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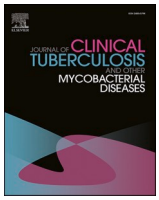

Discordance between genotypic and phenotypic methods for the detection of rifampicin and isoniazid resistant *Mycobacterium tuberculosis* and the correlation with patient treatment outcomes

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ABSTRACT

Background: Accurate drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB) is essential for proper patient management. We investigated discordance between genotypic (Xpert MTB/RIF and MTBDR*plus*) and phenotypic (MGIT 960) methods for the detection of rifampicin (RIF) and isoniazid (INH) resistant MTB and its correlation with patient treatment outcomes in Jimma, Southwest Oromia, Ethiopia.

Methods: A retrospective study was conducted on 57 stored MTB isolates with known Xpert RIF resistance status (45 RIF resistant and 12 RIF susceptible) at Jimma University Mycobacteriology Research Center from November 2, 2021, to December 28, 2022. We did MTBDR*plus* and phenotypic DST (using the Mycobacterial Growth Indicator Tube (MGIT) system). The Xpert and MTBDR*plus* results were compared using phenotypic DST as a reference standard method. The treatment outcome was determined as per national guideline. The discordance between the genotypic and phenotypic DST was calculated using GraphPad software.

Results: Among the 57 MTB isolates, six (10.5 %) had discordant results between the two DST methods. Xpert yielded five discordant results for RIF when compared with phenotypic DST (kappa coefficient (κ) = 0.76, 95 % confidence interval 0.56–0.96). The MTBDR*plus* compared with phenotypic DST gave three discordant results for RIF (κ = 0.86, 95 % confidence interval 0.71–1.00) and three for INH (κ = 0.86, 95 % confidence interval 0.70–1.00). Compared with Xpert, MTBDR*plus* yielded lower discordance with phenotypic DST for RIF. Out of six patients with discordant results, three had unfavorable outcomes while the other three were cured. Of the three patients with unfavorable outcomes, only one patient has received an inappropriate treatment regimen. There was no correlation between unfavorable outcomes and incorrect treatment regimens due to discordant results ($X^2 = 0.404$; P = 0.525).

Conclusions: Discordance between genotypic and phenotypic DST for RIF or INH occurred in 10.5 % of isolates. Only one patient with discordant results has received an inappropriate treatment regimen, resulting in an unfavorable outcome. The impact of parallel use of rapid molecular assay with phenotypic DST on patient treatment outcomes requires further study.

1. Introduction

Tuberculosis (TB) remains a significant global public health challenge, with 10.6 million people affected and 1.3 million deaths in 2022 alone; 167,000 of these deaths occurred among people co-infected with Human Immunodeficiency Virus (HIV) [1]. Over 80 % of TB morbidity and mortality occurred in low- and middle-income countries [2]. Ethiopia is one of the 30 high TB burden countries, with an estimated 156,000 incident TB cases and 22,700 deaths in 2022 [1].

Multidrug-resistant or rifampicin resistant TB (MDR/RR-TB) poses significant challenges to TB control efforts due to gaps in detection and treatment. MDR-TB refers to resistance to rifampicin and isoniazid, the two most effective first-line anti-TB drugs. Globally in 2022, of 410,000 of MDR/RR-TB cases estimated to occur, only 176,600 were detected and 175,650 were given treatment [1]. A systematic review of 24 studies conducted in Ethiopia revealed that 2.6 % of new TB cases and 11.5 % of previously treated cases had MDR/RR-TB [3].

