IJID Regions 2 (2022) 149–153

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Genotypic and phenotypic drug-resistance detection and prevalence of heteroresistance in patients with isoniazid- and multidrug-resistant tuberculosis in Ethiopia

Muluwork Getahun[a,b,*], Gobena Ameni[c,d], Helina Mollalign[a], Getu Diriba[a], Dereje Beyene[b]

[a] Ethiopian Public Health Institute
[b] Addis Ababa University, Department of Microbial, Cellular, and Molecular Biology
[c] Aklilu Lemma Institute of Pathobiology, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia
[d] Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, United Arab Emirates University, PO Box 15551, Al Ain, United Arab Emirates University

ARTICLE INFO

Key words:
LPA
heteroresistance
isoniazid- and multidrug-resistant TB

ABSTRACT

Objective: To assess the agreement between genotypic and phenotypic methods for detecting drug resistance, and examine the prevalence of heteroresistance among isoniazid (INH)- and multidrug/rifampicin-resistant (MDR/RR) TB.

Method: In total, 127 *Mycobacterium tuberculosis* (*Mtb*) isolates, including 65 MDR/RR and 62 INH resistant, were used. First-line drug susceptibility testing (DST) was performed using the LJ method to determine the percentage of resistant bacteria. All drug-resistant isolates underwent testing with LPA. Heteroresistance was defined as simultaneous detection of wild-type and resistance-conferring mutations using LPA.

Result: The sensitivity of LPA (compared with LJ DST) was 96% for any INH-resistant TB and 94% for any RR TB. The prevalence of heteroresistance among the 123. *Mtb* isolates was 9.8%. The percentage of resistant bacteria ranged from 1% to 10% for heteroresistant TB. Rifampicin heteroresistance was detected in 1.6% of MDR TB patients. INH heteroresistance was detected in 1.6% and 16.7% of MDR and INH-resistant TB patients, respectively. The proportion of INH heteroresistance was significantly higher ($p = 0.030$) in persons living with HIV.

Conclusion: Some phenotypic drug resistances were not captured by LPA. The prevalence and percentage of resistant bacteria among heteroresistant TB highlight the importance of LPA for early detection of heteroresistant TB.

Introduction

Tuberculosis (TB) continues to be a global public health threat. The effective management of TB and multidrug/rifampicin-resistant (MDR/RR) TB relies upon rapid diagnosis and treatment (WHO, 2011). Molecular diagnostic methods have led to the ability to detect drug-resistant TB more rapidly compared with phenotypic methods (i.e. culture and drug susceptibility testing [DST]), which usually require a minimum of 2 months (WHO, 2016a; WHO, 2016b). However, molecular diagnostic tests do not fully eliminate the need for phenotypic DST because the currently available rapid molecular methods do not detect all resistance mechanisms or the emergence of new mutations associated with resistance (WHO, 2015).

Molecular diagnostic methods have been also utilized to detect mixed infections (GLI, 2018). Mixed infections challenge the diagnosis and treatment of patients (Liang et al., 2018; Sergeev et al., 2011). A number of studies in countries with a high prevalence of TB and human immunodeficiency virus (HIV) coinfection have reported mixed infections (Dickman et al., 2010; Post et al., 2004; Stavrum et al., 2009; Warren et al., 2004). Various studies have reported mixed infections with and without heteroresistance (Cohen et al., 2011; Zheng et al., 2015). Line probe assays (LPAs) are designed to detect the most common resistance mutations for first-line and second-line drugs. LPAs can also simultaneously detect wild-type and resistance-conferring mutations in a single patient, which is referred to as heteroresistance (GLI, 2018).

Information on the agreement between phenotypic with genotypic resistance detection, and the prevalence of heteroresistance, has important implications for an individual patient's management as well as for TB control programs (Sergeev et al., 2011; WHO, 2015). In Ethiopia, there are limited data on the agreement between second-line phenotypic

* Corresponding author: Muluwork Getahun, Ethiopian Public Health Institute, P.O. Box 1242, Addis Ababa, Ethiopia, Tel: +251 911893728
E-mail address: mimishaget@yahoo.com (M. Getahun).

https://doi.org/10.1016/j.ijregi.2021.12.007
Received 22 September 2021; Received in revised form 28 November 2021; Accepted 19 December 2021

DST and LPA, or on the prevalence of heteroresistant TB (Damena et al., 2019; Mekonnen et al., 2015). To date, a population-based study in Ethiopia to investigate these particular issues has not been carried out.

