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TB or not TB? Definitive determination of species within the *Mycobacterium tuberculosis* complex in unprocessed sputum from adults with presumed multidrug-resistant tuberculosis

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Abstract

OBJECTIVES Differences among *Mycobacterium tuberculosis* complex (MTC) species may predict drug resistance or treatment success. Thus, we optimised and deployed the genotype MTBC assay (gMTBC) to identify MTC to the species level, and then performed comparative genotypic drug-susceptibility testing to anti-tuberculosis drugs from direct sputum of patients with presumed multidrug-resistant tuberculosis (MDR-TB) by the MTBDRplus/sl reference method.
METHODS Patients with positive Xpert® MTB/RIF (Xpert) results were consented to provide early-morning-sputum for testing by the gMTBC and the reference MTBDRplus/sl. Chi-square or Fisher's exact test compared proportions. Modified Poisson regression modelled detection of MTC by gMTBC.
RESULTS Among 73 patients, 53 (73%) were male and had a mean age of 43 (95% CI; 40–45) years. In total, 34 (47%), 36 (49%) and 38 (55%) had positive gMTBC, culture and MTBDR respectively. Forty patients (55%) had low quantity MTC by Xpert, including 31 (78%) with a negative culture. gMTBC was more likely to be positive in patients with chest cavity 4.18 (1.31–13.32, $P = 0.016$), high-quantity MTC by Xpert 3.03 (1.35–6.82, $P = 0.007$) and sputum smear positivity 1.93 (1.19–3.14, $P = 0.008$). The accuracy of gMTBC in detecting MTC was 95% (95% CI; 86–98; $\kappa = 0.89$) compared to MTBDRplus/sl. All *M. tuberculosis/canettii* identified by gMTB were susceptible to fluoroquinolone and aminoglycosides/capreomycin.
CONCLUSIONS The concordance between the gMTBC assay and MTBDRplus/sl in detecting MTC was high but lagged behind the yield of Xpert MTB/RIF. All *M. tuberculosis/canettii* were susceptible to fluoroquinolones, a core drug in MDR-TB treatment regimens.

keywords genotype MTBC, *M. tuberculosis* complex species, multidrug-resistant, sputum

Sustainable Development Goal: Good health and well-being

Introduction

Tuberculosis (TB) is a treatable disease yet is routinely the leading cause of mortality and morbidity from a single infectious pathogen worldwide [1]. TB in human and animals is caused by the species of the *Mycobacterium tuberculosis* complex (MTC), including *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. caprae*, *M. microti*, *M. pinnipedii*, *M. canettii* and *M. mungi* [2]. Previous comparative genomic studies for MTC have described important variations among species in the complex with differences in host association, drug resistance, virulence and epitope diversity [3]. Unfortunately, these studies are predominantly done in countries where TB burden is low but have high capacity for genomic testing and bioinformatics analyses [4]. This may partly limit the understanding of the global distribution of MTC species.

Tanzania is among the top 30 countries with high burden of TB in the world, accounting for 253 cases per 100 000 population incident rate, but a low rifampicin

and/or multidrug-resistant (R/MDR)-TB burden [5]. For instance, in 2018, an estimated 3.4% of new and 18% of previously treated patients for drug-sensitive (DS)-TB had R/MDR-TB globally compared to 1.1% and 3.9% respectively in Tanzania [5]. Of the MTC species, *M. tuberculosis* is the commonest causative agent for TB in humans globally. The *M. africanum* is historically predominant in West African countries like Ghana and Senegal [6]. In the past 20 years, one cross-sectional study reported *M. bovis* as the causative pathogen in 10% of patients with DS-TB in Tanzania [7], but there have been few or no further data, particularly in patients with R/MDR-TB. Unlike most strains of *M. tuberculosis*, lack of the pyrazinamidase enzyme in *M. bovis* leads to natural resistance to pyrazinamide, a key sterilising anti-TB drug for treating patients with DS- and R/MDR-TB [8]. Likewise, a study from West Africa showed that isoniazid resistance due to *katG* S315T mutation is more frequent in *M. tuberculosis* than *M. africanum* [6]. Furthermore, independent of drug resistance, certain species of MTC appear to respond more readily to anti-TB treatment than others [9]. Even with the alarming global trend of drug-resistant TB, it is scarcely known whether *M. tuberculosis*, or *M. bovis* or *M. africanum* causes R/MDR-TB. This information is not only important for tracking MTC species circulating in the region, but also in guiding clinical decisions in patients with R/MDR-TB.

