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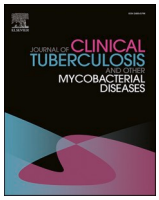

Resistance to pyrazinamide in *Mycobacterium tuberculosis* complex isolates from previously treated tuberculosis cases in Southwestern Oromia, Ethiopia

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ABSTRACT

Objective: Pyrazinamide (PZA) susceptibility testing is important to develop evidence-based algorithms for case management. We aimed to assess the prevalence of PZA-resistance and its impact on treatment outcomes in previously treated tuberculosis (TB) cases in southwestern Oromia, Ethiopia.

Methods: A Phenotypic Drug Susceptibility Testing (DST) of PZA with BACTEC MGIT 960 was conducted at the Mycobacteriology Research Center of Jimma University (MRC-JU) from June to November 2021 on sixty-six *Mycobacterium tuberculosis* complex (MTBC) isolates from previously treated TB cases. SPSS software package version 21 was used. The differences in the proportion of PZA resistance between the groups were compared using the chi squared test. Logistic regression was used to identify the association between PZA resistance and treatment outcomes.

Results: Among 66 MTBC isolates (49 rifampicin-resistant and 17 rifampicin-sensitive) included in this study, 31.8 % were resistant to PZA. The proportion of PZA resistance was almost three times higher in previously treated TB cases with rifampicin resistance than in rifampicin-sensitive patients (38.8 % vs. 11.8 %, $p = 0.039$). An unfavorable treatment outcome was documented for 23 % (15/65) of the participants. Patients with PZA resistance were almost four times more likely to have an unfavorable treatment outcome than patients with PZA sensitive (aOR 4.2, 95 % CI: 1.13–15.3).

Conclusions: The prevalence of PZA resistance was high compared to the pooled PZA resistance estimated worldwide. The majority of TB cases with PZA resistance had an unfavorable treatment outcome. PZA susceptibility testing should be included in the multidrug-resistant TB diagnostic algorithm to improve management of these patients.

1. Introduction

Tuberculosis (TB) is the world's leading cause of death from a single infectious agent [1]. Ethiopia is one of the 30 countries globally burdened with the highest TB and TB/HIV cases, with an estimated 143,000 incident cases [2]. According to the 2022 Global TB report, in Ethiopia, 12 % of previously treated TB cases were estimated to have multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB) [2]. Furthermore, evidence from selected TB treatment initiating centers in Ethiopia have showed high prevalence of multidrug-resistant TB (MDR-TB) among previously treated cases (64.5 %) [5]. Several studies indicate that the proportion of drug resistance is high in previously treated cases and is an independent predictor of the prevalence of MDR-TB [6–8].

Pyrazinamide (PZA) is one of the drugs recommended in the treatment of MDR-TB and non MDR-TB, both in the first line and second line treatment regimens. It kills non-replicating drug-tolerant bacilli that other TB drugs fail to kill and its inclusion in TB drug regimen enabled a reduction in treatment duration from 9 to 6 months among drug-susceptible TB patients [9,10]. It has been shown that having previous

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treatment history for TB could be a potential risk factor for resistance against common drugs including PZA [11]. A multi-country surveillance study found that PZA resistance is higher in previously treated TB cases (10.5 %) than in new TB cases (2.0 %) [12]. The most commonly identified risk factor for developing MDR-TB in Ethiopia has been shown to be prior exposure to anti-tuberculosis drugs [13], however there is a dearth of information regarding PZA resistance status among previously treated TB cases.

Several reports have shown that PZA resistance primarily results from mutations in the pncA gene, which encodes the enzyme pyrazinamidase [15,40]. These mutations lead to a loss of pyrazinamidase activity in the conversion of PZA to pyrazinoic acid (POA) [9]. Nevertheless, there are cases of PZA-resistant strains that do not exhibit pncA mutations [41], which suggests disparities between phenotypic resistance and pncA-based genotypic testing approaches [26]. When using sequencing as the reference standard, the phenotypic resistance of the MGIT 960 TB system demonstrated better concordance with sequencing for PZA susceptibility testing in comparison with the Wayne assay [3].

World Health Organization (W.H.O) recommended BACTEC MGIT 960 phenotypic drug susceptibility testing (DST) as the gold standard for determining PZA resistance [14]. But in Ethiopia, PZA DST has not been included in the TB DST algorithm for second-line regimens, and it is also not commonly performed. Hence, PZA is mostly added to treatment regimen empirically without having susceptibility testing results. This may amplify resistance and likely cause unnecessary outcome [15–17], and this would be worse for those who were treated previously for TB, as the likelihood of unsuccessful treatment outcome was more frequent in previously treated TB cases [18].

