for culture and speciation by HAIN®, Tuberculin Skin Testing (TST), HIV testing, and chest radiography. The American Thoracic Society Criteria (ATS) were applied to identify NTM disease. Among 927 (32% of 2900) with presumptive TB, 742 (80%) were investigated. NTM were isolated from 19/742 (2.6%) infants. *M. fortuitum* was most frequently speciated (32%). Total person-time was 3330 years. NTM incidence was 5.7/1,000 person-years, 95% CI (3.5, 8.7). Infants diagnosed with TB were more likely to have NTM isolation (odds ratio 11.5; 95% CI 3.25, 41.0). None of the infants with NTM isolated met the criteria for NTM disease. The incidence of NTM isolation was comparable to similar studies in Africa. NTM isolation did not meet ATS criteria for disease and could represent colonisation. TB disease appears to be structural lung disease predisposing to NTM colonisation.

1. Introduction

Non-Tuberculous Mycobacteria (NTM) are environmental saprophytes widely distributed in water and soil [1]. They are the genetic progenitors of *M. Tuberculosis* Complex (MTBC), after a series of gene deletions and gene acquisitions [2] with MTBC evolving to a more virulent pathogen. NTM rarely cause disease except when immune function is impaired [3], elderly patients and chronic lung disease. However, some NTM are pathogenic, and recently there has been a reported increase in NTM lymphadenitis [4, 5] and Buruli ulcers [6, 7].

The shared ancestry of NTM and MTBC is responsible for immune interference in BCG vaccination, via cross reactive immune responses [2]. This could be one of the reasons for low BCG efficacy where NTM are prevalent [2]. Absence of NTM sensitization was associated with higher efficacy of BCG against pulmonary and severe forms of tuberculosis

in a systematic review [8]. Surprisingly, the discontinuation of universal BCG vaccination in these countries has seen an increase of NTM lymphadenitis in children, suggesting BCG was also protecting against NTM in that setting [4]. NTM appear to be immune modulators influencing host interactions in BCG efficacy, TB burden, and NTM disease.

The antigen homologues [2] further decrease accuracy of biomarkers distinguishing latent TB infection (LTBI) and NTM exposure.

Pulmonary NTM disease is clinically and radiologically identical to TB and is so diagnosed, in the absence of microbiological confirmation in high TB burden settings. It is a relevant distinction to make as almost all NTM do not respond to anti-tuberculous therapy [9]. Isolation of NTM in sputum is not necessarily disease [9]. Data on NTM disease and prevalent subtypes is limited particularly in countries with a high TB burden. Most studies report on adults [10–12].

Few studies on NTM in children have been published on the continent [13–15]. Most document the proportion of NTM among those with presumptive TB. There is also a dearth of knowledge on risk and exposure factors. As infants are the target age group for TB vaccines in the pipeline, it is useful to describe the epidemiological landscape of NTM, given their role in tuberculosis incidence and possibly vaccine efficacy.

The objective of this study was to document the incidence and diversity of NTM disease or colonisation in sputum specimens from infants with presumptive TB, the risk factors, and clinical characteristics, in a high TB and HIV burden setting.

2. Study Population and Methods

The study took place in Siaya, Western Kenya, a predominantly rural community north of Lake Victoria. The area has a high prevalence of HIV, TB, and malaria. Most women delivered at home [21]. The NTM substudy was part of a prospective cohort study to document the incidence of TB ahead of TB vaccine trials in the same population. Presumably, infants are born uninfected; we present the incidence of NTM in this cohort.

Briefly, parents or guardians of 2900 infants aged zero to six weeks gave written permission for enrollment of their newborns between June 2009 and June 2010. Patients were followed up for at least one year and a maximum of two years. Through four monthly scheduled visits and ancillary care visits, infants were identified as having presumptive TB if they had history of TB contact, symptoms, or signs of pulmonary TB (failure to thrive, cough or night sweats or fever for more than two weeks, a history of hospitalization for HIV/AIDS related illness, lower respiratory tract infections, meningitis, or TB). Consequently, they were admitted into a case verification ward for three days. Two fasted sputum induction specimens and two gastric aspirates were collected on subsequent mornings. Tuberculin Skin Testing (TST) was done with two Tuberculin Units (2TU) from Statens Serum Institut (SSI). TST readings of 10mm and more or 5mm or more among HIV infected children were considered to be positive readings. Further, DNA PCR HIV (COBAS® HIV-1 Amplicor by ROCHE) tests and digital chest radiography were performed.

Patients received anti-tuberculous therapy if they had microbiological confirmation (definite TB) or clinically, based on the Keith Edward TB Score (KE Score) Chart of >7, or <7 if the chest radiograph was suggestive (probable TB). Mid-Upper Arm Circumference (MUAC) was used to determine nutritional status for children older than 6 months old at time of TB investigations. Weight for Age Z Score was used for those less than 6 months. HIV infected infants were referred for anti-retroviral treatment initiation and care. Patients vital status at last study contact was documented.

