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Can resistance to either isoniazid or rifampicin predict multidrug resistance tuberculosis (MDR-TB)

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Abstract

Background Previous studies have shown significant differences and lack clarity on whether resistance to either isoniazid or rifampicin can predict multidrug resistance tuberculosis (MDR-TB). Some consider rifampicin resistance to be a surrogate for MDR-TB. We, therefore, conducted this study to determine resistance to either isoniazid or rifampicin can predict MDR-TB.

Results A total of 315 *Mycobacteria tuberculosis* isolates were tested for resistance against isoniazid, rifampicin, ethambutol and streptomycin using the phenotypic proportion method on Lowenstein–Jensen media. Results showed most isolates (88.9%, 280/315) were not resistant to any anti-TB tested, 5.7% (18/315) were resistant to both isoniazid and rifampicin, 2.5% (8/315) were resistant to rifampicin only and 1.3% (4/315) were resistant to all four first-line anti-tuberculosis. Prediction of MDR TB basing on rifampicin results showed sensitivity of 100.0%, specificity of 96.3%, diagnostic accuracy of 96.5%, and positive and negative predictive values of 62.1% and 100.0%, respectively. Isoniazid had sensitivity of 100.0%, specificity of 98.3%, diagnostic accuracy of 98.4%, and positive and negative predictive values of 78.3% and 100.0%, respectively. Prediction of rifampicin resistance based on isoniazid results had sensitivity of 62.1%, specificity of 98.3%, diagnostic accuracy of 94.9%, a positive predictive value of 78.3% and a negative predictive value of 96.2%.

Conclusions Resistance to either rifampicin or isoniazid sub-optimally predicts MDR-TB. Despite having high sensitivity and specificity, the positive predictive value of rifampicin was only 62.1% and for isoniazid was 78.3%, suggesting that if either is tested in isolation both could result in false positives MDR-TB cases, resulting into patients being unnecessarily subjected to the more toxic and expensive second-line anti-TB drugs, which are less effective compared to first-line anti-TB drugs.

Keywords Prediction, Isoniazid, Rifampicin, Multidrug resistance tuberculosis (MDR-TB)

Background

Rifampicin and isoniazid are the two most powerful first-line anti-TB drugs (Mullerpattan et al. 2017; Saktiawati et al. 2019). Resistance to both of them, with or without resistance to other first-line anti-TB drugs is referred to as multidrug tuberculosis (MDR-TB) (Desissa et al. 2018; Seung et al. 2015). MDR-TB is more expensive and difficult to treat and threatens the control of TB world-wide (Paul 2018, Chakaya et al. 2021). Globally, approximately 600,000 cases of MDR-TB or rifampicin resistant TB occur annually (Global Tuberculosis report 2021;

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Chakaya et al. 2021). In East Africa, including Tanzania, the prevalence of MDR-TB among new and re-treatment cases is estimated to be 3.9% and 20.6%, respectively (Molla et al. 2022). Rapid diagnosis of MDR-TB is essential for proper management of patients and to minimize spread of resistant strains (Fox et al. 2017). Some studies have suggested rifampicin resistance predict MDR-TB (Liu et al. 2019). Indeed, Xpert MTB/RIF (Cepheid, Sunnyvale, CA), a molecular diagnostic test was developed on consideration that rifampicin resistance was surrogate for MDR-TB (Liu et al. 2019). The assumption is that there is high correlation of isoniazid and rifampicin resistance, thus not requiring independent testing of the former in diagnosis of MDR-TB (Bisimwa et al. 2021). It is taken, as matter of principal, that results that are positive for MTBC and for RIF resistance on Xpert MTB/RIF indicate a high probability of resistance to RIF (Bisimwa et al. 2021). However, previous studies have shown significant differences and lack clarity on resistance to either isoniazid or rifampicin predict multidrug resistance tuberculosis (MDR-TB) (Bisimwa et al. 2021). Assessing the ability of rifampicin resistance in predicting MDR-TB is particularly important to low and medium income countries (LMICs), where the burden of TB and MDR-TB is highest. We, therefore, conducted this study to determine (i) ability of resistance rifampicin/isoniazid in predicting MDR-TB, (ii) the relationship in the occurrence of isoniazid and rifampicin resistance, and (iii) whether resistance to either of them can predict resistance to the other. We used isolates whose resistance to first-line drugs was determined using the proportion method on Lowenstein–Jensen (LJ) media (Yu et al. 2016). Our working hypothesis was that *Mycobacteria tuberculosis* strains exhibiting resistance to rifampicin are also resistant against isoniazid, and can thus predict MDR-TB.