Our study utilized the availability of *Mycobacterium tuberculosis* (*Mtb*) strains collected throughout the country for national drug-resistance surveys (DRS). The goal of the study was to assess the agreement between genotypic with phenotypic resistance detection, and to determine the prevalence of heteroresistance in isoniazid (INH)-resistant and MDR/RR TB isolates collected for drug-resistance surveys in Ethiopia.

Methods

This study used stored MDR/RR and INH-resistant *Mtb* isolates collected during a population-based drug-resistance survey (DRS) carried out in Ethiopia between November 2011 and June 2013. The DRS was conducted at 32 health facilities. Among the 32 health facilities, 30 had been included in an earlier DRS (2003–2005) with the additional two purposely selected from regions that were not previously included, and to ensure that at least one health facility was included from each region. The target population of the DRS was newly diagnosed patients with sputum smear-positive TB. The estimated sample size of the DRS needed to determine the extent of MDR TB was 1420 new sputum smear-positive patients. Previously treated sputum smear-positive TB cases diagnosed during the study period were also included. All consecutive sputum smear-positive TB cases were included in the DRS, while those patients who were already receiving treatment for TB were excluded from the survey. The survey detected 67 MDR, five RR and 70 INH-resistant TB isolates using Löwenstein-Jensen (LJ) DST. Two sample sets of *Mtb* isolates were created. The first set was either INH-resistant, RR, or MDR *Mtb*, as defined by LJ DST, with LPA performed for 61 MDR, 4 RR, and 62 INH-resistant TB isolates. The second comprised *Mtb* isolates (N=2) resistant to fluoroquinolones (FQ) and/or second-line injectable agents (amikacin, kanamycin, and capreomycin), as defined using the Sensititre MYCOTB MIC plate (Hall et al., 2011; Heysell et al., 2015; Lee et al., 2014).

LJ DST

Phenotypic DST for first-line drugs, which include INH (0.2 μg/ml) and rifampicin (RIF) (40 μg/ml), was performed using the indirect proportion method on LJ (Kent, 1985). Interpretation of results was based on the proportions of growth on the control and drug-containing media. More than 1% growth on drug-containing media was interpreted as resistance (Canetti et al., 1969; Kent, 1985). The percentage of resistance was determined as described by Kent (Kent, 1985). Whenever possible, the actual growth count was recorded as follows: 1+ (50–100 colonies), 2+ (100–200 colonies), 3+ (200–500 colonies), and 4+ (> 500 colonies). The percentage of resistance was determined using the minimum (lower limit of drug-containing media and upper limit of drug-free media) and maximum (upper limit of drug-containing media and lower limit of drug-free media), reported as a range.

Sensititre MYCOTB MIC plate

DST for FQ and SLI (amikacin, kanamycin, and capreomycin) was performed for MDR/RR TB isolates (n = 48) using Sensititre MYCOTB MIC plates (MYCOTB; Trek Diagnostic Systems, Thermo Fisher Scientific, USA) as per the manufacturer's guidelines. Resistance was defined based on the critical concentration of each drug, as previously described (Hall et al., 2011; Heysell et al., 2015; Lee et al., 2014; WHO, 2018a). The results were read via inverted mirrors and interpreted on days 10 and 21 if growth was poor. MDR TB and any FQ resistance with and without SLI were defined as pre-extensive drug resistant (pre-XDR) TB (World Health Organization 2020).