Chest radiographs were read systematically and classified as abnormal probable TB, abnormal not TB, or normal [22]. The study was approved by Kenya Medical Research Institute Independent Ethics Committee (KEMRI-IEC) SSC 1465. The

data used to support the findings of this study are available from the corresponding author upon request.

We applied the American Thoracic Society's [23] criteria to establish clinical significance of positive NTM cultures.

2.1. Laboratory Methods and Sample Decontamination. Induced sputum and gastric aspirates were transported to the laboratory at 2 to 8°C, processed using freshly prepared N-acetyl L-cysteine (NALC)-4% sodium hydroxide (NaOH)-2.9% sodium citrate at a final concentration of 1%. Gastric aspirates with >5ml volume were concentrated by centrifugation and pellet resuspended with 5ml phosphate buffer saline (PBS). Digestion was stopped using pH 6.8 PBS after 20 minutes. Centrifugation was done at 3,000 x g for 15 minutes at 4°C. Supernatant was discarded and the pellet resuspended with 2ml PBS. This was used for inoculation of Lowenstein Jensen (LJ) [BD] media (0,2ml), fluorescent microscopy, and mycobacteria growth indicator tube (MGIT) [BD] (0.5ml). LJ were incubated in 37°C CO₂ incubators for 8 weeks, and MGIT was incubated in automated BACTEC™ MGIT™ 960 [BD] for 42 days. Artificial sputum was used as a negative control sample to check for cross-contamination with each batch processed.

MGIT cultures that turned positive were stained for acid fast bacilli (AFB) using Ziehl Neelsen (ZN). Contamination was checked by inoculation and incubation of blood agar plates at 37°C and read after 48 hours. Samples that tested ZN negative but Blood Agar Plate (BAP) positive ≥7 days later were discarded as contaminated. Those <7 days were redigested using 4% NaOH as described in MGIT™ procedure manual [24]. AFB positive cultures were tested by immunochromatographic assay (ICA) such as Capilia™ TB-Neo (TAUNS Laboratories, Numazu, Japan) or BD MGIT™ TBc identification kit ((BD, Franklin Lakes, NJ, USA) to identify whether NTM or MTBC.

For LJ cultures with visible growth, we assessed colony morphology. Those suggestive of mycobacteria were identified using ZN smear, and those AFB positive were tested with ICA.

NTM culture isolates were genetically identified to the species level using Genotype Mycobacterium Common Mycobacterium (CM) or Additional Species (AS) kits (HAIN Lifescience, Nehren, Germany). The procedure was done according to manufacturer's instructions.

2.2. Statistical Methods. Frequency methods were used to describe the baseline characteristics. Odds ratios were used to analyze whether differences between those with and without NTM were due to chance. T-tests were used to compare the mean age at TB investigations. To evaluate differences in clinical characteristics, known and potential risk factors, logistic regression was performed. NTM cases that had microbiologically confirmed or clinical TB were analysed as TB cases. A-priori risk factors included infant and maternal HIV infection, nutritional status, housing, and number of siblings.

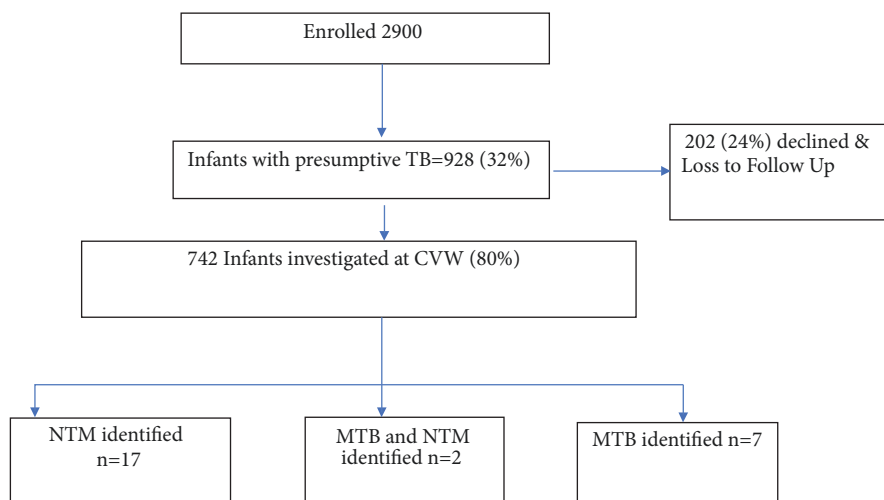


FIGURE 1: Study flow chart.