Diagnosis of MDR/RR-TB includes the use of both phenotypic and

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genotypic methods. Growth based phenotypic DST remains the golden standard. However, it is expensive, time consuming, require sophisticated laboratory infrastructure, qualified staff and strict quality control [4]. The genotypic method that include Xpert MTB/RIF (Xpert), Xpert MTB/RIF Ultra and MTBDR*plus* enables faster diagnosis by detecting mutations in the 81-bp hotspot region of the *rpoB* gene, conferring RIF resistance [5–7]. MTBDR*plus* can also detect additional mutations in the *katG* and *inhA* genes for INH resistance [8]. However, these genotypic tests all have limitations such as lack of sensitivity, unable to detect all resistance mechanisms, and thus can't provide full resistance profiles [9]. DNA sequencing may still be needed for confirmation, but its use is limited in low-income countries [10].

Phenotypic DST not routinely performed in Ethiopia. The genotypic (Xpert and MTBDR*plus*) are the most widely utilized for the detection of MDR/RR-TB [11]. However, studies have shown varying rates of discordant results between these assays and phenotypic DST. In Rwanda, 47 % of 121 patients with Xpert RR-TB were susceptible to RIF by phenotypic testing [12]. In Eastern Ethiopia, one isolate initially identified as RIF resistant by Xpert was found to be susceptible with phenotypic DST [13]. Wondale et al. compared the performance of MTBDR*plus* with phenotypic DST and found discrepancies between the two methods in three isolates, two for INH and one for RIF [14]. Additionally, MTBDR*plus* showed 11.68 % discordance rate in detecting INH resistance among MTB clinical isolates, emphasizing the need for caution when relying solely on MTBDR*plus* assays for the detection of INH resistance [15].

Investigation of discordance between genotypic and phenotypic DST methods for the detection of RIF and INH resistant MTB could provide evidence on the impact of discordant results on patient outcomes. Therefore, this study was conducted to investigate discordance between genotypic (Xpert and MTBDR*plus*) and phenotypic (MGIT 960) DST methods for the detection RIF and INH resistance in MTB and its correlation with patient treatment outcomes.

2. Materials and methods

A retrospective study was conducted at Jimma University Mycobacteriology Research Center (JUMRC), Jimma, Ethiopia, from November 2, 2021, to December 28, 2022. JUMRC is the only laboratory providing MTB culture and DST services for inpatients and outpatients from Jimma Medical Center and those referred from other health facilities in Southwest Oromia, Ethiopia [16]. Shanan Gibe Hospital (SGH) is one of the public hospitals located in Jimma Town. It is the only hospital that provides MDR-TB treatment in a Jimma zone. This study was conducted on stored MTB isolates at JUMRC for the previous study of DIAMA trial. All isolates were obtained from sputum Xpert positive patients visiting SGH between November 2017 and March 2021 who were ≥15 years old and diagnosed with RIF resistant (both new and previously treated cases) or RIF susceptible (previously treated cases only) TB. From a total of 60 stored isolates for the DIAMA trial, 57 (45 RIF resistant and 12 RIF susceptible) were successfully recovered and included into this study. All patients were treated with the nationally recommended regimen under routine program protocols at SGH. The 12 patients with RIF susceptible TB were treated with the standard first line regimen for six months. Among the 45 patients with RR-TB, 37 were treated with one of two WHO approved MDR-TB treatment regimens: the 9–12 month shorter regimen or the 18–24 month longer regimens [17].

Data were collected from patient records using standard data extraction sheet. The collected data includes Xpert result (RIF resistant or RIF susceptible), HIV status (positive or negative), previous TB treatment (new or previously treated), treatment outcomes. Treatment outcomes data were categorized into favorable outcome (the sum of cure and treatment completed) and unfavorable outcome (sum of treatment failure, loss to follow-up, and death) according to the national guideline [11]. The definitions of treatment outcome for drug susceptible and resistant TB used in this study is presented in supporting information (Supplemental Table 1).

The frozen suspension of stored isolates was thawed at room temperature and inoculated on Löwenstein-Jensen media according to the procedure described in the Global Laboratory Initiative (GLI) manual [18]. The BACTEC MGIT960 system was used to perform phenotypic DST on recovered isolates at critical concentrations of 1.0 μg/mL for streptomycin (STR), 0.1 μg/mL for INH, 1.0 μg/mL for RIF, and 5.0 μg/mL for ethambutol (EMB) [19]. In addition, the GenoType MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) was performed according to the manufacturer's instructions to detect RIF and INH resistance [20].