The current molecular methods like Xpert® MTB/RIF (Cepheid, USA) and the line probe assays (LPA, Hain LifeScience, Germany) such as the genotype MTBDRplus and MTBDRsl have been endorsed by the World Health Organization (WHO) for detecting MTC and susceptibility to rifampicin, isoniazid, fluoroquinolone and second-line injectable drugs like aminoglycosides/capreomycin [10]. While WHO and a recent clinical trial have recommended use of the MTBDRplus and MTBDRsl in detecting MTC and drug resistance in direct sputum samples collected from patients with R/MDR-TB, they do not detect MTC to the species level [11]. The genotype MTBC assay (gMTBC) is a commercial platform (Hain LifeScience, Germany) that can address this gap, but it is only validated for testing cultured MTC isolates [12,13]. Based on whether mutation(s) are present or absent in 23 rRNA, *gyrB* and RD1 genes, the gMTBC allows differential identification of the MTC to *M. tuberculosis*/*M. canettii, M. africanum, M. microti*, *M. bovis* subsp. *bovis, M. bovis* subsp. *caprae* and *M. bovis* BCG only [14]. Similarly, PCR amplification of regions of differences (RDs), spoligotyping or targeted DNA sequencing (i.e. *gyrB* and *hsp65*) and whole genome sequencing not only require MTC culture, but also are expensive and largely depend upon extensive bioinformatic expertise [4,15]. Nevertheless, MTC culture is laborious work and it delays results for up to 8 weeks, yet is prone to contamination in up to 15% of Tanzanian patients [16], and can miss the non-culturable strains [17], and thereby compromise patient care.

While building from the same principle of genotype MTBDRplus and MTBDRsl [11], we optimised and deployed the gMTBC assay to identify MTC to the species level from direct sputum samples of patients with presumed MDR-TB in Tanzania. We also performed comparative susceptibility testing of MTC species identified to rifampicin and isoniazid by using the genotype MTBDRplus, and to fluoroquinolones and second-line injectable drugs by using the genotype MTBDRsl.

Materials and methods

Study settings

This study was conducted from September 2018 through March 2019 at the Kibong'oto Infectious Diseases Hospital (KIDH) in Siha District, Kilimanjaro in northern Tanzania. KIDH is a public hospital with a bed capacity of 320. Each year, the hospital provides services to over 200 and 600 patients with drug-resistant and drug-sensitive TB respectively. It is the National Centre of excellence for clinical management of drug-resistant TB in the country. Patients are usually referred to KIDH if they have R/MDR-TB with or without comorbidity like diabetes or have confirmed pre/XDR-TB or those living far from R/MDR-TB ambulatory centres for advanced clinical management. The hospital mycobacteriology laboratory has capacity for testing MTC by smear microscopy for acid-fast bacilli (AFB), Lowenstein–Jensen (LJ) solid culture media, Xpert® MTB/RIF assay, the line probe assays including the genotype MTBDRplus and MTBDRsl [10], and the gMTBC kits which were added for this study.

Study design and patients

This was a cross-sectional design conducted among patients with presumptive MDR-TB. Presumed MDR-TB was referred to patients who presented with symptoms or signs and risk factors suggestive of R/MDR-TB including prior history of treatment for drug-sensitive TB and HIV with or without rifampicin resistance [18]. The study was approved by the National Ethics Health Review Committee at the National Institute for Medical Research (NIMR) in Tanzania (NIMR/HQ/R.8a/Vol. IX/2662). Permission to conduct the study was granted by KIDH authorities. Patients were included in the study if were aged ≥18 years old but presenting with clinical features

of TB. Prior to any study procedure, they signed a written or oral, witnessed informed consent. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request. Moribund patients and those unable to expectorate quality sputum were excluded. Eligible patients provided early-morning-sputum samples for laboratory procedures. Also, social-demographic and clinical characteristics such as age, gender, duration of sickness at enrolment, HIV status, previous history of TB treatment and working in mining, body weight in kilograms and height in metres were collected from patients. A chest radiograph was also taken to ascertain the presence or absence of lung disease including a cavitary lesion. All patients received anti-TB medications and those living with HIV received anti-retroviral according to the 2018 treatment guidelines in Tanzania.

Sample size estimation and sampling procedure

The minimum sample size for this cross-section study was estimated using 4% prevalence of R/MDR-TB among patients previously treated for DS-TB in Tanzania, with a two-sided type I error of 5%. Assuming that 90% of patients would have agreed to participate in this study, a minimum of 66 patients with presumed R/MDR-TB were required. These patients were conveniently recruited to participate in the study.