3. Results

Of 2900 infants enrolled, 927 (32%) were suspected to have TB (presumptive TB) during their 1-2-year follow-up. Of these 742 (80%) were admitted for investigations (Figure 1). There were 19 NTM identified following culture (2.6% of 742). Total person-time of follow-up was 3330.3 years. The incidence of NTM was 5.7 per 1,000 person-years (pyo) of follow-up (95% CI 3.5, 8.7), while all TB incidence (49 cases) was 15/1000 pyo (95% CI, 11-20) and microbiologically confirmed TB incidence was 2.7/1,000 pyo. At baseline, there were no statistically significant differences between those who had NTM identified versus all other infants (Table 1).

Upon bivariate comparison of clinical characteristics between presumptive TB patients and NTM cases, there were no statistically significant differences (Table 2(a)). However, odds of a positive NTM among infants with TB was eleven-fold that of infants with no TB (OR 11.6 (95% CI 3.25, 41.0)). NTM cases had forty-eight-fold higher odds of having microbiologically confirmed TB compared to all presumptive TB (OR 48.3 95% CI 9.3, 249) (Table 2(a)).

There were no differences between NTM cases and other presumptive TB cases in mean age at time of TB investigations (Table 2(b)).

Table 3 shows the NTM identified and the individual's clinical characteristics. *M. fortuitum* (6/19 32%) and *M. scrofulaceum* (2/19 11%) were most frequently isolated. Two of the 19 (11%) were unidentifiable. Two patients had MTBC and NTM coinfection.

Applying the ATS criteria for diagnosis of NTM disease, none of the NTM cases qualified as having NTM disease. Only 1/19 (5.3%) NTM case was HIV infected which had NTM cultured (*M. asiaticum*) while 3/19 (16%) were born to mothers who tested HIV positive but were themselves uninfected (HUE).

In our study, rapidly growing mycobacteria (RGM), which form colonies in less than seven days, were isolated most frequently (10/19) (Table 3). The most frequently isolated NTM in pediatric studies are shown in Table 4. *M.*

fortuitum was the most frequently isolated NTM among the identified studies.

4. Discussion

4.1. Burden of NTM. The proportion of NTM in pulmonary samples of presumptive TB cases in this infant cohort was relatively low (2.6%; 95% CI 1.5, 3.8). Standard sputum decontamination procedures were judiciously applied; hence it is unlikely that NTM yield was affected by this. A similar study among infants in Uganda and South Africa found 3.7% [14] and 6% [13], respectively. The epidemiology of exposure in this region could be nonlinear, where exposure in early childhood is minimal but increases rapidly in adolescents. A significantly higher proportion of NTM were identified among presumptive TB cases in adolescents in the study area (37.5%), at the time of the study (V. Nduba, Personal Communication). Nevertheless, the Mozambique cohort and a survey in Ethiopia had more NTM [15, 20], and the average prevalence in African adult pulmonary samples was 7.5% in a systematic review [10]. It is possible that BCG is protective against NTM colonisation. A twenty-year retrospective study of NTM notifications in children demonstrated increased odds of NTM disease when universal BCG vaccination was halted in Finland [4]. Therefore, BCG could also protect against colonisation. This can be evaluated conclusively in head to head comparisons of BCG and recombinant BCG vaccines presently in phase III clinical trials [25].

4.2. Colonisation or NTM Disease/Clinical Relevance. We did not find statistically significant differences in baseline characteristics between NTM cases and other presumptive TB patients suggesting widespread exposure across the study population. There were no differences in the clinical or radiological characteristics between presumptive TB and NTM cases.

NTM disease is clinically and radiologically indistinguishable from TB [9]. Two NTM cases were symptomatic

TABLE 1: Baseline characteristics of study sample, infants with presumptive TB and infants with NTM isolated; and comparison between infants with presumptive TB with and without NTM.