Descriptive summaries for tables were generated using IBM SPSS Statistics 20. Discordance between the genotypic and phenotypic DST was determined by online GraphPad software (San Diego, California, USA) [21]. Ethical approval was obtained from institutional review board (IRB) of Institute of Health. Official permission was obtained from the JUMRC to access the stored isolates and MDR-TB treatment initiating center of SGH to use patient's clinical and socio-demographic data. Informed consent was not obtained for this study as it is secondary analysis on stored isolates. However, written informed consent was obtained from each participant in the primary study (DIAMA trial).

3. Results

3.1. Baseline characteristics

Out of the 57 patients, 35 (61.4 %) were males, 10 (17.5 %) were TB/HIV co-infected, and 46 (80.7 %) were previously treated for TB. The mean age of participants was 30.4 years with a standard deviation of 13.6 years. Among the 57 MTB isolates, 45 (78.9 %) were RIF resistant and 12 (21.1 %) were RIF susceptible by Xpert (Table 1).

3.2. Discordant results between genotypic and phenotypic DST

Out of the 57 MTB isolates, six (10.5 %) had discordant genotypic (Xpert and MTBDR*plus*) and phenotypic (MGIT 960) DST results for RIF or INH. Three isolates exhibited discordant results for RIF between the two DSTs. Among these, two isolates had RIF resistant results by Xpert but susceptible by both the MTBDR*plus* and phenotypic DST. The remaining isolate was RIF resistant by both Xpert and MTBDR*plus*. However, phenotypic DST yielded susceptible result (Table 2).

Two isolates had discrepant results for both RIF and INH. Of these, one Xpert RIF susceptible isolate was found to be susceptible to both RIF and INH by the MTBDR*plus*. However, phenotypic DST indicated resistance to both RIF and INH. It was also resistant to STR and EMB. The other isolate had RIF resistant results by both genotypic method but susceptible by phenotypic DST. For INH, it was susceptible by the MTBDR*plus* but resistant by phenotypic DST. Similarly, another isolate

Table 1
Characteristics of the study population and MTB isolates (N = 57).

Characteristics	Categories	Number (%)
Sex	Male	35 (61.4)
	Female	22 (38.6)
Age (years), mean ± SD		30.4 ± 13.6
HIV status	Positive	10 (17.5)
	Negative	47 (82.5)
Previous TB treatment	Previously treated	46 (80.7)
	New	11 (19.3)
Xpert MTB/RIF result for RIF	Resistant	45 (78.9)
	Susceptible	12 (21.1)
Xpert MTB/RIF semi-quantitative grade (Ct value)	High (Ct < 16)	16 (28.1)
	Medium (16 < Ct < 22)	30 (52.6)
	Low (22 < Ct < 28)	8 (14.0)
	Very low (Ct > 28)	3 (5.3)

HIV, Human immunodeficiency virus; SD, standard deviation; TB, tuberculosis; RIF, rifampicin; Ct, cycle threshold.

Table 2
Xpert MTB/RIF, MTBDR*plus*, phenotypic DST, and treatment outcomes of six patients with discordant results for RIF or INH.