Methods

Isolates

This study involved all 315 isolates collected at the Central Tuberculosis Reference Laboratory (CTRL), Tanzania, between January 2016 and December 2019. These isolates have been described in a previous study (Mchaki et al. 2022).

Drug susceptibility testing on Lowenstein–Jensen media

Drug susceptibility testing was performed using proportional method (Rufai et al. 2014; Amini et al. 2019) on Lowenstein–Jensen (LJ) media containing 0.2 µg/mL and 1.0 µg/mL of isoniazid, 5 µg/mL streptomycin, 40 µg/mL of rifampicin, 2 µg/mL ethambutol and 500 ug/ml para-nitro benzoic acid (PNB) (Aricha et al. 2019). The proportion method determines the percentage of growth (number of colonies) of defined inoculums on a drug-free control medium versus growth on culture media containing the critical concentration of an anti-TB drug. The proportion method enables precise quantification of the proportion of organisms resistant to a given drug. First reading is done at 4 weeks (28 days). If resistant, no further reading, while if no growth seen, re-incubation was done up to 42 days (Charalampous et al. 2019).

Quality control

The reference strain H37Rv (ATTCC 25618) was used as a positive control in the Drug Susceptibility Testing on Lowenstein–Jensen Media (Werngren et al. 2017).

Statistical analysis

Data were analyzed using an open source statical programming language R (version 4.2.0) through its integrated development environments (IDEs) called JAMOV (version 2.2) and R Studio (release 2022.02.0) together with premium excel extension called Analyze—it (version 6.15.0). Cross tabulation between isoniazid drug susceptibility results and rifampicin results were used to show extents of correlation between these two results. Same apply to the drug susceptibility results of rifampicin and isoniazid were cross tabulated with the results of MD-TB to show extent of correlation between those results and MDR-TB. Sensitivity, specificity, diagnostic accuracy, as well as negative and positive predictive values were calculated and used to show how isoniazid resistance could predict rifampicin resistance, and isoniazid and rifampicin resistances could predict MDR TB. Comparison of sensitivities and specificities of rifampicin and isoniazid drug susceptibility results in predicting MDR-TB were carried out using Miettinen-Nurminen statistical test under hypothesis of equality. Comparison of positive and negative predictive values of rifampicin and isoniazid drug susceptibility results in predicting MDR-TB were carried out using generalized score statistic (gs), one of statically test in DT Com Pair R-package (version 1.03). Areas under receiver operating characteristics curve (ROC curve) were used to show discriminatory ability of Isoniazid susceptibility pattern in detecting rifampicin resistance, and discriminatory abilities of both isoniazid and rifampicin phenotypic susceptibility results in detecting MDR-TB.

Results

A total of 315 *Mycobacteria tuberculosis* isolates were tested for resistance to anti-TB drugs, and their drug susceptibility patterns are shown in Table 1. Most isolates (88.9%, 280/315) were not resistant to any of the anti-TB drug tested, 5.7% (18/315) were resistant to both isoniazid and rifampicin, 2.5% (8/315) were resistant to rifampicin only, and 1.3% (4/315) were resistant

Table 1 Phenotypic DST pattern of the isolates ($n=315$)

Drug susceptibility test pattern	Frequency (*n*)	Percent (%)
None	280	88.9
Rifampicin	8	2.5
Isoniazid	4	1.3
Streptomycin	1	0.3
Isoniazid and Rifampicin*	10	3.2
Rifampicin and streptomycin	2	0.6
Isoniazid and streptomycin	1	0.3
Rifampicin and ethambutol	1	0.3
Isoniazid, Rifampicin and streptomycin*	3	1.0
Isoniazid, Rifampicin and ethambutol*	1	0.3
Isoniazid, Rifampicin, ethambutol and streptomycin*	4	1.3

*MDR isolates

Table 2 Correlation between Isoniazid and rifampicin resistance ($n=315$)