Characteristic	Study Sample (n=2900)	Investigated for Presumptive TB (n =742) (N, column %)	NTM positive (n=19) (N, row%)	OR (95%CI) *
<i>Gender</i>				
Female	1412	358 (48%)	10 (2.8%)	1 (ref)
Male	1488	384 (52%)	9 (2.3%)	0.85 (0.35, 2.11)
<i>Enrolment weight</i>				
Normal	2674	667 (90%)	16 (2.4%)	1 (ref)
low	226	75 (10%)	3 (4.1%)	2.24 (0.65, 7.73)
<i>Place of birth</i>				
Home	1840	510 (69%)	11 (2.2%)	0.77 (0.31, 1.93)
Health facility	1038	229 (31%)	8 (3.5%)	1 (ref)
missing	22	3 (<1%)		
<i>Maternal HIV status</i>				
HIV negative	2451	598 (81%)	16 (2.7%)	1 (ref)
HIV positive	401	127 (17%)	3 (2.4%)	0.88 (0.25, 3.08)
Unknown	48	17 (2%)		
<i>Infant HIV status</i>				
HIV negative	2827	708 (95%)	18 (2.5%)	1 (ref)
HIV positive	73	34 (5%)	1 (2.9%)	2.17 (0.29, 16.5)
<i>Maternal age category</i>				
<19	635	152 (21%)	1 (0.7%)	1 (ref)
20-29	1533	384 (52%)	16 (4.2%)	6.69 (0.89, 50.5)
>29	732	206 (28%)	2 (1.0%)	1.74 (0.16, 19.2)
<i>Maternal Occupation</i>				
Unemployed	1676	409 (55%)	11 (2.7%)	1 (ref)
Farmer	864	250 (34%)	5 (2.0%)	0.88 (0.31, 2.54)
Business	260	61 (8%)	2 (3.3%)	1.17 (0.26, 5.32)
Salaried	71	13 (2%)	1 (7.7%)	2.16 (0.28, 17.0)
Unknown	29	9 (1%)		
<i>Housing Type</i>				
Mud House	1912	523 (71%)	11 (2.1%)	1(ref)
Semi-permanent	527	125 (17%)	4 (3.2%)	1.32 (0.42, 4.17)
Permanent	426	84 (11%)	4 (4.8%)	1.64 (0.52, 5.17)
Other	6	1 (0.1%)		
Unknown	29	9 (1.2%)		
<i>Number of Siblings</i>				
None	649	129 (17%)	3 (2.3%)	1 (ref)
One to three	1497	391 (53%)	14 (3.6%)	2.03 (0.58, 7.10)
>3	754	222 (30%)	2 (0.9%)	0.57 (0.10, 3.44)
<i>Vaccination Status at 6 weeks</i>				
Complete	2205	682 (92%)	16(2.4%)	0.48 (0.11, 2.10)
Incomplete	133	29 (4%)	2(7.0%)	1 (ref)
Missing	562	31 (4%)	1(3.2%)	

* Odds of being NTM case among those investigated for presumptive TB, given the category of baseline characteristic.

TABLE 2

(a) Comparative clinical characteristics of those investigated for presumptive TB and infants with NTM isolated (categorical).

Clinical Characteristics	Presumptive TB N (column %) (n=742)	NTM +ve N (row%) (n=19)	OR (95%CI)
<i>Any TB case (clinical or confirmed)</i>			
No	694 (94%)	16 (2.3%)	1 (ref)
Yes	48 (6.5%)	3 (6.3%)	11.6 (3.25, 41.0)
<i>MTBC +ve TB case</i>			
No	733 (99%)	17 (2.3%)	1 (ref)
Yes	9 (1%)	2 (22.2%)	48.3 (9.34, 249)
<i>Chest Radiograph</i>			
Normal	590 (80%)	13 (2.2%)	1(ref)
Abnormal not TB	110 (15%)	4 (3.6%)	1.71 (0.55, 5.35)
Abnormal TB	35 (5.0%)	2 (5.7%)	2.80 (0.61, 12.9)
missing	7 (0.9%)		
<i>Keith Edward TB score</i>			
<7	675 (90%)	17 (2.5%)	1 (ref)
>=7	32 (4.3%)	2 (6.3%)	2.62 (0.58, 11.9)
Missing	35 (4.7%)		
<i>Reason for TB suspicion</i>			
History of hospitalization			
No	283 (38%)	8 (3.4%)	1 (ref)
Yes	426 (57%)	11(2.6%)	0.78 (0.31, 1.97)
Missing	33 (5.0%)		
<i>TB Contact History</i>			
No	579 (78%)	13(2.3%)	1 (ref)
Yes	131 (18%)	6 (4.6%)	2.57 (0.96, 6.88)
Missing	32 (4.0%)		
<i>TST results</i>			
Negative	555 (76%)	14 (74%)	1 (ref)
Positive	172 (24%)	5 (26%)	1.15 (0.41, 3.25)
<i>TB symptoms</i>			
No	530 (71%)	15 (2.8%)	1 (ref)
Yes	180 (24%)	4 (2.2%)	0.85 (0.28, 2.58)
Missing	32 (4.0%)		
<i>Nutritional Status at admission</i>			
Healthy	379 (51%)	8 (2.1%)	1 (ref)
At risk	195 (27%)	6 (3.0%)	1.48 (0.51, 4.32)
Moderate Acute Malnutrition (MAM)	113 (15%)	2 (1.8%)	0.84 (0.18, 4.03)
Severe Acute Malnutrition (SAM)	40 (5%)	3 (7.5%)	3.73 (0.95, 14.7)
Missing	15 (2%)		

(b) Comparative clinical characteristics for those investigated for presumptive TB and infants with NTM isolated (continuous variable).