Culture, smear microscopy and Xpert® MTB/RIF assay

Each participant provided one early–morning-sputum samples for all laboratory tests. Sputum was decontaminated and cultured in two Lowenstein–Jensen (LJ) slants in according to the Clinical and Laboratory Standard Institute [19] and previous publications [20,21]. Decontaminated sputum was smeared and examined for AFB using light-emitting diode fluorescence microscopy as instructed [22]. All procedures and results interpretations of Xpert® MTB/RIF assay were performed as previously described [20,23].

Line probe assays (LPA)

DNA was extracted from decontaminated sputa sediments using the GenoLyse® kit in according to manufacturer instructions. The DNA was stored at −20°C until amplification, hybridisation and detection by the genotype MTBDRplus, MTBDRsl and gMTBC kits. Except for gMTBC assay, a multiplex amplification with biotinylated primers was performed on TC 4000 thermal cycler as per prior studies and manufacturer instructions of the genotype MTBDR*plus* and MTBDR*sl* assays. Unlike the previous assay's validation studies and manufacturer instructions for testing clinical isolates [13], the cycling conditions for the genotype MTBC assay were modified to allow testing using direct sputum samples. Briefly, one cycle at 95°C for 15 min, followed by 20 cycles at 95°C for 30 s and 58°C for 2 min in the first stage. In the second stage, this was followed by 30 cycles at 95°C for 25 s, 53°C for 40 s and 70°C for 40 s before a single extension cycle at 70°C for 8min. A *M. tuberculosis* H37RV DNA reference strain and sterile molecular grade water were run together with DNA extractants, as positive and negative control for MTC respectively. As instructed by manufacturer and a recent reported by Ahmed *et al*, genotype Mycobacterium CM VER 2.0 kit (Hain LifeScience, Nehren, Germany) was used to test direct sputa samples and cultured isolates that had negative MTC by the MTBDRplus/sl and gMTBC [24]. Amplicons were finally held at 4°C until the DNA strip-based hybridisation and downstream detection steps on twin-incubator, and results were interpreted according to manufacturer instructions and previous publication [25]. Like in Xpert® MTB/RIF assay, LPA results are usually available between 24–48 h.

Data management and statistical analysis

A clinical report form was used to collect patient's socio-demographic and clinical data. Bacterial load was categorised as low (sum of low and very low MTC) if MTC was detected at the quantification cycle (Cq) of 23 to >28, and high (sum of high and medium MTC) if MTC was detected at the Cq of < 23 by the Xpert® MTB/RIF assay [23]. Chi-square or Fisher's exact test compared proportions. Accordingly, continuous variables were either reported using mean and a 95% confidence interval (CI) or median and their 25th and 75th interquartile range (IQR). Sensitivity, specificity, positive and negative predictive values and accuracy of the genotype MTBC in detecting MTC were calculated as previous [26], using the genotype MTBDRplus/sl, or smear microscopy and LJ culture reference methods. An accuracy was computed as the ratio of the total number of correct results by both test over the total number of patients. The weighted Cohen's kappa (κ) statistic measured the level of agreement between genotype MTBC assays with other tests [27]. Modified Poisson regression was used to model detection of MTC by gMTBC and was adjusted against gender, age, presence of cavitary disease, HIV status, prior history of anti-TB exposure and working in mining with radiological features suggestive of silicosis, smear results for AFB and bacterial load. A 95% confidence interval of test performance was included. A *P* value

<0.05 was considered significant. All analyses and visualisation were performed in R programming language, version 4.0.2 (http://www.R-project.org).

Results

Socio-demographic and clinical characteristics of patients

Among 73 patients, 73% were male and had an overall mean age of 43 (95% CI: 40–45) years. The socio-demographics and clinical characteristics of patients are presented in Table 1. In total, 39 (53%) had cavity on chest radiograph. Of 39 patients with cavity, 29 (74%) had high MTC quantity compared to 4 (12%) of 34 patients without cavity on chest radiograph (Figure 1a, $P < 0.001$). Moreover, 27 (37%) were people living with HIV/AIDS (PLWHA) and had a mean CD4 count of 244 (95% CI 184–304) cells/μl. Among PLWHA, 17 (63%) had low MTC quantity compared to 23 (50%) of 46 patients without HIV infection ($P = 0.381$). Also, 43 (59%) patients had prior history of exposure to first line anti-TB drugs compared to 30 (41%) of anti-TB naive ($P = 0.030$). However, 20 (67%) of anti-TB naïve patients had low MTC quantity compared to 20 (47%) of those who had prior treatment ($P = 0.084$), but there was no difference in proportion of culture positivity between patients who had prior treatment and anti-TB naïve [51% (22/43) vs. 47% (14/30), $P = 0.887$]. Distribution of test positivity/negativity among patients with high and low MTC detected by Xpert MTB/RIF is displayed in Figure 1.