Table 1
Background characteristics of patients with drug-resistant TB

Variables		Total	MDR/RR		INH resistant	
			N	%	*N*	%
Total		127	65	51.2	62.0	48.8
Age	Mean age, years	30	28	–	32	–
Sex	Male	76	33	43	43	57
	Female	51	32	63	19	37
HIV status	Positive	34	21	32.3	13	20.9
	Negative	84	41	63.1	43	69.3
	Unknown	9	3	4.6	6	9.7
History of Treatment	New	74	27	41.5	47	75.8
	Previously treated	53	38	58.5	15	24.2

MDR: multidrug resistant; RR: rifampicin resistant; INH: isoniazid

Molecular DST and interpretation

GenoType MTBDR*plus* and MTBDR*sl* assays were performed on *Mtb* isolates as recommended by the manufacturer (HainLifescience, Nehren, Germany). DNA was extracted using a GenoLyse® kit (HainLifescience GmbH, Nehren, Germany); amplification and detection were performed using GenoType®MTBDR*plus* version 2.0 and GenoType®MTBDR*sl* version 1.0 (HainLifescience GmbH, Nehren, Germany). Simultaneous detection of wild-type and resistance-conferring mutations using LPA in a single patient was referred to as heteroresistance (GLI, 2018; WHO, 2016a; WHO, 2016b).

Quality control

All tests, including LJ DST, Sensititre MYCO TB MIC plate, and LPA (MTBDR*plus* and MTBDR*sl*) used positive and negative controls, which were within the target values. LPA results with uncommon patterns, i.e less frequent mutations, heteroresistance, or faint bands, were retested and confirmed. Any discrepant results as obtained by both DST procedures were retested and confirmed.

Statistical analysis

The sensitivity and specificity of LPA were compared with phenotypic DST for both first- and second-line drugs. The frequencies and proportions of heteroresistance were stratified by sex, age, history of treatment, HIV, and DST results. A chi-square test was used to assess the association, and a p-value < 0.05 was considered significant.

Results

Patient characteristics

The mean age of the patients with drug-resistant TB isolates (n = 127) was 30 years; 76 (60%) were male, and 53 (42%) of the cases had a previous history of TB treatment. Newly diagnosed TB cases made up 41% (27/65) of MDR/RR TB and 74% (47/62) of INH-resistant TB cases. HIV status was available for 118 patients, and 34 (27%) were HIV seropositive (Table 1).

Phenotypic and genotypic DST concordance

In total, 142 drug-resistant TB isolates (MDR = 67, RR = 5, and INH = 70) were detected using LJ DST; data were available for 127 isolates, including 61 MDR, 4 RR, and 62 INH-resistant. Unavailable MDR/RR and INH-resistant TB data were either not retrieved (MDR = 5, RR = 1, and INH = 8) or invalid LPA (MDR = 1). The sensitivity of LPA (MTBDR*plus*) was 96% (95% CI 84.9–98.2) for any INH and 94% (95% CI 90.7–98.6) for any RIF, when compared with LJ DST results. LPA did not detect RIF resistance in 6% of MDR/RR TB isolates and in 4.1% of

Table 2
Agreement of first-line phenotypic and genotypic results among multidrug/rifampicin (MDR/RR) and isoniazid (INH)-resistant TB isolates

LJ DST result	Total tested	First-line probe assay		Sensitivity (95% CI)
		Resistant	Susceptible	
Rif-R among MDR	61	58	3	95% (86–99)
Rif-R among RR	4	3	1	75% (19–99)
Overall Rif-R	65	61	4	94% (85–98)
INH-R among MDR	61	58	3	95 % (86–99)
INH-R among mono-INH	62	60	2	97% (89–99)
Overall INH	123	118	5	96% (91–99)

Rif-R: rifampicin resistant; INH-R: isoniazid resistant

Table 3
Percentage of resistance for drug susceptibility discordant and heteroresistance cases

		Frequency	RIF	INH	History of treatment
Discordant	MDR	1	5.9–11.8	5.9–11.9	Retreatment
		1	16.6–41.6	_	Retreatment
		1	11.8–29.4	_	Retreatment
		1	_	3.3–6.6	Retreatment
		1	_	6.2–12.5	Retreatment
	RIF	1	10.0–25.0	_	Retreatment
	INH	1	_	10.0–20.0	New
		1	_	1.0–2.0	New
Heteroresistance	MDR	1	1.0–2.0	_	New
		1	_	1.0–2.0	New
	INH	1	_	2.0–5.0	New
		1	_	2.0–4.0	New
		1	_	1.0–4.0	Retreatment
		1	_	2.4–5.0	New
		1	_	2.0–10.0	Retreatment
		1	_	1.6	New
		1	_	2.0–10.0	New
		1	_	1.0–5.0	Retreatment
		2	_	2.0–10.0	New

