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Prevalence and Drug Susceptibility of *Non-tuberculous mycobacteria* Among Presumptive MDR-TB Patients Admitted at KIDH in Kilimanjaro, Tanzania

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Abstract

Background

The reports on *Non-tuberculous mycobacteria* (NTM) pulmonary infections associated with multidrug-resistant tuberculosis are increasing worldwide. Despite a significant increase in knowledge about NTM infections, they present a diagnostic and therapeutic challenge in resource-limited settings. This study aimed to determine the prevalence and drug susceptibility of NTM among presumptive multidrug-resistant tuberculosis patients in Tanzania.

Materials and Methods

A cross-sectional study was conducted from April to October 2021 on presumptive Multidrug-resistant tuberculosis patients admitted at KIDH in Kilimanjaro. All isolated mycobacterial growth was characterized into MTBC and NTM and finally tested for susceptibility on the first-line anti-tuberculosis drugs. The data were analyzed using Stata version 16 (Stata Corp, College Station, TX, USA), and presented using frequency and tables. A p-value less $\leq$ 0.05 was considered statistically significant.

Results

The study population included 160 presumptive MDR-TB patients and 75% were males, NTM infections were found in 14 (8.75 %). In 17 patients that were HIV positive, 13 were infected by NTM, the *Mycobacterium avium complex* was the most frequently isolated NTM (9/14 patients, 64.3 %). Almost all isolates of NTM were resistant to majority of the first-line anti TB drugs.

Vol. 6, No. 03; 2022

ISSN: 2581-3366

Conclusion

Findings from this study indicate that HIV infected individuals who were regarded as presumptive MDR-TB patients are at high risk of NTM infections. High proportion of NTM isolates were MAC, which included *M. avium* and *M. in tracellulare*. Additionally, NTM isolates showed to be resistant to majority of the recommended first-line anti-TB drugs used in the country indicating challenging treatment outcome.

Keywords: *Non-tuberculous mycobacteria*, anti TB drugs, HIV, immune compromised, immune competent.

Introduction

The *Non-tuberculous mycobacteria* (NTM) are environment organisms commonly isolated from air, soil, water, plants, foods, and milk product (1,2). NTM are mycobacteria other than *Mycobacterium tuberculosis* complex (MTBC) and *Mycobacterium leprae* that cause human TB and leprosy, respectively, that remain a major public health problem worldwide (3). A substantial increase in the number of pulmonary NTM infections among immune compromised individuals living with HIV/AIDS has been reported worldwide (4). However, due to improved molecular typing of Mycobacterial species, an increase of reported NTM isolations among immune competent individuals has been reported in countries with a low incidence of TB (5,6).To date, epidemiological reports on NTM infections are still limited in resource-limited countries like Tanzania.

According to the report by World Health Organization (WHO), it was estimated that in 2020 a total of 9.9 million people fell ill from TB, among them 1.5 million died and 214,000 were co-infected with HIV. Furthermore, it was anticipated that during the 2021 and 2022 periods there will be an increase in the number of people at risk of dying of TB due to the COVID-19 pandemic (7). Tanzania is among the 22 countries with a high TB burden globally (8) and a pandemic with HIV. It was estimated in 2020 that the incidence of TB cases was 222 per 100,000 population and the number of individuals who were TB/HIV co-infected was 47 per 100,000 population. HIV prevalence was estimated to be 5% in Tanzania and 22% knew their HIV serostatus(7).

Diagnosis for the NTM infections remains a challenge in resource-limited countries where direct sputum smear microscopy for acid fast bacilli (AFB) and Gene Xpert in some countries have remained the main diagnostic methods (9). Absence of rapid and correct methods to identify AFB positive pulmonary infections due to NTM ends up in misidentified and mishandling of pulmonary TB in such situations. Accurate and appropriate identification of NTM is mostly urgent for both treatment and epidemiology, since infections with different mycobacterial species requires different handling methods(10). The identification of NTM requires molecular methods that are not widely available (11). Isolation and antimicrobial susceptibility testing of NTM from biological materials are difficult or not done, which brings up a challenge for the treatment of NTM infections. Consequently, patients with direct sputum smear samples testing