Table 1 Socio-demographic and clinical characteristics ($N = 73$)

Patient's characteristics	Results
Male gender, *n* (%)	53 (73)
Mean age (95% CI) in years	43 (40–45)
Age groups in years	
18–30	10 (14)
31–50	47 (64)
Above 50	16 (22)
Median (IQR) days of sickness to enrolment	92 (58–224)
Cough, *n* (%)	73 (100)
Difficulty in breathing, *n* (%)	53 (73)
Chest pain, *n* (%)	58 (79)
Fever and excessive night sweet, *n* (%)	54 (74)
Weight loss of 3 Kg per month, *n* (%)	58 (79)
Mean BMI (95% CI) in Kg/m^2	18 (17–20)
Prior history of worked in mining, *n* (%)	27 (37)
HIV positive, *n* (%)	27 (37)
Mean (95% CI) CD4 count ($n = 27$)	244 (184–304)
Prior history of TB treatment, *n* (%)	43 (59)
Had cavity on chest radiograph, *n* (%)	39 (53)
Low MTC detected by Xpert® MTB/RIF, *n* (%)	40 (55)

BMI, body mass index; gMTBC, genotype MTBC v1.x; IQR, Interquartile range; MTBDRplus/sl, genotype MTBDRplus or MTBDRsl; MTC, *M. tuberculosis* complex.
Patients with low MTC included those who had low (5/40) and very low (35/40) MTC at the Xpert® MT/RIF assay cycle quantification (Cq) of 23–28 and >28 respectively. MTC was categorised as high (33/73) if it was detected at the Cq of <23.

Detection of MTC and non-tuberculous mycobacteria to the species level

Among 73 patients with positive Xpert® MTB/RIF assay, 40 (55%) had low MTC quantity. Of these 73 patients, 26 (36%) and 36 (49%) had positive acid-fast bacilli (AFB) on smear microscopy and LJ culture respectively. Also, 38 (52%) and 34 (47%) had MTC detected by genotype MTBDRplus/sl and gMTBC assays, respectively (Figure 2). Overall, 33 (45%) of patient's specimens had positive Xpert® MTB/RIF but negative culture and no MTC detection by genotype MTBDRplus/sl and gMTBC assays. In total, 31 (78%) of 40 patients with low MTC quantity had negative culture, and any of the genotype MTBDRplus/sl and gMTBC compared to 1 (3%) of 33 patients with high MTC quantity (Figure 1d, $P < 0.001$). In multivariate Poisson regression model, patients with chest cavity, high MTC quantity and smear positive were 4.18 (1.31–13.32, $P = 0.016$), 3.03 (1.35–6.82, $P = 0.007$) and 1.93 (1.19–3.14, $P = 0.008$) times more likely to have detectable MTC from direct sputa by the gMTBC compared to patients without chest cavity, low MTC and smear negative (Table 2).

Of 36 patients with positive culture, 34 (94%) were identified as MTC and further speciated to *M. tuberculosis/canettii* by the gMTBC test on both cultured isolates and direct sputa. Neither *M. bovis* nor *M. africanum* was detected. The genotype MTBDRplus/sl and gMTBC assays were negative in the remaining 2 (6%) of 36 positive cultures (Figure 2, Figure 1d) and were identified using the genotype Mycobacterium CM VER 2.0 kit as non-tuberculous mycobacteria with one being *M. intracellulare* and the other one being *M. kansasii*.