MDR: multidrug resistant; RIF: rifampicin; INH: isoniazid

INH-resistant TB isolates (Table 2). The percentage of resistant bacteria ranged from 5.9% to 41.6% in seven out of nine discordant cases. All RIF discordant cases were from previously treated TB patients (Table 3). Second-line phenotypic DST results were available for 48 MDR/RR TB isolates using the Sensititre MYCOTB MIC plate. One TB isolate was capreomycin monoresistant and the other ones were FQ and SLI resistant. LPA (MTBDR*sl*) did not detect resistance mutations for one isolate with FQ resistance and one with capreomycin monoresistance.

Heteroresistance

The prevalence of heteroresistance among MDR and INH-resistant TB was 9.8% (95% CI 5.2–16.4). The coexistence of wild-type and mutant isolates is shown in Supplementary Figure 1. The percentages of resistant bacteria among 12 heteroresistant TB isolates were between 1% and 10%. Of the INH-heteroresistant TB cases, 70% had been newly diagnosed TB patients (Table 3). Table 4 shows the frequencies of heteroresistance by demography, clinical data, and drug-resistance profiles for 123 isolates. Rifampicin heteroresistance was detected in 1.6% of MDR TB patients. INH heteroresistance was detected in 1.6% of MDR TB and 16.7% of INH-resistant TB patients. Heteroresistance was significantly higher among INH-resistant TB isolates ($n = 10$, 16.7%) compared with MDR/RR TB ($n = 2$, 3.2%) (p = 0.012). The proportion of INH heteroresistance was significantly higher ($p = 0.030$) in persons living with HIV.

Discussion

LPA involves targeted genotypic DST assays designed to detect the most common mutations, and is used for rapid diagnosis in order for patients to receive appropriate treatment (WHO, 2019). LPA is recommended for the rapid detection of resistance to first-line drugs (RIF and INH) and second-line drugs (FQ and SLI) (WHO, 2016a; WHO, 2016b). In our study, LPA failed to detect RIF- or INH-resistance mutations in 6% and 4.1% of the *Mtb* isolates, respectively. This difference could be due to resistance mutations that are not detected by the currently available LPAs (Feuerriegel et al., 2009; Kang et al., 2019; Ocheretina et al., 2014; Takawira et al., 2017). Moreover, in our study, one FQ-resistant and one capreomycin-resistant TB were not detected by SL-LPA. Similarly, this discordance could be due to mutations outside the LPA-targeted gene (Feuerriegel et al., 2009; Jugheli et al., 2009; Zaunbrecher, 2010).

Table 4
Heteroresistance by demography, clinical data, and drug sensitivity results

Variables		Total	Heteroresistance	
			N	%
Total		123	12	9.8
Sex	Female	48	7	14.6
	Male	75	5	6.7
Age	< 15	3	0	0.0
	15–24	45	5	11.1
	25–34	38	3	7.9
	35–44	18	1	5.6
	45–54	12	3	25.0
	55–64	6	0	0.0
	65+	1	0	0.0
HIV status	Negative	82	7	8.5
	Positive	33	5	15.2
Treatment history	Newly diagnosed	72	9	12.5
	Retreatment	51	3	5.9
Drug profiles	INH resistant	60	10	16.7
	Overall MDR TB	63	2	3.2
	Rifampicin among MDR	63	1	1.6
	INH among MDR	63	1	1.6
	HIV negative INH resistant	42	5	11.9
	HIV Positive INH resistant	13	5	38.5