Clinical Characteristic	Categories	n	Mean age (95% CI)	Rank sum p- value/t-test p value
Mean age at TB investigation (months)	NTM negative Presumptive TB	718	9.34 (8.95, 9.74)	0.20
	NTM case	19	11.0 (8.02, 13.9)	
	Missing	5		

TABLE 3: NTM identified; clinical and radiological profile of cases.

Number	Age (months) at admission	NTM species	MTBC +ve	Infant HIV status	Nutritional status at admission	TST reading (mm)	KE Score	Vital Status	CXR	Siblings	Housing
<i>Rapidly Growing Mycobacteria</i>											
1	52452	<i>M. peregrinum</i>	No	Negative	At risk	0	0	alive	Normal	4	Mud
2	50170	<i>M. smegmatis</i>	No	Negative	Healthy	4	0	alive	Normal	1	Semi
3	50220	<i>M. smegmatis</i>	No	Negative	At risk	3	1	alive	Normal	unknown	Semi-
4	51388	<i>M. chelonae</i>	No	Negative	Healthy	0	0	Alive	Normal	2	mud
5	52696	<i>M. fortuitum1</i>	No	Negative	Healthy	0	0	Alive	Abnormal not TB	6	mud
6	52727	<i>M. fortuitum1</i>	No	Negative	SAM	10	6	alive	Normal	3	mud
7	50206	<i>M. fortuitum1</i>	No	Negative	Healthy	12	3	Alive	Normal	1	stone
8	50523	<i>M. fortuitum1</i>	No	Negative	At risk	0	0	Alive	Normal	1	stone
9	51104	<i>M. fortuitum1</i>	No	Negative	Healthy	1	1	Alive	Normal	1	mud
10	52024	<i>M. fortuitum2</i>	No	Negative-HUJE	Healthy	0	1	alive	Normal	1	mud
<i>Slow Growing Mycobacteria</i>											
11	51599	<i>M. asiaticum</i>	Yes	Positive	SAM	7	10	Died	Abnormal not TB	unk	stone
12	50049	<i>M. celatum</i>	No	Negative	MAM	0	0	Alive	Normal	3	mud
13	51598	<i>M. gordonae</i>	No	Negative	At risk	1	1	alive	Abnormal not TB	3	mud
14	52683	<i>M. intracellulare</i>	No	Negative	Healthy	0	0	alive	Abnormal not TB	3	Semi-
15	51119	<i>M. malmoense</i>	No	Negative	At risk	2	1	alive	Normal	2	Semi-
16	50380	<i>M. scrofulaceum</i>	No	Negative-HUJE	At risk	12	4	alive	Abnormal TB likely	3	Mud
17	50108	<i>M. scrofulaceum</i>	No	Negative	Healthy	3	3	alive	Normal	1	mud
<i>Unidentified Mycobacteria</i>											
18	50178	Unidentified	No	Negative-HUJE	Healthy	0	0	alive	Normal	unk	mud
19	51706	Unidentified	Yes	Negative	SAM	12	13	alive	Abnormal TB likely	2	stone

TABLE 4: Pediatric NTM studies in Africa between years 2000 and 2018.

Authors, Country, Year of Publication	Study Type	Study Population	NTM proportion of Presumptive TB	Most frequently isolated NTM	Clinical Relevance*	MTBC-NTM co-infection	Proportion of participants with TB	National/local TB prevalence per 100,000 at time of study
Present Study (Kenya)	Prospective Cohort Study	<2 years	2.6%	<i>M. fortuitum</i> (32%)	Colonisation	2/19	1.5%	600 [16]
Asimwe B, Uganda 2013 [14]	Prospective Cohort Study	<1 year	3.7%	<i>M. fortuitum</i> (64%)	Not specified	0	Not specified	193 [17]
Hatherill M, South Africa 2006 [13]	Prospective Cohort Study	<2 years	6%	<i>M. intracellulare</i> (41%)	7/109-NTM disease	5/109	11%	960 [18]
Lopez-Varela E, Mozambique 2017 [15]	Prospective Cohort Study	<2 years	26%	<i>M. intracellulare</i> (68%)	Colonisation	0	>1.4%	>544 [19]
Workalemahu B, Ethiopia 2013 [20]	Cross sectional Hospital Survey	<15 years	9.9%	<i>M. fortuitum</i> (29%)	Not specified	0	15%	237 [20]

* Based on authors' description of suggestive clinical and radiological features.

with a suggestive radiological picture and would have qualified as NTM disease, but MTBC was also isolated from their sputum. The remainder had no combination of suggestive clinical or radiological features. We therefore conclude the NTM cases represent colonization. There is a possibility that these are laboratory contaminants; however this is unlikely since we checked for contaminants by having negative controls.