	Rifampicin		Total
	Resistant	Sensitive	
Isoniazid			
Resistant	18	5	23
Sensitive	11	281	292
Total	29	286	315

Table 3 Prediction of rifampicin resistance using isoniazid results ($n=315$)

Decision statistics	Estimate (%)	95% Confidence interval	
		Lower (%)	Upper (%)
Sensitivity	62.1	42.3	79.3
Specificity	98.3	96.0	99.4
Diagnostic accuracy	94.9	91.9	97.1
Positive predictive value	78.3	56.3	92.5
Negative predictive value	96.2	93.4	98.1

to all four 1st line anti-TB drugs; isoniazid, rifampicin, ethambutol and streptomycin. A small number 1.0% (3/315) were resistant to isoniazid, rifampicin, and streptomycin. In total 18/315 (5.1%) isolates were MDR.

Table 2 shows isoniazid resistance in 18 isolates among 29 isolates found to be resistant to rifampicin, while 281 isolates showed sensitivity to rifampicin among 292 isolates found to be sensitive to isoniazid.

Prediction of rifampicin resistance based on isoniazid results had sensitivity of 62.1% (95% CI of 42.2–79.3%), specificity of 98.3% (95% CI of 96.0–99.4%), diagnostic accuracy of 94.9% (95% CI of 91.9–97.1%) a positive predictive value of 78.3% (95% CI of 56.3–92.5%) and a negative predictive value of 96.2% (95% CI 93.4–98.1%) (Table 3).

As depicted in area under curve in Fig. 1, the discriminatory ability of isoniazid susceptibility pattern in predicting rifampicin resistance was found to be 0.802 (80.2%).

Table 4 summarizes correlation between rifampicin resistance and MDR-TB. Among 29 isolates found to be resistant to rifampicin, 18 were MDR-TB, and among 286 rifampicin susceptible isolates, all were found to be among the 297 non MDR-TB isolates.

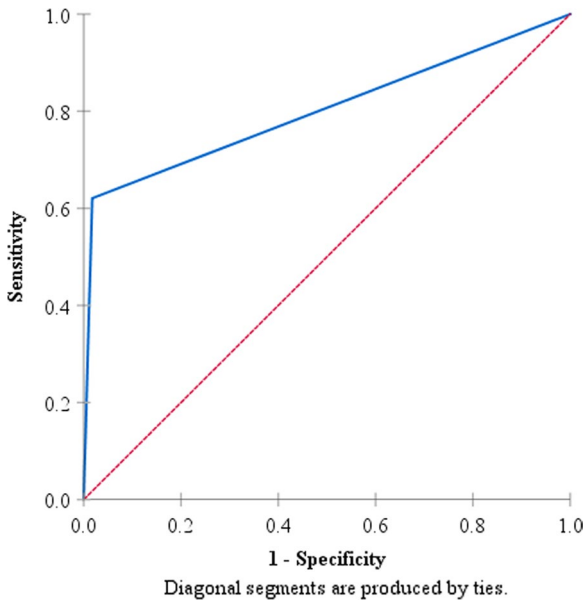

Fig. 1 RoC curve showing prediction of isoniazid resistance using rifampicin results ($n=315$)

Table 4 Correlation between rifampicin resistance and MDR-TB ($n=315$)

	MDR-TD		Total
	Resistant	Sensitive	
Rifampicin			
Resistant	18	11	29
Sensitive	0	286	286
Total	18	297	315

Table 5 Correlation between Isoniazid and MDR-TB ($n=315$)

	MDR-TD		Total
	Resistant	Sensitive	
Isoniazid			
Resistant	18	5	23
Sensitive	0	292	292
Total	18	297	315

Table 5 shows the correlation between isoniazid resistance and MDR-TB. Among 23 isolates found to be resistant to isoniazid, 18 were MDR-TB, and among 292 isoniazid susceptible isolates, all were found to be among 297 non MDR-TB isolates.