Performance of the genotype MTBC assay in patient's sputum samples

Compared to genotype MTBDRplus/sl, the accuracy of gMTBC in detecting MTC from direct sputa samples was 95% (95% CI; 86–98; Cohen's kappa (κ) = 0.89). Sensitivity, specificity, positive predictive value (PPV), negative predictive (NPV) value and accuracy of the genotype

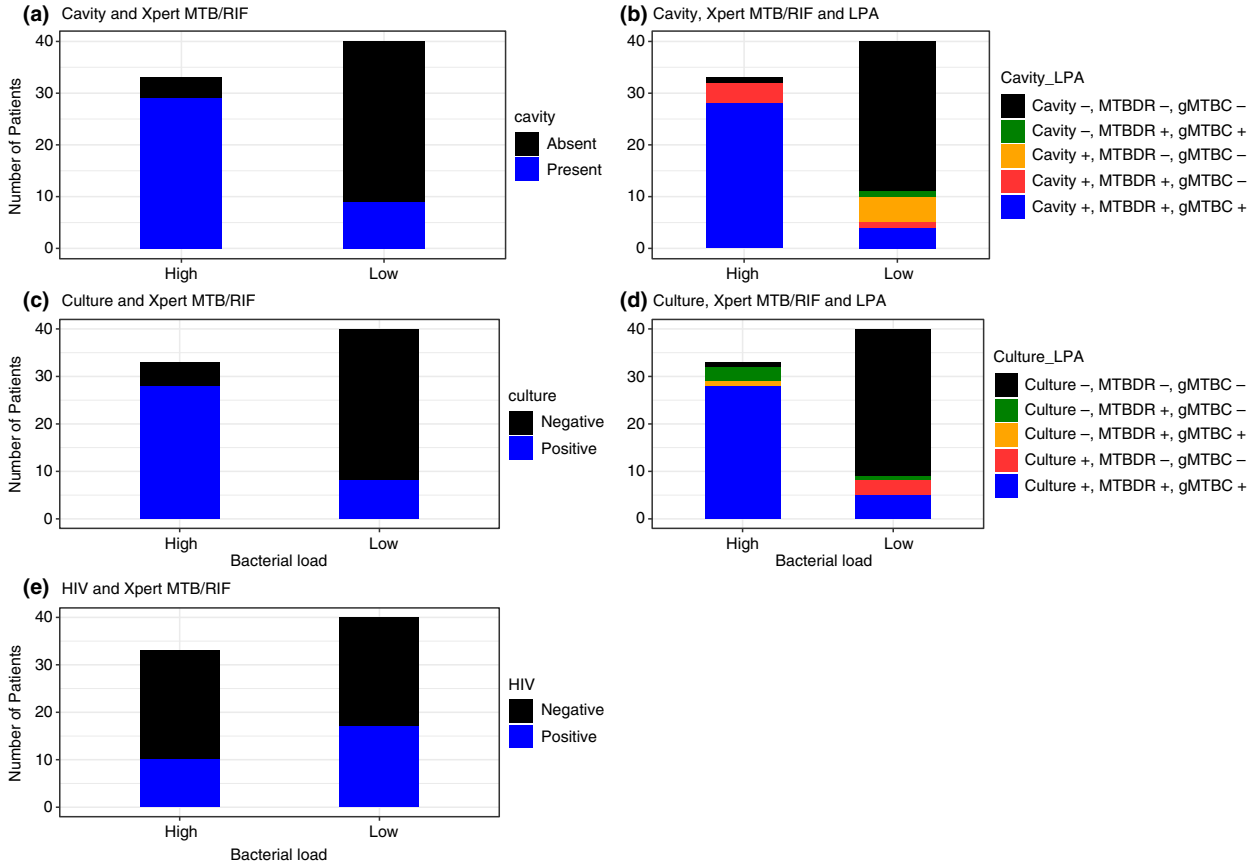

Figure 1 Distribution of bacterial load measured by Xpert® MTB/RIF among patients with chest cavity and HIV and test positivity/negativity. Bacterial load was quantified as low and high at the Xpert® MTB/RIF quantification cycle of 23 to >28, and <23, respectively. Patients were tested using culture and line probe assay (LPA) including the genotype MTBC (gMTBC) and genotype MTBDRplus/sl (MTBDR).

MTBC assay in detecting MTC from direct patient's sputa were summarised in Table 3. There was a strong concordance between the gMTBC assay and both LJ culture and MTBDR*plus/sl* ($\kappa \geq 89\%$), but was moderate when compared to smear microscopy ($\kappa = 61\%$, Table 3). Except for 2 patients with non-tuberculous mycobacteria, the gMTBC detected MTC in 33 patients with positive culture at an accuracy of 95% (95% CI; 86–98) compared to culture (Figure 2 and Table 3). Moreover, gMTBC detected 7 patients more than smear microscopy (Figure 2).

