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RESEARCH ARTICLE

CHARACTERIZATION OF MYCOBACTERIAL ISOLATES USING BIOLINE TB AGMPT64 ASSAY AT CENTRAL TUBERCULOSIS REFERENCE LABORATORY IN DAR ES SALAAM, TANZANIA.

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ABSTRACT

Background: Mycobacterium other than tuberculosis (MOTT) can cause lung diseases with clinical and radiological features similar to the Mycobacterium tuberculosis complex, (MTBC) leading to potential misdiagnosis in the absence of specific diagnostic tests. This study aimed to identify MOTT among Mycobacterial culture isolates using the Bioline TB AgMPT64 assay at the Central Tuberculosis Reference Laboratory in Dar es Salaam. ***Methods:*** This retrospective cross-sectional study was conducted at Central Tuberculosis Reference Laboratory. We analyzed Mycobacterial isolates to distinguish MOTT from MTBC using the Bioline TB AgMPT64 assay, which detects the secreted Mycobacterium Tuberculosis protein 64 antigen. ***Results:*** A total of 150 Mycobacterial isolates were characterized and of these, 92% were identified as Mycobacterium tuberculosis complex, while 8% were classified as Mycobacteria Other Than Tuberculosis. Among the MOTT isolates, 35% were found among People living with HIV. Furthermore, none of the MOTT isolates were detected by the GeneXpert/Ultra diagnostic assay. A significant association was found between HIV status and the occurrence of MOTT, with a p-value of 0.001. ***Conclusion:*** This study has shown that a significant proportion of MOTT isolates are misidentified as MOTT, which may impact the treatment outcome, and management of the patients infected with these strains.

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INTRODUCTION

Tuberculosis is a major public health problem worldwide, mainly causing high morbidity and mortality in developing countries. According to the World Health Organization (WHO) the pandemic scope of the disease is associated with low income, low immune status, and malnutrition. Additionally, lack of diagnostic capabilities causes an increase in transmissions in lower and middle-income countries (1). Mycobacteria that cause tuberculosis are called *Mycobacterium tuberculosis* complex, which includes *Mycobacterium africanum*, *Mycobacterium bovis*, and *Mycobacterium tuberculosis* while other Mycobacteria strains that do not cause tuberculosis are known as non-tuberculous Mycobacteria (NTM) or Mycobacterium other than tuberculosis (MOTT) that include *Mycobacterium xenopi*, *Mycobacterium fortuitum complex, Mycobacterium kansasii* and *Mycobacterium abscessus* (2). Several studies have shown the proportions of MTBC and MOTT differ across various geographic regions, including those with high and low TB burdens. According to the Indian report, MOTT and MTBC proportion were 6.4% and 93.6%, respectively, compared to 4.2% and 95.8% in the Indonesia report (3,4). The MOTT was reported to be high at 10.3% and *Mycobacterium tuberculosis* complex was reported to be present at 89.6% in Ethiopia (5). WHO recommends the use of culture for diagnosis due to its accuracy in the growth of the MTBC strains, however, it takes a longer time to grow and is not readily available in many limited resource settings. Furthermore, when growth occurs, it requires discrimination to distinguish MTBC from MOTT (6). Bioline TB AgMPT64 test was developed for the discrimination of MTBC from MOTT grown on the culture medium with a sensitivity of 98% and specificity of 100% (7,8). MTBC are known to secrete more than 33 different proteins, one of these proteins is MPT64. It is a protein antigen secreted by the MTBC, which has a role in the inhibition of apoptosis of macrophages in vitro by up-regulation of Bcl-2, the involvement of increased miRNA-21, and the control of the transcription factor NF-kB (7). It is a protein secreted by only strains of MTBC and not found in other Mycobacteria (9).