Vol. 6, No. 03; 2022

ISSN: 2581-3366

microscopically positive for AFB have undoubtedly been regarded as MTBC infections and received unnecessary empirical anti-TB treatment (12). For the first time Malawi attempted to quantify NTM prevalence in assumed MTBC cases in 2020 (13), it means in the past time, all patients were treated with empirical anti TB drugs. TB patients including those of unrecognized NTM receiving and failing first-line anti-TB drugs based on National TB and Leprosy Programme (NTLP) guidelines are regarded as presumptive MDR-TB. The information on the resistance profile for the NTM species from reported cases from developed countries with low TB incidences has shown poor response to treatment and frequent relapses occurring after stopping the treatment (14). The previous reports on the prevalence of NTM in the country indicated diverse isolates were found among humans, livestock and wildlife, in the Serengeti ecosystem, (15-18). The present study aimed to determine the prevalence and drug susceptibility of NTM isolates suspected to be MDR-TB in Tanzania.

Materials and methods

The cross-sectional hospital-based study was conducted from April to October 2021at Kibong`o to Infectious Disease Hospital (KIDH), a national referral hospital for patients failing first-line anti-TB drugs, presumptive MDR-TB, in the country. The hospital has a public health laboratory supported by East African Public Health Laboratory Networks through the World Bank Scheme.

Population of the study

All patients with clinical signs and symptoms suggestive of MDR-TB (prolonged cough, patient not responding to first line anti TB etc) who were -referred to KIDH were eligible for the study. Demographic information: age, gender, occupation, education were collected only after provision of informed consent. A careful cross examination of patient history for previous anti-TB treatment using a structured questionnaire was used to classify patients as ‘failure’, ‘relapse’ or ‘defaulter’ TB cases. A patient was considered as treatment failure if a patient is a sputum smear or culture positive at 5 months or later during treatment, also a patient is considered as Defaulter if a patient whose treatment was interrupted for 2 consecutive months or more and a patient is considered as Relapse if a patient have previously been treated for TB, were declared cured or treatment completed at the end of their most recent course of treatment, and are now diagnosed with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection). Presumptive TB refers to a patient who presents with symptoms or signs suggestive of TB (previously known as a TB suspect)(16).

Sampling method, data collection procedure and sample collection

We used a census study approach method whereby all reports were considered and we determined the sample size by using the Kish and Leslie formula(17) and the minimum sample size for the study found to be 185.

A piloted structured questionnaire was used to collect the demographic and clinical profiles of the study participants including the HIV serostatus of the study participants. The data were collected by trained clinicians working in the hospital. Study participants without known HIV serostatus were counselled and tested following the National comprehensive guidelines on HIV

Vol. 6, No. 03; 2022

ISSN: 2581-3366

testing services (18). Two sputum samples, one spot during enrolment and the second early next morning were collected from each of 185 enrolled presumptive MDR-TB patients aged 18 years and above admitted at KIDH. Direct sputum smear microscopy using Ziehl-Nielsen (ZN) stain was conducted and microscopy was performed by competent Laboratory Technologists following the NTLP guidelines (19).

MGIT 1st line antibiotic susceptibility test.

Culture and drug susceptibility testing were conducted by using automated liquid culture on Bactec MGIT™ 960 (Becton Dickinson, Maryland, USA). Briefly, susceptibility testing in the MGIT 960 system is centred on the similar principles as isolation from sputum (detection of growth). DST is done using an AST (antibiotic susceptibility testing) set and TB exit, which comprises of a Growth Control tube and one tube for every drug. A known concentration of a drug was added to the MGIT tube, along with the specimen, and growth was compared with a drug-free control of the similar specimen. The indorsed critical concentration of each drug were: isoniazid (INH) 0.1µg/ ml, rifampicin (RIF) 1.0 µg/ml, streptomycin (STR) 2.0 µg/ml, ethambutol (EMB) 5.0µg/ml and pyrazinamide (PZA) 100 µg/ml. If the drug was active against the mycobacterial isolate, growth were inhibited and fluorescence were suppressed in the drug-containing tube; in the meantime, the drug-free control grow and show increasing fluorescence. If the isolate was resistant, growth and its corresponding increase in fluorescence were evident in both the drug-containing and the drug-free tube. The MGIT 960 system monitors those growth patterns and automatically interpret results as susceptible or resistant. An isolate was defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug (20).