Susceptibility profile of MTC to anti-TB drugs

Among 38 patients with MTC, 28 (74%) had rifampicin resistance detected by both Xpert® MTB/RIF and genotype MTBDRplus assays. Eight (29%) of 28 patients with rifampicin-resistant MTC strains had isoniazid resistance, suggestive of MDR-TB. One patient had isoniazid resistance in MTC but rifampicin was susceptible. All 38 patients were susceptible to fluoroquinolones and injectable aminoglycosides/peptides as shown by genotype MTBDRsl.

Discussion

This study showed strong concordance between the genotype MTBC and MTBDRplus/sl as well as the LJ culture, in detecting MTC from sputum samples of patients with presumed R/MDR-TB. The assay achieved a ≥97% sensitivity compared to LJ culture and genotype MTBDRplus/sl which is higher than 93% sensitivity reported by Somoskovi *et al.*, from patients with culture and smear-positive sputa [13]. Molecular methods including the gMTBC and sequencing technologies have been optimised for testing high burden samples such as cultured isolates

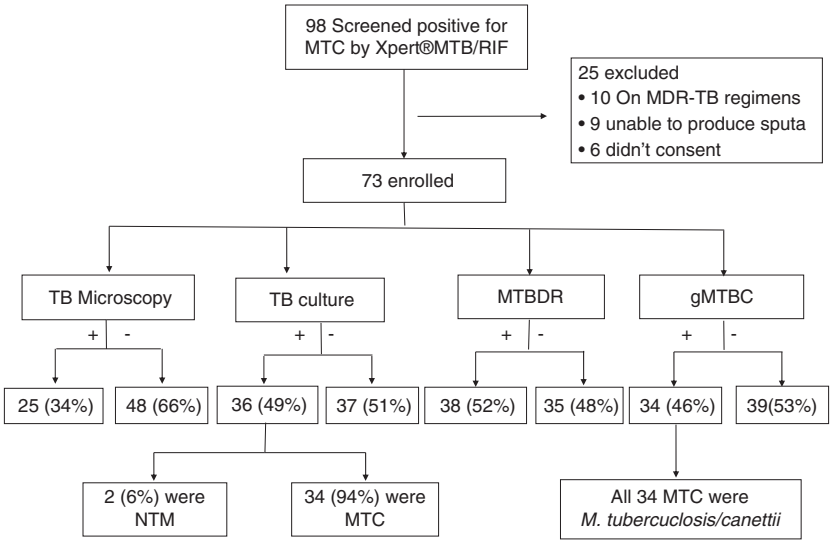

Figure 2 Detection of *M. tuberculosis* complex (MTC) from patients presumed for multidrug-resistant (MDR)-TB. MTC are the causative agents for tuberculosis (TB), and were identified using the line probe assays including the genotype MTBC (gMTBC), and any of the genotype MTBDRplus or MTBDRsl assays (MTBDR), and were further speciated to *M. tuberculosis/canettii* by gMTBC. Non-tuberculous mycobacteria (NTM) were identified to *M. intracellulare and M. kansansii* by using the genotype Mycobacterium CM VER 2.0 kit.

[13,25]. Despite that the genotype MTBDRplus and MTBDRsl assays have recently been tested on unprocessed sputa [11], this is to our knowledge the first report to compare the performance with the gMTBC.

Patients with cavitary disease had high MTC compared to those without chest cavity. Presence of cavity can also result in an local environment of high bacterial replication within the cavity which can later distribute into the airway, consistent with a higher sputum bacterial burden [28,29]. Patients with chest cavity, HIV negative, smear positive, and high bacterial load measured by Xpert® MTB/RIF harboured detectable MTC from direct sputa by the gMTBC compared to patients without chest cavity, low MTC and smear negative. Sensitivity improves in people with conditions of known higher bacterial burden and expectedly decreases in paucibacillary conditions particularly in patients with atypical features of TB on chest radiograph [30,31]. Moreover, the high yield of gMTBC compared to smear microscopy supports its use during screening of participants in clinical trials [11].