With regard to prediction of MDR TB basing on rifampicin results we found sensitivity of 100.0% (95% CI 81.5–100.0%), specificity of 96.3% (95% CI 93.5–98.1%), diagnostic accuracy of 96.5% (95% CI 93.8–98.2%), and positive and negative predictive values of 62.1% (95% CI 42.3–79.3%) and 100.0% (95% CI 98.7–100.0%), respectively. On the other hand, isoniazid results had sensitivity of 100.0% (95% CI 81.5–100.0%), specificity of 98.3% (95% CI 96.1–99.5%), diagnostic accuracy of 98.4% (95% CI 96.3–99.5%), and positive and negative predictive values of 78.3% (95% CI 56.3–92.5%) and 100.0% (98.7% CI 98.7–100.0%) respectively, for predicting MDR-TB, as shown Table 6.

As shown in Tables 7 and 8, the differences in specificities and positive predictive values between rifampicin and isoniazid susceptibility results in predicting MDR TB were not found to be statistically significant (p-values of 0.134 and 0.122 respectively),

Table 7 Comparison of sensitivities and specificities of rifampicin and Isoniazid drug susceptibility results in detecting MDR-TB

	Proportion of difference	Tango 95% CI	Z Statistic	*p*—Value
Sensitivity	0.000	− 0.176 to 0.176	–	–
Specificity	0.020	− 0.007 to 0.051	1.50	0.1336[$]

[$] Do not reject the null hypothesis (equality) at the 5% significant level

Table 8 Comparison of positive and negative predictive values of rifampicin and Isoniazid drug susceptibility results in detecting MDR-TB

	Difference	Test statistics	*p*—Value
Positive predictive value	0.162	2.4	0.122[$]
Negative predictive value	0	–	–

[$] Do not reject the null hypothesis (equality) at the 5% significant level

As shown on area under ROC curve in Fig. 2, the discriminatory ability of the rifampicin and Isoniazid drug susceptibility patterns in predicting MDR-TB was found to be 0.981 (98.1%) and 0.992 (99.2%), respectively. This difference in areas under ROC curves between rifampicin and isoniazid was not statistically significant; (AUC difference = − 0.010 with 95% CI − 0.023–0.003, $p=0.133$).

Discussion

In this study, we evaluated the ability of rifampicin and isoniazid in predicting MDR-TB. Furthermore, we determined association between occurrence of rifampicin and isoniazid resistance, to find out whether the degree of correlation between the two, and whether testing of isoniazid is not needed in determination of MDR-TB.

Our results indicate that both isoniazid and rifampicin had very good and comparable sensitivities,

Table 6 Prediction of MDR-TB using rifampicin and isoniazid results

Decision statistics	Rifampicin results		Isoniazid results		*p*—value
	Estimate (%)	95% CI	Estimate (%)	95% CI	
Sensitivity	100.0	81.5–100	100.0	81.5–100.0	–
Specificity	96.3	93.5–98.1	98.3	96.1–99.5	0.134
Diagnostic accuracy	96.5	93.8–98.2	98.4	96.3–99.5	–
Positive predictive value	62.1	42.3–79.3	78.3	56.3–92.5	0.122
Negative predictive value	100.0	98.7–100.0	100.0	98.7–100.0	–