Therefore, Bioline TB Ag MPT64 test an immunological assay that specifically detects the MPT64 protein antigen secreted by MTBC (10). Mycobacterium Other Than Tuberculosis are found in different environments and have been drastically causing TB-like infections worldwide that are difficult to diagnose and treat (2,11). Due to heavy reliance on direct sputum smear microscopy for the diagnosis of TB in limited resource settings, the diagnosis of MOTT is frequently missed (12). MOTT discrimination from MTBC on culture isolates is important because false identification as MTBC can even be misdiagnosed as multidrug-resistant TB (MDR TB) when discrimination is not available (13). However, it has been reported in Iran that among those classified as multidrug-resistant tuberculosis patients, 30% were identified as patients infected by MOTT, this shows that patients who were known to have multidrug-resistant tuberculosis had infections with other mycobacteria (14). Therefore, determination of MOTT frequency is important, since, it ensures better treatment management to the patient because some non-tuberculous Mycobacteria usually do not respond to anti-tuberculous medication. Identifying MOTT will help in reducing the number of hospitalization days, lowering healthcare costs and aiding in the control of transmissions.

MATERIALS AND METHODS

Study Design and Study Site: The study was a retrospective cross-sectional hospital-based study that was conducted on the archived Mycobacterial isolates from January to May 2023 at the Central Tuberculosis Reference Laboratory (CTRL in Dar es Salaam). With all the Mycobacterial isolates, the corresponding demographic information, GeneXpert test results and HIV status were collected.

Data collection: A laboratory request form was used to collect Bioline TB AgMPT64 assay results and GeneXpert results from January to May 2023.

Internal quality control check: Bioline TB AgMPT64 test device has letters T and C on the surface of the cassette as "Test line" and "Control line" respectively. Both the "Test line" and "Control line" are not visible before applying any sample. The control line is used for procedural control, and it shows that the diluent has been applied successfully and that the active ingredients of the main components on the strip were still functional.

Data analysis: The data obtained was entered into Excel based on the identification number of the sample size of the Mycobacterial isolates. Data was cleaned, summarized, and analyzed using SPSS version 20. A descriptive statistic was done where categorical variables were summarized using frequency and proportion. Numerical variables were summarized using the mean and their respective measures of dispersion. The results were presented using tables, and simple frequencies were run to determine the socio-demographic variables of the patient whose Mycobacterial isolates were used

RESULTS

Characteristics of the patients corresponding to the Mycobacterial isolates with their results: A total of 150 Mycobacterial isolates were characterized in the present study and majority were isolated from 119 (79%) e males, and 20 (13%) were among people living with HIV. The mean age was 38 years (SD±15) and the majority 90 (60%) of the patients were aged between 18-36 years group (Table 1). The proportion of MOTT among patients aged 40-59 was 16% and the proportion of MOTT among females was observed to be 13%. Also, among people living with HIV, the proportion of MOTT was 35% (Table 1).

Table 1. Demographic characteristics of the 150 mycobacterial isolates at CTRL in Dar es Salaam

Variable	Frequency n (%)	MTBC n (%)	MOTT n (%)	GeneXpert n (%)		P-value
Age Group				**Positive**	**Negative**	
18-39	90(60)	85(94)	5(**6**)	85(94)	5(6)	0.063
40-59	45(30)	38(84)	7(**16**)	36(80)	9(20)	
> 60	15(10)	15(100)	0(0)	15(100)	0(0)	
Mean(±SD)	38 (±15)					
Gender						
Male	119(79)	111(93)	8(**7**)	110(92)	9(8)	0.259
Female	31(21)	27(87)	4(**13**)	26(84)	5(16)	
HIV status						
Negative	130(87)	125(96)	5(**4**)	123(95)	7(5)	<0.001
Positive	20 (13)	13(65)	7(**35**)	13(65)	7(35)	
Total	150	138	12	136	14	

Bioline TB Ag MPT64 test on Mycobacterial Isolates: A total of 150 Mycobacterial isolates archived from January to May 2023 were included in the study. All 150 Mycobacterial isolates were subjected to Bioline TB AgMPT64 assay and 138 (92%) of the Mycobacterial isolates were identified as MTBC while 12 (8%) of the isolates were identified as MOTT (Table 2).

Table 2. Bioline TB Ag MPT64 characterization of 150 Mycobacterial isolates at CTRL in Dar es Salaam.