4.3. Risk Factors

4.3.1. Environmental Exposure. We did not identify any environmental risk factors for NTM incidence. Unlike MTBC which is transmitted from person to person, NTM transmission occurs via repeated environmental exposure. In infants, this would be through handling by parents and siblings. The study area is rural. Risk for acquiring NTM is significantly higher in communities engaged in occupations that generate aerosols and are exposed to soil for prolonged periods such as agriculture [26]. It is not clear what the environmental source of these NTM is.

4.4. Host Factors. Host factors predisposing to NTM isolation were intercurrent MTBC disease and severe undernutrition, although the latter did not reach statistical significance. Past history of TB has been known to be a risk factor for NTM disease [27, 28], since we studied infants that could not be confirmed. Interestingly, in this study, MTBC isolation increased the odds of NTM isolation almost fifty-fold. NTM-MTBC coinfection in the same infant host has been observed [13, 15], and in adults in high TB burden countries [29]. TB appears to be a preexisting lung condition predisposing to NTM colonisation [9].

Low Body Mass Index and poor nutrition are other possible host factors, even predicting risk of disseminated NTM disease in other studies [30, 31], our study seemed to show the same trend.

Only in one case was the NTM case HIV infected, indicating among infants in this region, immunodeficiency is not a factor in NTM isolation in sputum.

4.5. NTM Isolated. The spectrum of organisms identified in this NTM study is similar in type and frequency to those reported in Uganda [14], Ethiopia [20], and Saudi Arabia [28]. *M. fortuitum* was most frequently isolated in these studies. There could be geographic and climatic factors in the distribution. All the regions have warm climates. Increase in latitude and polarity has been shown to be associated with higher isolation rates of more pathogenic, slow growing mycobacteria [4, 13, 15].

4.6. TB Diagnostics. There was no detectable difference in TST positivity between NTM cases and other patients whereas NTM sensitization is known to be responsible for false positive TST readings. Indeed false positive TSTs due to NTM are infrequent and mainly relevant in areas with low TB endemicity [32].

4.7. BCG Efficacy. NTM influence the relative efficacy of BCG vaccines [33]. The nature and type of NTM isolated in TB endemic countries are critical to an efficient vaccination campaign [2]. The relative frequency of isolated species may correlate with the prevalence of skin sensitivity to their antigens, as was shown in Malawi [11]. RGMs have been shown to be protective against leprosy and TB [11]. This could not be confirmed in the current study due to the low numbers of NTM isolated.

As there was no unvaccinated control group, it is not possible to assess efficacy of BCG. Thus, it appears that the risk of exposure to NTM as a covariate of vaccine efficacy, as has been previously suggested, is quite low in the target age group.

4.8. Limitations. Our analysis was limited due to the small proportion of NTM isolated in this age group. Nevertheless, it forms a baseline assessment for future studies including future vaccine trials.

Also, not all infants could be tested for NTM; this was not the primary objective, and it is challenging to obtain samples from children without presumptive TB. Therefore, the NTM incidence may be an underestimate of the NTM burden in the population.

5. Conclusions

This study has attempted to document the incidence of NTM among infants thought to have TB. The clinical relevance of NTM isolated points to colonisation and not disease, as all the infants from whom NTM were isolated did not meet the ATS criteria for disease. Our data shows that a patient presenting with features of TB is less likely to have NTM disease, in similar settings.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

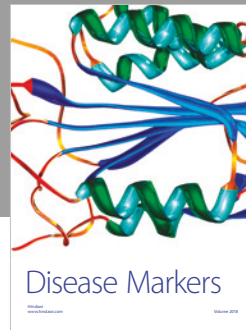
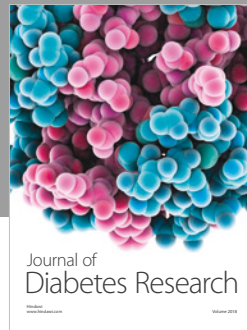
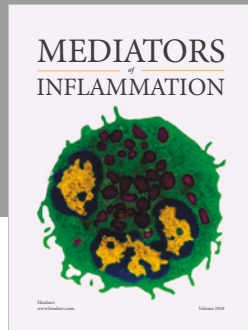
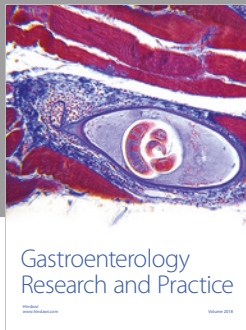
Aeras and European and Developing Countries Clinical Trials' Partnership (EDCTP) [Identifier: IP_07_32080_003] funded the study. Grace Kaguthi and Videlis Nduba were funded by KEMRI and EDCTP for the study and manuscript, Suzanne Verver through KNCV. Grace Kaguthi performed the analysis and wrote the manuscript; Wilfred Murithi wrote the manuscript and performed the laboratory assays. Videlis Nduba, Suzanne Verver, and Grace Kaguthi designed the study, reviewed the manuscript, and approved the final version.