Surprisingly in this study, approximately 45% of patients with a positive Xpert®MTB/RIF result for MTC had a negative LPA and LJ culture, as compared to only 20% from other studies [32]. Under expected circumstances, culture should be more sensitive than Xpert® MTB/RIF assay and LPA [33]. A similar discordance was recently reported in a clinical trial in which 34% of paucibacillary samples re-tested negative for active TB by Xpert® MTB/RIF assay [34,35]. Similar to our findings, a paucibacillary state was among the potential sources of discrepancy [34,35]. Despite the similar culture positivity rate between patients with prior history of treatment with first-line anti-TB drugs and those who were anti-TB drug naïve, approximately half of those with prior treatment had low detectable MTC by Xpert® MTB/RIF assay, which may have represented a false-positive signal [31]. Moreover, in patients with prior history of TB treatment, detecting low level of MTC DNA by Xpert® MTB/RIF or the more sensitive Xpert® MTB/RIF Ultra may represent a subpopulation without active infection but rather chronic lung disease, and residual cavitary changes and/or bronchiectasis [36,37]. In such patients, the differential diagnosis should also include non-tuberculous mycobacteria and fungal pathogens such as *Aspergillus* species that are known to colonise those with residual lung disease following otherwise successful TB treatment [37,38]. We and others have reported on the importance of accurately detecting non-tuberculous mycobacteria such as *M. intracellulare* and *M. kansasii* among people living with HIV/AIDS in Tanzania for whom multiple rounds of TB treatment were performed before alternative diagnoses and post-TB chronic lung disease were considered [39,40]. To enhance more accurate TB diagnosis, our findings

Table 2 Modified Poisson regression modelling of MTC detection by gMTBC ($N = 73$)

	Univariate		Multivariate	
Variable	Crude incident rate ratio (95% CI)	*P* value	Adjusted incident rate ratio (95% CI)	*P* value
Gender				
Female	Ref:		Ref:	
Male	0.79 (0.38–1.61)	0.518	1.10 (0.66–1.82)	0.725
Age group in years				
18–30	Ref:		Ref:	
31–50	0.61 (0.35–1.08)	0.093	1.58 (1.11–2.27)	0.012
Above 50	0.96 (0.49–1.88)	0.899	0.49 (0.31–0.78)	0.003
HIV status				
Negative	Ref:		Ref:	
Positive	0.93 (0.46–1.88)	0.838	0.53 (0.36–0.79)	0.002
Cigarette smoking				
Non-smokers	Ref:		Ref:	
Smokers	1.04 (0.53–2.03)	0.918	0.81 (0.55–1.19)	0.283
Chest cavity				
Absent	Ref:		Ref:	
Present	9.52 (2.91–31.13)	<0.001	4.18 (1.31–13.32)	0.016
MTC quantity				
Low	Ref:		Ref:	
High	7.03 (2.72–18.16)	<0.001	3.03 (1.35–6.82)	0.007
Chest silicosis				
Absent	Ref:		Ref:	
Present	1.05 (0.53–2.11)	0.88	0.96 (0.63–1.47)	0.863
Prior TB treatment				
New	Ref:		Ref:	
Retreatment	1.13 (0.56–2.25)	0.735	0.84 (0.61–1.17)	0.231
Smear microscopy				
Negative	Ref:		Ref:	
Positive	3.78 (1.84–7.75)	<0.001	1.93 (1.19–3.14)	0.008

gMTBC, genotyope MTBC ver 1.x; HIV, human immunodeficiency virus; MTC, *M. tuberculosis* complex measured by Xpert® MTB/RIF; TB, tuberculosis.

warrant consideration of modifying the current Xpert® MTB/RIF testing algorithm to include an assessment of active/replicating bacillary load with tools such as the Molecular Bacterial Load assay, which detects and quantifies the rRNA of viable *M. tuberculosis* within 24 h, as well as other conventional techniques such as culturing the *M. tuberculosis* in liquid and solid media for those with prior TB treatment, and expanding access to non-sputum assays such as urine TB-LAM for people living with HIV/AIDS, and suspicion of concomitant extrapulmonary TB [41–43]. In doing so, patients with active TB can be treated with anti-TB drugs, while those with non-tuberculous mycobacteria or other manifestations of post-TB lung disease can be effectively triaged for chronic lung disease pharmacotherapy and pulmonary rehabilitation [44]. These approaches support recent observations such as those by Costantini *et al* (2020) who reported a favourable outcome after withholding anti-TB medication in a patient with positive Xpert® MTB/RIF but negative culture results [45].