INH: isoniazid; MDR: multidrug resistant; TB: tuberculosis

Heteroresistant TB challenges the diagnosis and treatment of patients (Liang et al., 2018; Sergeev et al., 2011). In our study, rifampicin heteroresistance was detected in 1.6% of MDR TB using LPA. Patients with heteroresistant TB are more likely to be undetected using the frontline molecular detection tools (Folkvardsen et al., 2013a; Folkvardsen et al., 2013b; Liang et al., 2018). The Xpert MTB/RIF assay can detect rifampicin heteroresistance when the resistant *Mtb* subpopulation accounts for > 50% of bacterial populations (Shin et al., 2018). Our results suggest that a certain proportion of MDR TB isolates could not be detected using the Xpert MTB/RIF assay. Furthermore, the risk of poor treatment outcomes is higher in heteroresistant MDR TB patients (Baffoe-Bonnie et al., 2019; Zetola et al., 2014). Those patients might be also misclassified as having acquired drug resistance (Sergeev et al., 2011). This highlights the importance of heteroresistant MDR TB detection at baseline, and the follow-up of MDR TB patients.

Isoniazid is one of the key first-line drugs for the treatment of active TB, as well as latent TB infection (WHO, 2018b). Undetected INH-resistant TB increases the risk of treatment failure or relapses, and would also have a role in the continued transmission of INH resistance (Gegia et al., 2017). In our study, the prevalence of heteroresistance was significantly higher among INH-resistant isolates than in MDR/RR TB ($p = 0.012$), which was consistent with the results reported in previous studies (Gupta et al., 2018; Shin et al., 2018). Most (70%) of INH heteroresistance in our findings was detected in newly diagnosed TB patients. A significantly higher prevalence of INH heteroresistance could be the result of simultaneous infection with drug-susceptible and resistant TB strains. Furthermore, the prevalence of INH-resistant TB, the detection method used, and the treatment strategy can also influence the heteroresistance prevalence. In Ethiopia at the time of the survey (2011–2013), there was no national strategy to detect and treat

1.	CC
2.	AC
3.	TUB
4.	*rpoB* Locus Control
5.	*rpoB* WT1
6.	*rpoB* WT2
7.	*rpoB* WT3
8.	*rpoB* WT4
9.	*rpoB* WT5
10.	*rpoB* WT6
11.	*rpoB* WT7
12.	*rpoB* WT8
13.	*rpoB* MUT1
14.	*rpoB* MUT2A
15.	*rpoB* MUT2B
16.	*rpoB* MUT3
17.	*katG* Locus Control
18.	*katG* WT1
19.	*katG* MUT1
20.	*katG* MUT2
21.	*inhA* Locus Control
22.	*inhA* WT1
23.	*inhA* WT2
24.	*inhA* MUT1
25.	*inhA* MUT2
26.	*inhA* MUT3A
27.	*inhA* MUT3B
	Colored marker