SD Bio line TB Antigen MPT64 Rapid test assay

Identification of NTM was performed by using an immune-chromatographic assay, SD Bioline TB Ag MPT64®(Standard Diagnostics, Yongin-si, Gyeonggi-do, Republic of Korea)assay was performed by following manufacturer instructions (21). Briefly, MTBC species secrete MPT64 protein that is detected by SD Bioline assay and NTM is non-reactive. AFB positive liquid cultures were used and for each sample, a total of 100 µL were placed in sample wells of the test device and incubated at room temperature for 15 min. The test results were visually read based on colour development. A positive result for MTBC was indicated by a red band on the test window of the device in addition to the control band. Standard reference strain MTBC H_{37}Rv ATCC 27294 was used as a positive control.

GenoType CM/AS assay

NTM speciation was performed on AFB positive liquid cultures that were subjected to definitive speciation by performing GenoType Mycobacterium CM/AS(Hain Life science, Nehren, Germany) assay according to the manufacturer's instructions(22). About 1 ml of the AFB positive liquid culture was centrifuged for 15 min in a standard tabletop centrifuge with an aerosol tight rotor at approximately 10,000xg. The mycobacterial pellet was used for DNA extraction and PCR was performed using primers included in the Primer Nucleotide Mix (PNM)

Vol. 6, No. 03; 2022

ISSN: 2581-3366

provided with the kit for the amplification of specific regions of the mycobacterial chromosome. The DNA amplificates were incubated with the membrane strips coated with specific probes complementary to the amplified nucleic acids for hybridization. Each strip contains three bands Conjugate Control zone (CC) Universal Control zone (UC) and Genus Control zone (GC) for quality control to control the correct performance of the test and the proper functioning of kit constituents. The developed strips were aligned in the designated fields by aligning the bands' CC and UC with the respective lines on the sheet. An evaluation sheet included in the kit was used to determine species with the help of the interpretation chart.

Ethical Consideration

The identification numbers were used and confidentiality was observed in all the patient's information. Permission to conduct the present study was issued by KIDH and an ethical clearance Certificate was obtained from the Kilimanjaro Christian Medical University College Research and Ethical Review Committee (CRERC).

Statistical analysis

Data were collected, entered, and analyzed using Stata version 16 (Stata Corp, College Station, TX, USA). Data were cleaned and checked for completeness Data were presented in form of frequencies, percentages, and tables. A p-value less than 0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics of Presumptive MDR-TB Patients Admitted at KIDH.

A total of 185 presumptive MDR-TB patients with ages ranging from 22 to 55 years and a mean age of 36 years admitted at KIDH were enrolled. All samples were subjected to liquid culture, 25(13.5%) samples didn`t grow, thus we remained with 160 samples. Majority were males (75%), with primary education level (80%) and failures to first-line drugs (82.5%). A total of 152 (95%) participants were AFB positive on direct sputum microscopy. Seventeen (10.6%) participants were HIV sero positive and among them, 13 (76.5 %) were infected by NTM, and 4 (23.5%) were infected by MTBC. Majority of the isolates from 160 presumptive MDR-TB patients were MTBC (91.25%) and the remaining were NTM (8.75%) isolates, all from failures to first line anti-TB drugs. The pastoralist were predominant (64%) among presumptive MDR-TB patients who were infected by NTM. All the patients were categorized in three groups: failures, defaulters and relapse (Table 1).

Vol. 6, No. 03; 2022

ISSN: 2581-3366

Table 1: Demographic Characteristics of Presumptive MDR-TB Patients infected by MTBC and NTM admitted at KIDH. N= 160