References

- [1] T. P. Primm, C. A. Lucero, and J. O. Falkinham III, "Health impacts of environmental mycobacteria," *Clinical Microbiology Reviews*, vol. 17, no. 1, pp. 98–106, 2004.
- [2] A. O. Jenkins, A. Michel, and V. Rutten, "Original Mycobacterial Sin, a consequence of highly homologous antigens?" *Veterinary Microbiology*, vol. 203, pp. 286–293, 2017.
- [3] M. A. Lake, L. R. Ambrose, M. C. I. Lipman, and D. M. Lowe, "“Why me, why now?” Using clinical immunology and epidemiology to explain who gets nontuberculous mycobacterial infection," *BMC Medicine*, vol. 14, p. 54, 2016.
- [4] A. Kontturi, H. Soini, J. Ollgren, and E. Salo, "Increase in childhood nontuberculous mycobacterial infections after bacille calmette-guerin coverage drop: a nationwide, population-based retrospective study, Finland, 1995–2016," *Clinical Infectious Diseases*, vol. 67, no. 8, pp. 1256–1261, 2018.
- [5] P. Zimmermann, A. Finn, and N. Curtis, "Does BCG vaccination protect against nontuberculous mycobacterial infection? a systematic review and meta-analysis," *The Journal of Infectious Diseases*, vol. 218, no. 5, pp. 679–687, 2018.
- [6] D. P. O'Brien, I. Jeanne, K. Blasdel, M. Avumegah, and E. Athan, "The changing epidemiology worldwide of Mycobacterium ulcerans," *Epidemiology and Infection*, pp. 1–8, 2018.
- [7] M. J. Loftus, E. L. Tay, M. Globan et al., "Epidemiology of buruli ulcer infections, Victoria, Australia, 2011–2016," *Emerging Infectious Diseases*, vol. 24, no. 11, pp. 1988–1997, 2018.
- [8] P. Mangtani, P. Nguipod-Djomo, R. H. Keogh et al., "The duration of protection of school-aged BCG vaccination in England: A population-based case-control study," *International Journal of Epidemiology*, vol. 47, no. 1, pp. 193–201, 2018.
- [9] E. López-Varela, A. L. García-Basteiro, B. Santiago, D. Wagner, J. van Ingen, and B. Kampmann, "Non-tuberculous mycobacteria in children: Muddying the waters of tuberculosis diagnosis," *The Lancet Respiratory Medicine*, vol. 3, no. 3, pp. 244–256, 2015.
- [10] C. Okoi, S. T. B. Anderson, M. Antonio, S. N. Mulwa, F. Gehre, and I. M. O. Adetifa, "Non-tuberculous Mycobacteria isolated from Pulmonary samples in sub-Saharan Africa - A Systematic Review and Meta Analyses," *Scientific Reports*, vol. 7, no. 1, p. 12002, 2017.
- [11] P. E. M. Fine, S. Floyd, J. L. Stanford et al., "Environmental mycobacteria in northern Malawi: implications for the epidemiology of tuberculosis and leprosy," *Epidemiology and Infection*, vol. 126, no. 3, pp. 379–387, 2001.
- [12] P. C. A. M. Buijtsels, M. A. B. van der Sande, C. S. de Graaff et al., "Nontuberculous mycobacteria, Zambia," *Emerging Infectious Diseases*, vol. 15, no. 2, pp. 243–249, 2009.
- [13] M. Hatherill, T. Hawkridge, A. Whitelaw et al., "Isolation of non-tuberculous mycobacteria in children investigated for pulmonary tuberculosis," *PLoS ONE*, vol. 1, p. e21, 2006.
- [14] B. B. Asiimwe, G. B. Bagyenzi, W. Sengooba et al., "Species and genotypic diversity of non-tuberculous mycobacteria isolated from children investigated for pulmonary tuberculosis in rural Uganda," *BMC Infectious Diseases*, vol. 13, p. 88, 2013.
- [15] E. Lopez-Varela, A. L. Garcia-Basteiro, O. J. Augusto et al., "High rates of non-tuberculous mycobacteria isolation in mozambican children with presumptive tuberculosis," *PLoS ONE*, vol. 12, no. 1, Article ID e0169757, 2017.
- [16] A. H. Van't Hoog, K. F. Laserson, W. A. Githui et al., "High prevalence of pulmonary tuberculosis and inadequate case finding in rural Western Kenya," *American Journal of Respiratory and Critical Care Medicine*, vol. 183, no. 9, pp. 1245–1253, 2011.