Labels for each line

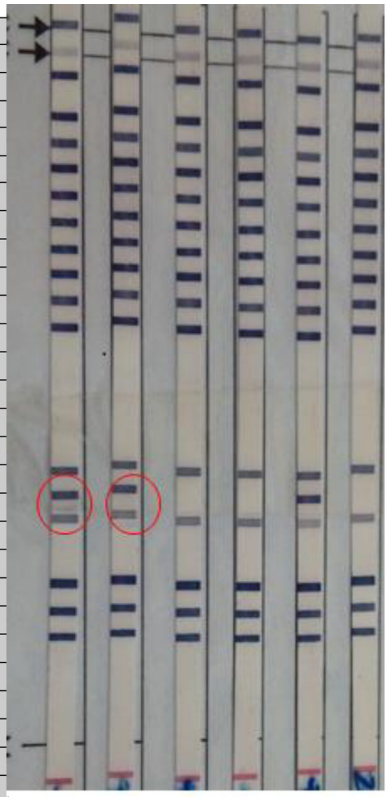

Image 1

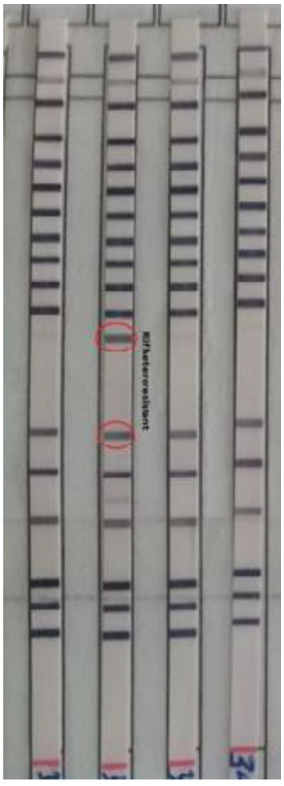

Image 2

Figure 1. The label for each line indiates the corresponding probe desription. Image 1 includes isonazid heteroresistance (circled in red). Image 2 includes rifampicin heteroresistance (circled in red).

INH-resistant TB, which also increased the prevalence of INH-heteroresistant TB in our findings.

HIV infection increases the susceptibility to infection or reinfection with TB (El-Sadr and Tsiouris, 2008; Guerra-Assunção et al., 2015). Previous studies have reported that persons living with HIV can be infected with more than one *Mtb* strain (Baffoe-Bonnie et al., 2019; Chaves et al., 1999; Cohen et al., 2011; Dickman et al., 2010; Stavrum et al., 2009; Zetola et al., 2014). Our study found a significantly higher prevalence ($p = 0.03$) of INH-heteroresistant TB isolates recovered from persons living with HIV. In line with our findings, some studies have reported heteroresistance in HIV/TB coinfected individuals (Baffoe-Bonnie et al., 2019; Cohen et al., 2011; Zetola et al. 2014). Treating mixed infections with conflicted drug profiles can lead to patients responding poorly to the treatment by allowing the drug-susceptible strain to re-emerge or outnumber the drug-resistant strains (Sergeev et al., 2011; Tolani et al., 2012; van Rie et al., 2005). Furthermore, heteroresistance in HIV-infected patients has been associated with longer times to culture conversion (Zetola et al., 2014).

Our study had certain limitations. The use of culture isolate had a lower likelihood of identifying heteroresistant TB. The percentage of resistant bacteria was calculated using minimum and maximum colony ranges. The proportion of heteroresistance cases that had ≥ 5% resistant bacteria was not explicitly reported. Moreover, there were no sequencing data to confirm the heteroresistant TB cases.

Figure 1

Conclusion

This study demonstrated a general agreement between genotypic and phenotypic drug-resistance detection, and the prevalence of heteroresistance in MDR and INH-resistant *Mtb* isolates from newly diagnosed TB patients. The results revealed that some drug-resistance cases might not be captured by LPA, which suggests that a proportion of rifampicin- and isoniazid-resistant TB might continue as a source of infection unless these mutations are captured by molecular diagnostic tools or phenotypic DST. Furthermore, the occurrence of heteroresistant TB in newly diagnosed patients with MDR/RR and INH-resistant TB is not rare. The prevalence of resistant bacteria among heteroresistant TB highlights the importance of LPA for the early detection of heteroresistance, which can assist in tailoring the treatment regimen and prevent further transmission.

Conflicts of interest

There are no conflicts of interest.

Author contributions

MG conceived and developed the protocol, conducted the study, and drafted the manuscript. DB and GA reviewed and edited the draft manuscript. HM and GD conducted laboratory analysis and quality control. All authors read the manuscript.

Funding sources

This work was supported in part by the Ethiopian Public Health Institute (EPHI) and a grant from the National Institutes of Health (NIH) Fogarty International Center (D43 TW009127).

Ethical approval statement

The study was approved by the Addis Ababa University Ethics Committee/Institutional Review Board.

References

Baffoe-Bonnie A, Houpt ER, Turner L, et al. Drug-susceptible and multidrug-resistant Mycobacterium tuberculosis in a single patient. Emerg Infect Dis 2019;25(11):2120–1.

Canetti G, Fox W, Khomenko A, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 1969;41(1):21–43.

Chaves F, Dronda F, Alonso-Sanz M, et al. Evidence of exogenous reinfection and mixed infection with more than one strain of Mycobacterium tuberculosis among Spanish HIV-infected inmates. AIDS 1999;13(5):615–20.