Variable	Attribute	MTBC n=146 (91.25%)	NTM n=14 (8.75%)	TOTAL (100%)
Gender	Male	110 (68.75%)	10 (6.25%)	120(75%)
	Female	36 (22.5%)	4 (2.5%)	40(25%)
Age	22 - 35	74 (46.25%)	10 (6.25%)	84(52.5%)
	36 - 45	55 (34.37%)	2 (1.25%)	57(35.63%)
	46 - 55	17 (10.63%)	2 (1.25%)	19(11.87)
Level of Education	No formal education	2 (1.25%)	2 (1.25%)	4(2.5%)
	primary school education	116 (72.5%)	12 (7.5%)	128(80.0%)
	Secondary school education	18 (11.25%)	0	18(11.25%)
	Tertiary education	10 (6.25%)	0	10(6.25%)
HIV status	Positive	4 (2.5%)	13 (8.13%)	17(10.62%)
	Negative	142 (88.75%)	1 (0.62%)	143(89.38%)
Occupation	Laborers	54 (33.75%)	1 (0.63%)	55(34.37%)
	Peasants	40 (25%)	4 (2.5%)	44(27.5%)
	Mining	14 (8.75%)	0	14(8.75%)
	Pastoralists	4 (2.5%)	9 (5.63%)	13(8.13%)
	Business	11 (6.88%)	0	11(6.88%)
	Primary school teachers	6 (3.75%)	0	6(3.75%)
	Secondary school teachers	3 (1.87%)	0	3(1.88%)
	Taxi drivers	5 (3.1%)	0	5 (3.1%)
	Mining brokers	4 (2.5%)	0	4 (2.5%)
	Gate keeper	1 (0.63%)	0	1 (0.63%)
	Barmaid	3 (1.88%)	0	3 (1.88%)
	Office secretary	1 (0.63%)	0	1 (0.63%)
Case types	Failures	118 (73.75%)	14(8.75%)	132(82.5%)
	Relapse	22 (13.75%)	0	22 (13.75%)
	Defaulters	6 (3.75%)	0	6 (3.75%)

Vol. 6, No. 03; 2022

ISSN: 2581-3366

All 14 NTM isolates were subjected to GenoType Mycobacterium CM/AS assay for typing to species level and we successfully identified *M. avium*7 (50%), *M. kansasii*3 (21.4%), *M. fortuitum*2 (14.3%) and *M.intracellulare*2 (14.3%).*M. avium and M. intracellulare* compose a *Mycobacterium avium complex* (MAC) group.

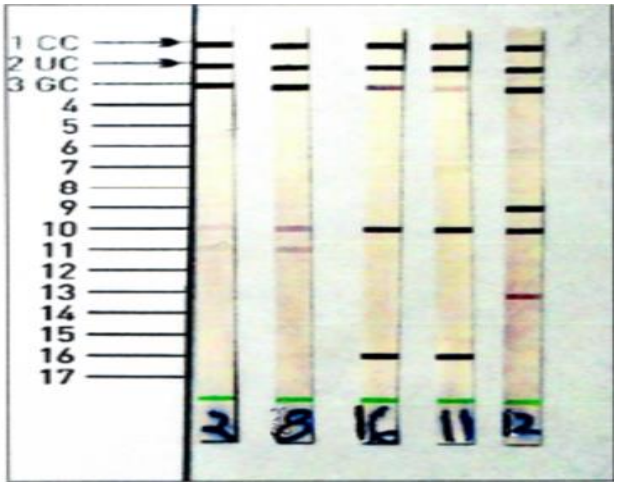

Figure 1: GenoType® CM/AS assay for rapid differentiation of different species of Myco bacteria. Strain number 2, 8, 12 were identified as NTM and 16, 11 as MTBC by GenoType® Mycobacterium CM/AS

Susceptibility testing was reconducted on the 14 NTM on the five first-line anti- TB drugs and found 10 (6.25%) were resistant to RIF, 8 (5%) were resistant to INH, 9 (5.63%) were resistant to EMB and all NTM species were resistant to PZA, however, streptomycin was sensitive to all NTM species. (Table2).

Table 2: Presumptive MDR-TB Anti-TB Drug Susceptibility testing pattern N=160

	MTBC (n = 146)		**NTM (n = 14)**		
Anti-TB drug	**Resistant n (%)**	**Susceptible n (%)**	**Resistant n (%)**	**Susceptible n (%)**	**p-value**
RIF	120 (75%)	26 (16.25%)	10 (6, 25%)	4(2.5%)	0.659
INH	134 (83.75)	12 (7.5%)	8 (5%)	6(3.75%)	<0.001
EMB	136 (85%)	10 (6.25%)	9 (5.63%)	5(3.12%)	<0.001
STR	89 (55.62%)	57 (35.63%)	0	14(8.75%)	<0.001
PZA	145 (90.63%)	1 (0.62%)	14(8.75%)	0	0.756

Key: RIF – Rifampicin, INH – Isoniazid, Emb – Ethambutal, STR – Streptomycin, and PZA - Pyrazinamide