- [17] J. Waako, S. Verver, A. Wajja et al., "Burden of tuberculosis disease among adolescents in a rural cohort in Eastern Uganda," *BMC Infectious Diseases*, vol. 13, p. 349, 2013.
- [18] M. Claassens, C. van Schalkwyk, L. den Haan et al., "High prevalence of tuberculosis and insufficient case detection in two communities in the western cape, South Africa," *PLoS ONE*, vol. 8, no. 4, Article ID e58689, 2013.
- [19] A. F. Auld, F. Mbofana, R. W. Shiraishi et al., "Incidence and determinants of tuberculosis among adults initiating antiretroviral therapy - Mozambique, 2004–2008," *PLoS ONE*, vol. 8, no. 1, Article ID e54665, 2013.
- [20] B. Workalemahu, S. Berg, W. Tsegaye et al., "Genotype diversity of Mycobacterium isolates from children in Jimma, Ethiopia," *BMC Research Notes*, vol. 6, p. 352, 2013.
- [21] F. O. Odhiambo, K. F. Laserson, M. Sewe et al., "Profile: The KEMRI/CDC health and demographic surveillance system-Western Kenya," *International Journal of Epidemiology*, vol. 41, no. 4, pp. 977–987, 2012.
- [22] G. Kaguthi, V. Nduba, J. Nyokabi, F. Onchiri, R. Gie, and M. Borgdorff, "Chest Radiographs for Pediatric TB Diagnosis: Interrater Agreement and Utility," *Interdisciplinary Perspectives on Infectious Diseases*, vol. 2014, Article ID 291841, 2014.
- [23] D. E. Griffith, T. Aksamit, B. A. Brown-Elliott et al., "An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases," *American Journal of Respiratory and Critical Care Medicine*, vol. 175, no. 4, pp. 367–416, 2007.
- [24] S. Rüsck-Gerdes, G. E. Pfyffer, M. Casal, M. Chadwick, and S. Siddiqi, "Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of Mycobacterium tuberculosis to classical second-line drugs and newer antimicrobials," *Journal of Clinical Microbiology*, vol. 44, no. 3, pp. 688–692, 2006.
- [25] N. E. Nieuwenhuizen, P. S. Kulkarni, U. Shaligram et al., "The recombinant bacille calmette-guerin vaccine VPM1002: ready for clinical efficacy testing," *Frontiers in Immunology*, vol. 8, p. 1147, 2017.
- [26] S. Hamada, Y. Ito, T. Hirai et al., "Impact of industrial structure and soil exposure on the regional variations in pulmonary nontuberculous mycobacterial disease prevalence," *International Journal of Mycobacteriology*, vol. 5, no. 2, pp. 170–176, 2016.
- [27] S. Simons, J. van Ingen, P. Hsueh et al., "Nontuberculous mycobacteria in respiratory tract infections, eastern Asia," *Emerging Infectious Diseases*, vol. 17, no. 3, pp. 343–349, 2011.
- [28] B. Varghese, Z. Memish, N. Abuljadayel, R. Al-Hakeem, F. Alrabiah, and S. A. Al-Hajjo, "Emergence of clinically relevant non-tuberculous mycobacterial infections in Saudi Arabia," *PLOS Neglected Tropical Diseases*, vol. 7, no. 5, Article ID e2234, 2013.
- [29] J. Y. Chien, C. C. Lai, W. H. Sheng, C. J. Yu, and P. R. Hsueh, "Pulmonary infection and colonization with nontuberculous mycobacteria, Taiwan, 2000–2012," *Emerging Infectious Diseases*, vol. 20, no. 8, pp. 1382–1385, 2014.
- [30] S. Ikegame, S. Maki, K. Wakamatsu et al., "Nutritional assessment in patients with pulmonary nontuberculous mycobacteriosis," *Internal Medicine*, vol. 50, no. 21, pp. 2541–2546, 2011.
- [31] M. Fujita and T. Kikuchi, "Immunological background on nontuberculous mycobacteriosis," *Kekkaku*, vol. 88, no. 12, pp. 797–814, 2013.
- [32] M. Farhat, C. Greenaway, M. Pai, and D. Menzies, "False-positive tuberculin skin tests: What is the absolute effect of BCG

and non-tuberculous mycobacteria?" *The International Journal of Tuberculosis and Lung Disease*, vol. 10, no. 11, pp. 1192–1204, 2006.

- [33] G. F. Black, R. E. Weir, S. Floyd et al., "BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: Two randomised controlled studies," *The Lancet*, vol. 359, no. 9315, pp. 1393–1401, 2002.



Hindawi

Submit your manuscripts at
www.hindawi.com