The *M. tuberculosis/canettii* was the only member of MTC identified as the pathogen among patients with presumptive MDR-TB. Because of the close genetic relatedness, the gMTBC assay cannot fully differentiate *M. tuberculosis* from *M. canettii*, even with the recent 894 diverse genomes of *M. canettii* and major phylogenetic groups of MTC that were typed to guide the new revised interpretation of the assay's result [46]. The *M. tuberculosis/cannetii* predominance in this study is similar to a prevalence survey in sub-Sahara Africa, that at least 95% of TB was due to *M. tuberculosis* and 2% due to *M. africanum* [25]. Our findings confirm prior epidemiological studies that had suggested the geographic distribution of *M. africanum* shows it to be an important cause of TB in West Africa, but that it is rarely detected in East African countries, including Tanzania [47]. In

Table 3 Performance of the genotype MTBC assay in detecting *M. tuberculosis* from direct sputum samples

	Genotype MTBC assay, *n* % (95% confidence interval)							
Reference methods	Positive	Negative	Sensitivity	Specificity	PPV	NPV	Accuracy	Cohen's kappa
Smear Microscopy								
Positive	23	3	68 (49–83)	92 (78–98)	88 (69–97)	77 (62–87)	81 (70–89)	61 (43–79)
Negative	11	36						
LJ culture								
Positive	33	3	97 (83–100)	92 (78–98)	92 (66–99)	97 (84–100)	95 (86–98)	89 (79–99)
Negative	1	36						
Genotype MTBDRplus/sl								
Positive	34	4	100 (87–100)	90 (75–97)	89 (74–97)	100 (88–100)	95 (86–98)	89 (79–99)
Negative	0	35						

LJ, Lowenstein–Jensen solid media; MTBDRplus/sl, genotype MTBDRplus or MTBDRsl assays for detecting *M. tuberculosis* complex and drug resistance; NPV, negative predictive value; PPV, positive predictive value.

these settings, this species dominance underscores the treatment, prevention and control strategies for *M. tuberculosis*.

Isoniazid resistance in patients with rifampicin resistant was uncommon, accounting for about 29% compared to over 64% in South Africa [48] and 78% in China [49]. In the present study, isoniazid monoresistance was categorised from direct sputum samples which was negative in a large number of patients [30], as compared to MTC isolates in two studies [48,49]. However, this is contrary to a global genomic analysis of over 5000 *M. tuberculosis* strains by Manson *et al.*, who documented that mutations conferring resistance to isoniazid evolve first before rifampicin resistance across all lineages, geographic regions and time periods [50], and therefore our findings likely represent the incomplete coverage of isoniazid resistance targets on the genotype MTBDRplus assay [51]. We were not able to perform conventional culture-based isoniazid susceptibility testing to interrogate this observation. Favourably in the present study, all *M. tuberculosis/canettii* identified were susceptible to fluoroquinolone and aminoglycosides/capreomycin, similar to findings by Mpagama *et al.*, among patients with MDR-TB in Tanzania [52] and in studies from Kenya [53]. Our findings continue to support the empirical use of fluoroquinolones in the treatment of R/MDR-TB in Tanzania while awaiting treatment modification from culture-based susceptibility testing.

This study has limitations. The relatively small sample size does not allow for generalisability beyond the referral patterns to the hospital of study, but do provide a confirmatory snapshot of circulating MTC species in patients with MDR-TB in Tanzania to inform further the current approach to optimal diagnosis and clinical management. However, the main comparison was made in the performance characteristics in gMTBC from unprocessed sputa that did not require a diversity of samples. Furthermore, we included patients with previous history of treatment or those with rifampicin-resistant TB rather than anti-TB naïve, which could have contributed to discrepancies among tests as previously outlined [54]. Nonetheless, these patients represent an important population at risk for acquiring drug resistance or other circulating strains given their exposure to the healthcare setting and as evidenced by our findings of non-tuberculous mycobacteria.

In conclusion, the concordance between the gMTBC and MTBDRplus/sl in detecting MTC from direct sputum was considerably high but lagged behind the yield of Xpert MTB/RIF. High bacterial loads such as those found in patients with chest cavity and sputum smear positive were independent predictors of MTC detection. Modification by integrating biomarkers for active TB, such as the TB molecular bacterial load assay, urine LAM and selective use of mycobacterial culture for non-tuberculous mycobacteria into Xpert® MTB/RIF testing algorithm for those with prior TB treatment can be pivotal not only in addressing potentially false-positive Xpert® MTB/RIF results, but also in differentiating active TB from the sequelae of post-TB lung disease. Importantly, all *M. tuberculosis/canettii* detected were susceptible to fluoroquinolones, a core drug in MDR-TB treatment regimens.

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Data availability statement

The data used in this manuscript are available from the corresponding author upon request and permission from Kibong'oto Infectious Diseases Hospital (KIDH).

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