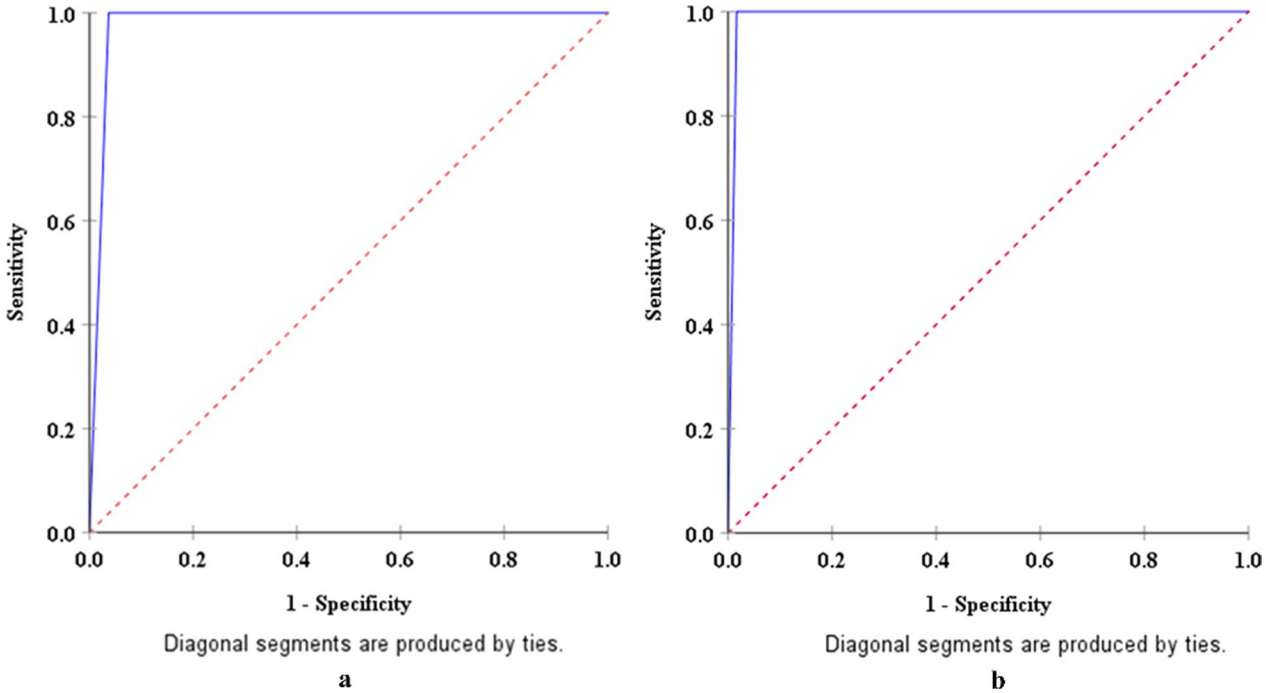

Fig. 2 Depicting area under ROC curve of rifampicin (**a**) and isoniazid (**b**) results in detecting MDR-TB

specificities, diagnostic accuracy and NPVs, which is in line with previous observations (Bisimwa et al. 2021). Respective ROCs were also impressive for both anti-TB drugs. However, their positive predictive values, for MDR-TB, were less impressive, especially for rifampicin (62.1%), meaning that testing each of them in isolation will results into false positive MDR cases, who will be wrongly subjected to second anti-TB drugs. These drugs are more costly and toxic and less effective than first-line TB drugs, with a risk of reverting to XDR-TB (Prasad et al. 2017; Lange et al. 2018). Surprisingly, the positive predictive value for isoniazid was higher at 78.3%, which is contrary to many studies as well as general thinking. The level of positive prediction seen in this study has also been reported in other studies, where testing for rifampicin alone resulted in PPV of around 60% (Aricha et al. 2019; Mchaki et al. 2022). The reason for the low positive predictive value is due the moderate correlation between isoniazid and rifampicin resistance. Isoniazid resistance was observed in 18 among 29 isolates found to be resistant to rifampicin, equivalent of 62.1%, while isoniazid was able to predict rifampicin resistance by 80.2%.

Our results are in keeping with those of others, showing rifampicin-based tests, such as Xpert MTB/Rif, did not accurately predict phenotypic MDR-TB (Feliciano et al. 2019; Rigouts et al. 2013), emphasizing the need for isoniazid testing (Zaragoza et al. 2017). It is known that mechanisms of resistance against the two anti-TB drugs are different and complex, with multiple mutations, and insertion events (Ghajavand et al. 2019). For example, some isolates showing resistance to rifampicin have showed no mutations in *rpoB*, suggesting that other mechanisms of resistance, possibly efflux pumps, may exist (Ghajavand et al. 2019). Likewise, *multiple mutations* in *katG* have been observed in strains showing resistance against isoniazid *(*Ghajavand et al. 2019*)*.

Results of our study have several implications of particular importance to the diagnosis and management of MDR-TB cases. It is clear that rifampicin is not a surrogate marker of MDR-TB as alluded by others. As a matter of fact, isoniazid performed significantly better than rifampicin. Secondly, by implication, the GeneXpert MTB/RI, which is the most commonly rolled out molecular technology in high TB burden LMICs, cannot be relied upon for detection of MDR-TB. Based on results of the current study, and on our previous study showing better performance of LPA (Mchaki et al. 2022), PPV of 90.1% versus 58.5% of the GeneXpert MTB/RIF, we urge that rifampicin resistance detected on GeneXpert MTB/RIF should be subjected to LPA test for confirmation (Mchaki et al. 2022). Unlike GeneXpert MTB/RIF that test for only rifampicin, LPA tests for both rifampicin and isoniazid (Yadav et al. 2021). When compared with BACTEC MGIT 960 system, LPAs such as The Genotype ®MTBDR*plus* (version 2) have been found to be efficient