Vol. 6, No. 03; 2022

ISSN: 2581-3366

Discussion

Pulmonary infections resembling TB caused by NTM infections are increasingly reported in HIV endemic regions and those with improved laboratory diagnostic facilities(23). In resource limited settings, pulmonary infections with AFB positive direct sputum microscopy results are regarded as MTBC infections. Reports from regions with advanced laboratory diagnostic services indicate that most of the NTM species are not susceptible to anti-tuberculous drugs and when patients are infected by these species show failure on first-line anti-tuberculous drugs. There is less awareness and attention on NTM infections among clinicians in resource-limited setting and hence missed during diagnosis because they are regarded as presumptive MDR-TB. Additionally NTM infections are not reportable in any country hence awareness is lacking among treating physicians and microbiologists and due to this situation, NTM is recognized in late stage whereby infection with NTM is not considered until late in the course of disease(24), also Laboratory infrastructure is lacking for culture and identification of NTM in many countries (25). High burden of TB and HIV attracts the bulk of the attention of the health care system; governmental fiscal inputs toward the costs of these neglected infections continue to be neglected, likewise, standardized or accepted criteria to define NTM respiratory disease are lacking (25). According to the report on NTM infection, it is indicated to be the main source of TB like syndrome in people suffering from pulmonary infection and have been documented globally (4,5,26). In the absence of culture and species identification capacity, pulmonary NTM infection is possible to be mistaken as pulmonary TB infection based on AFB microscopy(27). NTM ailments share clinical signs and symptoms with TB, causing a clinical dilemma with a concern to therapy for patients (25). In parts of the world where infection by acid-fast bacilli could be due to either *M. tuberculosis* or NTM and due to the need to establish proper public health and chemotherapeutic measures immediately, the causative agent must be known. The present study has shown that among presumptive MDR-TB suspects, the prevalence of NTM infections was 8.75% (Table 1), This finding indicates that, the 8.75% of NTM patients were classified and treated as TB cases using anti TB drugs as a result they showed unfavourable response to anti TB therapy, hence they were referred to a referral hospital as presumptive MDR-TB patients. In this study MAC was predominant (9/14), this is in agreement with previous report(28).

A considerable number of patients with NTM infections in the high TB burden countries and HIV pandemic with limited diagnostic capabilities are incorrectly diagnosed with TB, leading to incorrect treatment management using anti-TB which do not respond, thus may end up with increased morbidity and mortality (29). All the study participants who were infected by NTM species and did not respond to the first-line anti-TB drugs, finally were regarded as presumptive MDR-TB patients. Among 14 patients infected with NTM 13 were HIV infected, which indicate that in regions with high HIV prevalence these patients are at high risk of the infections and may be contributing to the HIV/TB co-infections.

A number of predisposing factors for NTM infections are known, such as older age and immune suppressive conditions. Additionally, sufferer with acquired immunodeficiency syndromes

Vol. 6, No. 03; 2022

ISSN: 2581-3366

including AIDS and haematological malignancies, hairy cell leukaemia in particular, are also identified as susceptible to NTM infection(30). The latter groups of patients however, usually develop disseminated NTM infection (DNTM) preferably than isolated pulmonary NTM infection (PNTM) which is noticed in patients with structural lung disease and are considered as a separate risk group. NTM are opportunistic organisms, when the person is weak, malnourished, with lung problem is high risk for NTM infection(5).

The susceptibility testing of the NTM showed large proportions of resistant to the five first-line anti TB drugs and even other drugs such as PZA were completely resistant to all isolated NTM in our study. A large proportion of MTBC isolates were resistant to PZA as well and this demonstrates the rethinking of including PZA in the TB treatment regimens and the need for the improvement of the laboratory facilities to be able to perform susceptibility testing and type mycobacterial isolates to species level for better management and control of the infectious NTM. The MPT 64 test was easy to perform, quite inexpensive, and rapid to be afforded in laboratories with routine TB liquid culture. It is there fo re, imperative in low-resource settings, where improved diagnostics are available such as referral laboratories attending specimens from presumptive MDR-TB patients to establish and include routinely the MPT 64 test for valuable impact on patient management and outcome.

Conclusion

Our findings from this study showed a high prevalence of NTM especially among HIV infected individuals who were regarded as presumptive MDR-TB patients. High proportion of NTM isolates were MAC, which included *M. avium and M. intracellulare.* Additionally, NTM isolates showed to be resistant to majority of the recommended first-line anti-TB drugs used in the country indicating poor treatment out come. Future studies should focus on the non-culture methods for the identification and speciation of NTM as well as susceptibility testing for accurate and timely treatment of individuals at high risk of NTM infection.

Conflict of Interests

None declared.

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