No.	Age	Sex	HIV status	Xpert result	Ct values of *rpoB* probes					MTBDR*plus*		Phenotypic DST		Treatment regimen	Treatment outcome
					A	B	C	D	E	RIF	INH	RIF	INH		
1*	50	Male	Negative	R	14.1	16	14.8	0	15.4	R	S	S	R	Shorter MDR-TB regimen	Cured
2**	35	Female	Negative	S	28.2	28.2	28.2	28.7	29.8	S	S	R	R	First-line regimen	Dead
3	22	Female	Negative	R	28.6	17.4	15.9	0	16.4	R	S	R	R	shorter MDR-TB regimen	Dead
4	25	Female	Negative	R	17.8	19.3	18.5	0	19	S	S	S	S	longer MDR-TB regimen	Cured
5***	23	Male	Negative	R	15.2	0	16.3	15.8	16.5	R	S	S	S	Shorter MDR-TB regimen	Cured
6****	21	Male	Positive	R	31.9	30.1	32.5	0	33.6	S	S	S	S	Shorter MDR-TB regimen	Dead

Three isolates showed discordance with phenotypic DST by both MTBDR*plus* and Xpert. Two isolates showed discordance only between Xpert and phenotypic DST, and one between MTBDR*plus* and phenotypic DST. *, identified as RIF resistant by MTBDR*plus* by the absence of rpoB wild type 7 hybridization; **, additional resistance to streptomycin and ethambutol; ***, Identified as RIF resistant by MTBDR*plus* by the absence of rpoB wild type 3 and 4 hybridization. ****, no previous TB treatment; no, number; INH, isoniazid; RIF, rifampicin; Ct, cycle threshold; R, resistant; S, susceptible; HIV, human immunodeficiency virus; DST, drug susceptibility testing.

showed INH discordance with genotypic susceptibility and phenotypic resistance to INH (Table 2).

Overall, five (8.8 %) isolates showed discordance between Xpert and phenotypic DST for RIF (kappa coefficient (κ) = 0.76, 95 % confidence interval 0.56–0.96). Four Xpert RIF resistant isolates were found to be susceptible by phenotypic DST. One Xpert RIF susceptible isolate was found to be resistant by phenotypic DST (Table 2). Four (7 %) isolates gave six discordant results between MTBDR*plus* and phenotypic DST: three for RIF (κ = 0.86, 95 % confidence interval 0.71–1.00) and three for INH (κ = 0.86, 95 % confidence interval 0.70–1.00). Among these four isolates, one isolate had discordant results for RIF, two for both RIF and INH, and one for INH. Genotypic susceptibility with phenotypic resistance to INH was the most common pattern of discordance. Although the number is low, MTBDR*plus* yielded lower discordance with phenotypic DST for RIF when compared to Xpert (κ = 0.86 versus 0.76) (Table 3).

3.3. Treatment outcomes

Of the 57 patients, 44 (77.2 %) had favorable outcomes (43 cured and one treatment completed), and 11 (19.3 %) had unfavorable outcomes (three treatment failed, three loss to follow up, and five died). Two (3.5 %) patients were transferred to another health facility, and treatment outcome was not evaluated (Table 4). Among six patients with discordant results, five patients were given appropriate treatment regimens. Two patients (number 4 and 6) were treated with WHO approved MDR-TB regimens when both MTBDR*plus* and phenotypic DST showed susceptible results. When one test shows RIF resistance, without DNA sequencing, many clinicians would treat it as MDR-TB. Thus, patients number 4 and 6 received appropriate treatment regimens. When two tests show RIF resistance, without DNA sequencing, almost all clinicians treat it as MDR-TB. Hence, patients number 1, 3, and 5 received appropriate treatment regimens. Only one patient (number 2) was inappropriately treated with a standard first-line regimen when the phenotypic DST showed MDR-TB, and died as a result (Table 2). There was no correlation between death and inappropriate regimens based on discordant results ($X^2 = 0.404$; P = 0.525).

Table 3
Drug susceptibility testing results for RIF and INH as determined by Xpert MTB/RIF and MTBDR*plus* in comparison with phenotypic DST (N = 57).