Mycobacterial isolates - n (%)	**GeneXpert n (%)**		
	Positive	**Negative**	**Total**
MOTT - 12 (8)	0 (0)	**12 (100)**	12
MTBC-138(92)	**136(98.5)**	2 (1.5)	138
Total (100)	136	14	150

GeneXpert results on MTBC and MOTT characterized among the 150 Mycobacterial isolates: A total of 14 samples were negative by GeneXpert, 12 were identified as MOTT and 2 were MTBC. Similarly, out of 138 MTBC isolates, 136 were positive on the GeneXpert assay, while two were negative. All 12 MOTT isolates were negative on GeneXpert assay (Table 2).

DISCUSSION

This study revealed that a significant proportion of Mycobacterial isolates initially identified as MTBC were MOTT, leading to a misdiagnosis of TB. Using the Bioline TB AgMPT64 test to characterize archived Mycobacterial isolates at CTRL, the results showed that 92% were MTBC and 8% were MOTT. The findings highlight those common diagnostic methods available in limited resource settings, such as direct sputum microscopy, culture on LJ medium, and the GeneXpert test for TB, successfully identify acid-fast bacilli and mycobacterial cultures. However, these methods may not exclusively indicate MTBC infections, as in some cases involving MOTT infections. Nonetheless, some MOTT patients were treated according to routine TB management guidelines which may lead to drug resistance. The observed

frequency of MOTT in this study is consistent with a study conducted in South America, which reported a similar proportion (15), but lower than studies in India, which reported the proportion of MOTT to be 31.1% (16) and 12.5% (17). These differences may be attributed to variations in environmental factors and the timing of the studies. Our findings suggest that non-tuberculous mycobacterial infections are relatively less prevalent in limited resource settings, but they have significant implications for healthcare economics, epidemiology, antimicrobial resistance, and patient health improvement. The burden of tuberculosis caused by MTBC has not yet declined, possibly due to delayed diagnoses, misdiagnoses associated with inadequate diagnostic tools, and delays in culture results, which contribute to increased transmission, particularly among HIV-infected individuals (18,19). In this study, MOTT infections were found in 35% among people living with HIV, a higher proportion than a study in Bahrain, Asia, which reported MOTT in only 2.5% of HIV patients (20). These discrepancies may be due to differences in the timing and location of data collection. Therefore, it is essential to consider MOTT infections among people living with HIV when AFB and culture-positive results are obtained from their sputum samples. Additionally, our study found that 1.5% of MTBC isolates were not detected by GeneXpert, potentially due to low bacilli levels or contamination. Furthermore, all MOTT isolates in our study were undetected by GeneXpert, these results are consistent with another study in India, likely due to GeneXpert's specificity for detecting MTB-specific DNA (21). This presents a challenge in controlling the transmission of MOTT, which could be misdiagnosed when not isolated on LJ medium. Policymakers must consider including routine MOTT screening among Mycobacterial culture isolates in national TB diagnostic guidelines. The study involved a relatively small sample size which may limit generalizability. Future studies involving a larger sample size should be conducted.

CONCLUSION

This study has shown that a significant proportion of MOTT isolates are misidentified as MTBC, which may impact the treatment, and management of the patients infected with these strains. Likewise, MOTT infections are more prevalent among people living with HIV/AIDS.

RECOMMENDATION

We recommended further additional diagnostic techniques for identifying MOTT and consider incorporating MOTT screening into routine patient screening.

Ethical Considerations: Ethical clearance for the study was granted by the KCMUCo Research Ethics Committee with the registration number PG 182/2023.

Competing Interest: The author declares no conflict of interest

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Abbreviations

AFB	Acid-fast bacillus
Ag	Antigen
Bcl-2	B-cell lymphoma
CTRL	Central Tuberculosis Reference Laboratory
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
KCMC	Kilimanjaro Christian Medical Centre
LJ medium	Lowenstein-Jensen medium
miRNA21	mammalian microRNA
MNH	Muhimbili National Hospital
MPT64	Mycobacterium Tuberculosis Protein 64
MOTT	Mycobacteria other than tuberculosis
MTBC	*Mycobacteria Tuberculosis* complex
NTM	Non -Tuberculous Mycobacteria
NF-kB	Nuclear factor kappa light chain enhancer of activated B cell
PCR	Polymerase Chain Reaction
PG	Post Graduate
QC	Quality Control
RIF	Rifampicin
SPSS	Statistical package for the social sciences
TB	Tuberculosis
WHO	World Health Organization
ZN	Ziehl Nielsen

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