Cohen T, Wilson D, Wallengren K, et al. Mixed-strain Mycobacterium tuberculosis infections among patients dying in a hospital in KwaZulu-Natal, South Africa. J Clin Microbiol 2011;49(1):385–8.

Damena D, Tolosa S, Hailemariam M, et al. Genetic diversity and drug susceptibility profiles of Mycobacterium tuberculosis obtained from Saint Peter's TB specialized hospital, Ethiopia. PLoS One 2019;14(6).

Dickman KR, Nabyonga L, Kateete DP, et al. Detection of multiple strains of Mycobacterium tuberculosis using MIRU-VNTR in patients with pulmonary tuberculosis in Kampala, Uganda. BMC Infect Dis 2010;10:349.

El-Sadr WM, Tsiouris SJ. HIV-associated tuberculosis: diagnostic and treatment challenges. Semin Respir Crit Care Med 2008;29(5):525–31.

Feuerriegel S, Cox HS, Zarkua N, et al. Sequence analyses of just four genes to detect extensively drug-resistant Mycobacterium tuberculosis strains in multidrug-resistant tuberculosis patients undergoing treatment. Antimicrob Agents Chemother 2009;53(8):3353–6.

Folkvardsen DB, Svensson E, Thomsen VØ, et al. Can molecular methods detect 1% isoniazid resistance in Mycobacterium tuberculosis? J Clin Microbiol 2013a;51(5):1596–9.

Folkvardsen DB, Thomsen VØ, Rigouts L, et al. Rifampin heteroresistance in Mycobacterium tuberculosis cultures as detected by phenotypic and genotypic drug susceptibility test methods. J Clin Microbiol 2013b;51(12):4220–2.

Gegia M, Winters N, Benedetti A, et al. Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. Lancet Infect Dis 2017;17(2):223–34.

Global Laboratory Initiative. Line probe assays for drug-resistant tuberculosis detection. Interpretation and reporting guide for laboratory staff and clinicians. Geneva, Switzerland: World Health Organization; 2018.

Guerra-Assunção JA, Houben RM, Crampin AC, et al. Recurrence due to relapse or reinfection with Mycobacterium tuberculosis: a whole-genome sequencing approach in a large, population-based cohort with a high HIV infection prevalence and active follow-up. The Journal of Infectious Diseases 2015;211(7):1154–63.

Gupta R, Thakur R, Kushwaha S, et al. Isoniazid and rifampicin heteroresistant Mycobacterium tuberculosis isolated from tuberculous meningitis patients in India. Indian J Tuberc 2018;65(1):52–6.

Hall L, Jude KP, Clark SL, et al. Antimicrobial susceptibility testing of Mycobacterium tuberculosis complex for first and second line drugs by broth dilution in a microtiter plate format. J Vis Exp 2011(52):3094.

Heysell SK, Pholwat S, Mpagama SG, et al. Sensititre MycoTB plate compared to Bactec MGIT 960 for first- and second-line antituberculosis drug susceptibility testing in Tanzania: a call to operationalize MICs. Antimicrob Agents Chemother 2015;59(11):7104–8.

Jugheli L, Bzekalava N, de Rijk P, et al. High level of cross-resistance between kanamycin, amikacin, and capreomycin among Mycobacterium tuberculosis isolates from Georgia and a close relation with mutations in the rrs gene. Antimicrob Agents Chemother 2009;53(12):5064–8.

Kang JY, Hur J, Kim S, et al. Clinical implications of discrepant results between genotypic MTBDRplus and phenotypic Löwenstein-Jensen method for isoniazid or rifampicin drug susceptibility tests in tuberculosis patients. Journal of Thoracic Disease 2019;11(2):400.

Kent PT. Public health mycobacteriology: a guide for the level III laboratory. US Department of Health and Human Services, Public Health Service, Centers for Disease Control; 1985.

Lee J, Armstrong DT, Ssengooba W, et al. Sensititre MYCOTB MIC plate for testing Mycobacterium tuberculosis susceptibility to first- and second-line drugs. Antimicrob Agents Chemother 2014;58(1):11–18.