Genotypic Methods	Drug	Phenotypic DST		Total (N = 57)	Kappa Value (95 %CI)
Xpert*	**RIF**	**R**	**S**		
	Resistant	41	4	45	0.76 (0.56–0.96)
	Susceptible	1	11	12	
MTBDR*plus*	**RIF**				
	Resistant	41	2	43	0.86 (0.71–1.00)
	Susceptible	1	13	14	
	INH				
	Resistant	42	0	42	0.86 (0.70–1.00)
	Susceptible	3	12	15	

*, only rifampicin resistance is detected by this assay; CI, confidence interval; DST, drug susceptibility testing; RIF, rifampicin; INH, isoniazid.

Table 4
Treatment outcomes of patients with discordant and non-discordant DST results for RIF or INH (N = 57).

Treatment outcomes	Total (N = 57)	Non-discordant cases (n = 51)	Discordant cases (n = 6)
Cured	43	40	3
Completed	1	1	0
Failed	3	3	0
Loss to follow up	3	3	0
Died	5	2	3
Not evaluated *	2	2	0

* Both cases were RIF susceptible TB cases.

4. Discussion

Accurate DST of MTB is essential for proper patient management. The WHO recommended both genotypic and phenotypic DST methods for the detection of RIF and INH resistant MTB [4]. However, both methods have shortcomings, and discordances between the two DST methods occur. This could lead to inappropriate patient management. In Ethiopia, phenotypic DST is not routinely performed, and genotypic method are used to make treatment decisions [16].

In this study, a total of six (10.5 %) isolates had discordant results between the genotypic and phenotypic DST for RIF or INH. Our study found that genotypic resistance and phenotypic susceptibility to RIF were the most frequent patterns of discordance between Xpert and phenotypic DST. Discrepancies due to Xpert RIF resistance but susceptible by phenotypic DST were also reported from studies done in Ethiopia [13] and Rwanda [12]. In our study, four (7 %) isolates showed discordance between MTBDR*plus* and phenotypic DST for RIF or INH, which is lower than findings of 2.4 % in Southern Ethiopia [14] and 6.2 % in Northeastern Ethiopia [21].The difference might be due to sample size, study settings, and bacterial strains. Most of discordance between MTBDR*plus* and phenotypic DST was genotypic susceptibility and phenotypic resistance to INH. This is probably due to the presence of INH resistance conferring mutations other than *KatG* and *inhA* genes, such as *ahpC, fabG*1, and *ndh* genes. The MTBDR*plus* cannot detect these resistant subpopulations and may yield false susceptible results [22].

Our study found that one patient with discordant results had received an inappropriate treatment regimen, resulting in unfavorable outcomes. Treatment regimen is chosen based on DST results. As a result, discordant DST results would lead to either unnecessary toxicity or inadequate treatment. For instance, false detection of RIF resistance

from patients with drug sensitive MTB would lead to unnecessary treatment of TB patients with second-line drugs, which are toxic and less effective [23,24]. At the same time, misdiagnosis of patients with RR-TB as RIF susceptible TB would leads to inadequate treatment with first-line drugs, resulting in higher rates of treatment failure and mortality [23]. A multicenter study conducted in high TB burden countries reported higher mortality rate among patients with discordant results than patients with concordant results (25 % versus 16 %) [25]. On the other hand, deaths in TB/HIV co-infected patients might be associated with drug toxicity, side effects, and poor adherence [26,27]. However, this is a retrospective study and we were unable to collect these data elements.

In our study, two Xpert RIF resistant isolates were found to be susceptible by both MTBDR*plus* and phenotypic DST. Both isolates showed no signal for probe D hybridization on Xpert. One of these isolates was obtained from a TB/HIV co-infected patient who had no previous TB treatment. It had a very low Xpert semi-quantitative grade. The respective patient died before treatment completion with shorter MDR-TB regimen. In some cases, the absence of probe binding in Xpert might be caused by insufficient mycobacterial DNA rather than the *rpoB* mutation. This would lead to false RIF resistant results on Xpert [12].