and reliable rapid molecular DST assay for rapid susceptibility screening of MDR and even XDR-TB and are particularly useful in high MDR/XDR burden countries (Maningi et al. 2017). Likewise a study conducted in Ethiopia by Yigzaw et al. 2021, comparing phenotypic and LPA susceptibility results, showed excellent concordance (98.77%) of MDRTB plus to all INH, RIF and MDR TB, with very low discordance value of 1.23%). Furthermore, Rufai et al. (2014) showed 100% agreement between MGIT960 and LPA results, but only 64.4% agreement with rifampicin-based Xpert MTB/RIF results, while sequencing analysis of discrepant samples showed 91.3% concordance with LPA but only 8.7% concordance with the Xpert MTB/RIF assay. Collectively these results support that LPA can be a good alternative method for detection of INH and RIF resistance, especially where phenotypic DST is not available and a fast treatment decision is needed. This allows for timely and appropriate treatment, reducing transmission rates, morbidity and improve treatment outcomes. However, it should be noted that even with good performance, the PPV value of LPA is around 90% (Mchaki et al. 2022; Singh et al. 2017), implying that some few cases will be missed. Thus, there is a need of developing a model that can be used predict the status of MDR-TB that could give guidelines to physicians in classifying high-risk patients (Ali et al. 2021; Koo et al. 2020). Of late, nomograms have been successfully used for individualized, simple and precise prediction of incident multidrug-resistant tuberculosis (Cheng et al. 2020). Such approaches provide avenue for monitoring, estimating and intervening the risk of incident MDR-TB.

We acknowledge, as a limitation, that this study did not characterize mutations conferring resistance to either rifampicin or isoniazid. Development of sensitive, rapid, and economical genotypic test for MDR TB requires detailed knowledge of the prevalent mutations among MDR TB isolates. Secondly, being a cross-sectional study, we were not able to follow TB treatment outcomes of either drug-resistant and susceptible cases.

Conclusions

Our findings have important clinical, diagnostic, and treatment guideline implications. Based on our results, we conclude that neither rifampicin nor isoniazid resistance is a surrogate marker for MDR-TB. Testing for rifampicin resistance alone will lead to approximately 40% false positive MDR cases, while isoniazid will lead into 22% false positive MDR cases. This will result into patients being unnecessarily subjected to the more toxic and expensive second-line anti-TB drugs. We recommend that resistance to either of them should be followed by further screening with other tests such as LPA or culture methods. In addition, facilities should be encouraged to develop nomograms, which have been successfully used for individualized, simple and precise prediction of incident multidrug-resistant tuberculosis.

Abbreviations

ATTCC	The American Type Culture Collection
AUC	Area under the curve
CTRL	Central Tuberculosis Reference Laboratory
IDEs	Integrated Development Environments
INH	Isoniazid
LJ	Lowenstein–Jensen
LMICs	Low and Middle Income Countries
LPA	Line probe assay
MDR-TB	Multi-drug-resistant tuberculosis
PPV	Positive predictive value
NPV	Negative predictive value
RIF	Rifampicin
NPV	Negative predictive value
ROC	Receiver operating characteristic
TB	Tuberculosis
XDR-TB	Extensively drug-resistant TB

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Author contributions

Study conceptualization, BRM; and MIM; data collection, BRM; data analysis, PPK; review of first draft, FXM; supervision and verification of data, MIM All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate

The ethical consideration was obtained from the Directorate of Research and Publications (DRP) of Muhimbili University of Health and Allied Sciences (MUHAS) in Dar es Salaam, Tanzania (Ref. No. DA.282/298/01.C/named MUHAS-REC-08-2020-344 on 12 August 2020). Permission to use the archived isolates was obtained from the National Tuberculosis and Leprosy Program (NTLP) and Central TB Research Laboratory (CTRL).

Consent for publication

Not applicable.

Competing interests

The authors do not have any potential, perceived or real competing interests relating to this work.

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