Liang B, Tan Y, Li Z, Tian X, et al. Highly sensitive detection of isoniazid heteroresistance in Mycobacterium tuberculosis by DeepMelt Assay. J Clin Microbiol 2018;56(2):e01239-17.

Mekonnen D, Admassu A, Mulu W, et al. Multidrug-resistant and heteroresistant Mycobacterium tuberculosis and associated gene mutations in Ethiopia. Int J Infect Dis 2015;39:34–8.

Ocheretina O, Escuyer VE, Mabou MM, et al. Correlation between genotypic and phenotypic testing for resistance to rifampin in Mycobacterium tuberculosis clinical isolates in Haiti: investigation of cases with discrepant susceptibility results. PLoS One 2014;9(3):e90569.

Post FA, Willcox PA, Mathema B, et al. Genetic polymorphism in Mycobacterium tuberculosis isolates from patients with chronic multidrug-resistant tuberculosis. J Infect Dis 2004;190(1):99–106.

Sergeev R, Colijn C, Cohen T. Models to understand the population-level impact of mixed strain M. tuberculosis infections. J Theor Biol 2011;280(1):88–100.

Shin SS, Modongo C, Baik Y, et al. Mixed Mycobacterium tuberculosis-strain infections are associated with poor treatment outcomes among patients with newly diagnosed tuberculosis, independent of pretreatment heteroresistance. J Infect Dis 2018;218(12):1974–82.

Stavrum R, Mphahlele M, Ovreås K, et al. High diversity of Mycobacterium tuberculosis genotypes in South Africa and preponderance of mixed infections among ST53 isolates. J Clin Microbiol 2009;47(6):1848–56.

Takawira FT, Mandishora RSD, Dhlamini Z, et al. Mutations in rpoB and katG genes of multidrug resistant mycobacterium tuberculosis undetectable using genotyping diagnostic methods. Pan Afr Med J 2017;27:145.

Tolani MP, D'souza DT, Mistry NF. Drug resistance mutations and heteroresistance detected using the GenoType MTBDRplus assay and their implication for treatment outcomes in patients from Mumbai, India. BMC Infect Dis 2012;12:9.

van Rie A, Victor TC, Richardson M, et al. Reinfection and mixed infection cause changing Mycobacterium tuberculosis drug-resistance patterns. Am J Respir Crit Care Med 2005;172(5):636–42.

Warren RM, Victor TC, Streicher EM, et al. Patients with active tuberculosis often have different strains in the same sputum specimen. Am J Respir Crit Care Med 2004;169(5):610–14.

World Health Organization. Towards universal access to diagnosis and treatment of multidrug-resistant and extensively drug-resistant tuberculosis by 2015: WHO progress report 2011. World Health Organization. 2011.

World Health Organization. Implementing tuberculosis diagnostics: policy framework. World Health Organization; 2015.

World Health Organization. The use of molecular line probe assay for the detection of resistance to isoniazid and rifampicin: policy update. World Health Organization; 2016.

World Health Organization. The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance. World Health Organization; 2016.

World Health Organization. Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. World Health Organization; 2018a.

World Health Organization. WHO treatment guidelines for isoniazid-resistant tuberculosis: supplement to the WHO treatment guidelines for drug-resistant tuberculosis. World Health Organization; 2018b.

World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. World Health Organization; 2019.

World Health Organization. Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27–29 October, 2020.

Zaunbrecher MA. Low-level kanamycin resistance in Mycobacterium tuberculosis: molecular mechanisms and clinical implications. Emory University; 2010 PhD diss..

Zetola NM, Modongo C, Moonan PK, et al. Clinical outcomes among persons with pulmonary tuberculosis caused by Mycobacterium tuberculosis isolates with phenotypic heterogeneity in results of drug-susceptibility tests. J Infect Dis 2014;209(11):1754–63.

Zheng C, Li S, Luo Z, et al. Mixed infections and rifampin heteroresistance among Mycobacterium tuberculosis clinical isolates. J Clin Microbiol 2015;53(7):2138–47.