The other isolate had a medium semi-quantitative grade of bacilli on Xpert. The respective patient received the longer MDR-TB regimen and had a favorable outcome. Xpert testing was performed on sputum, while MTBDR*plus* and phenotypic DST were performed on isolates from culture. In some cases, acquisition of RIF resistance may reduce the fitness of the bacilli and allows overgrowth of susceptible populations [28].

Another two isolates showed RIF resistance by both Xpert and MTBDR*plus* but susceptible by phenotypic testing. In one isolate, resistance to RIF was detected by the absence of probe B binding on Xpert and wild type 3 and 4 on MTBDR*plus*, both targeting codon 516 of the *rpoB* gene. The other isolate showed RIF resistance with failure of probe D binding on Xpert and wild type 7 on MTBDR*plus*, both covering codon 526 of the *rpoB* gene. In both isolates, no *rpoB* mutation band was detected by the MTBDR*plus*. Both patients had favorable outcomes with shorter MDR-TB regimen. In some cases, discordance between genotypic and phenotypic DST might be caused by the presence of silent mutations in the *rpoB* gene, which are not associated with RIF resistance. The genotypic DST detects these mutations as resistance, while phenotypic testing shows susceptibility [29,30]. Alternatively, the observed discrepancy could be attributed to disputed *rpoB* mutations, mutations conferring low-level RIF resistance. These mutations have slightly increased minimum inhibitory concentration below critical concentration used in MGIT. Xpert and MTBDR*plus* indicate RIF resistance in the presence of disputed mutations, while phenotypic DST shows RIF susceptible [31,32].

In this study, one isolate had genotypic susceptibility and phenotypic resistance for both RIF and INH. It had RIF susceptible result by Xpert but resistance to both RIF and INH by phenotypic DST. The MTBDR*plus* analysis yielded susceptible results for both RIF and INH. The respective patient died with standard first-line regimen including RIF and INH. Another two isolates had also genotypic susceptibility and phenotypic resistance to INH. Both patients were treated with shorter MDR-TB regimen, with one patient having unfavorable outcome. Genotypic DST detects resistance-conferring mutations in a selected target. For this reason, genotypic DST fails to detect new and rare mutations occurring outside the selected target and indicate susceptibility. Phenotypic DST on the other hand, can detect these mutations and indicate resistance. According to studies, 12.5 % isolates from Ethiopia [33] and 52.8 % from South Africa [34], had *rpoB* mutations outside RRDR. Additionally, it was found that 20 % of INH resistance was caused by mutations occurring outside *KatG* and *inhA* genes [35]. Parallel use of both genotypic and phenotypic DST is important to overcome these challenges.

Our study is not without limitations. First, we didn't perform DNA sequencing for isolates with discordant DST results for RIF or INH. Second, the WHO revised critical concentration for low-level RIF resistance was not utilized for this study. The small sample size, incomplete data on toxicity, and retrospective nature of this study were also considered as limitation.

5. Conclusions

Discordance between genotypic and phenotypic DST for RIF or INH occurred in 10.5 % of isolates. Although the numbers are low, our study found relatively lower discordance between the MTBDR*plus* and phenotypic DST for RIF when compared to Xpert. There was no correlation between death and inappropriate regimens based on discordant results. Further studies with larger number of isolates and a prospective study design are needed to determine whether adding phenotypic DST will aid in better patient treatment outcomes due to the presence of discordant phenotypic/genotypic DST results.

Authors' contributions

ZB, MT, WK, and GA were involved in the conception and design of the study. ZB and GB performed data extraction, laboratory experiments, data analysis, and interpretation. ZB was also involved in the drafting of the manuscript. MT, WK, GB, and GA critically reviewed the manuscript. GA also supervised the overall project activities. All authors made a significant contribution to the reported work in conception, design, analysis, and drafting. All authors have agreed to take responsibility and be accountable for the contents of the article.

CRediT authorship contribution statement

Zegeye Bonsa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Mulualem Tadesse:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Getu Balay:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Wakjira Kebede:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing. **Gemeda Abebe:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jctube.2023.100410.

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