Furthermore, the study setting lacks information on PZA resistance among previously treated TB cases, making it crucial to assess the drug resistance status of PZA at various sites for the establishment of the best regimen. Therefore, this study aimed to assess the prevalence and distribution of PZA resistance between rifampicin (RIF)-resistant and RIF-sensitive MTBC isolates obtained from previously treated TB cases and the effect of PZA resistance on patient treatment outcome in Southwest Oromia, Ethiopia.

2. Material and methods

2.1. Study design, area and period

A retrospective cross-sectional study on Mycobacteria tuberculosis specimens collected between May 2016 and May 2021 was conducted during June-November 2021 at the Mycobacteriology Research Center of Jimma University (MRC-JU). MRC-JU is serving as a TB referral laboratory for Southwestern part of Ethiopia mainly for MDR-TB diagnosis and treatment follow-up. Positive MTBC isolates have been stored and kept in a deep freezer (-80℃) as per the standard operating procedure of the laboratory.

2.2. Ethical consideration

Ethical clearance was obtained from Jimma University, Institute of Health-Institutional Review Board (Ref. No. IHRPGn/168/21). The official letter and ethical clearance were presented to the management of Mycobacteriology Research Center of Jimma University and other concerned bodies for permission to collect patient data and for processing patient isolates. The confidentiality of the data was guaranteed. The collected information was only used for the purpose of the study.

2.3. Patients, MTBC isolates and inclusion criteria

This study sought to recover stored isolates collected from TB patients who had previous anti-TB treatment histories, attend routine clinical services at TB treatment initiation centers (TIC) in Southwestern Oromia between May 2016 and May 2021, and look for their PZA resistance status (Fig. 1). Patients' sputum samples were collected at TIC and transported by using a cold chain (2–8 °C) to MRC-JU for routine diagnostic services. The current Ethiopian guideline for clinical and programmatic management of TB recommends first line-line probe assays (FL-LPA) and Xpert tests as a baseline test for all patients who have a history of prior anti-TB treatment [19]. For all RIF-susceptible DST results, it is recommended to initiate treatment with 2(RHZE)/4(RH). If the Xpert test confirms or the patient is suspected of having drug-resistant TB, the patient is referred to a drug-resistant TB treatment center, which provides referral services for the transportation of samples for diagnosis and treatment follow-up.

This study included sixty-six TB cases with the following conditions: a history of receiving anti-TB therapy; MTBC isolate stored at MRC-JU; registered medical information on socio-demographic characteristics (age, sex, and residence); registered HIV co-infections status; registered patient previous treatment category; registered patient treatment outcomes within the last five years (May 2016 to May 2021); and whose isolate had successfully recovered after sub-culturing on liquid media (BACTEC MGIT 960).

2.4. Study procedures

2.4.1. Data collection procedure

Patient data were obtained from the culture and DST laboratory registration book of TB cases using a pre-structured data extraction tool. The data for RIF-susceptibility status were obtained from either GeneXpert MTB/RIF assay or Line probe assay (LPA) laboratory registration book, and the corresponding isolates were then retrieved from the freezer. The data for treatment outcome status were obtained from patient medical records monitored over time at the TIC; by using the patient medical registration number on request paper and the contact address of the TB focal person working at the respective TIC.

2.4.2. Laboratory procedures

2.4.2.1. Sub-culturing of MTBC isolates and seed MGIT tube preparation. Sub-culturing of stored MTBC isolates and seed MGIT tube preparation was performed according to the procedure described in the manual of the BD BACTEC^{TM} MGIT^{TM} 960 system at the MRC-JU [21]. After being removed from a −80 °C freezer and thawed at room temperature, each isolate was added (100 μL) to 2 mL of phosphate-buffered solution. The suspension was then decontaminated by adding an equal volume of NaOH/NALC-Na citrate solution (1:1 vol), according to the Global Laboratory Initiative Manual [20]. After 15 min of adding digestion-decontamination solution, phosphate buffer (pH 6.8) was added to the 50-ml mark on the centrifuge tube and centrifuged at 3,000g for 15 min at 4 °C. Thereafter, after decanting the supernatant, the sediment was re-suspended in phosphate buffer, and 0.5 mL of a well-mixed suspension was inoculated into MGIT (7 mL of a PANTA enrichment mix), which was then incubated in the BACTEC MGIT 960 instrument.

For MGIT-positive isolates, a smear was prepared and stained with carbol fuchsin. In addition to the smear, a loopful of MGIT-positive isolates was subcultured on a brain–heart infusion (BHI) agar plate to rule out contamination [21]. AFB positive with no growth seen on BHI were sub-cultured on a Lowenstein-Jensen (LJ) slant media. The inoculums were distributed evenly over the surface of the slant and incubated at 37 °C for 3 days with a loose lid to facilitate the complete evaporation of the liquid on medium surface. The LJ slants then read weekly for growth check.

When the LJ slants became positive, 3 drops of a 0.5 McFarland turbidity bacterial growth suspension were inoculated on 0.5 mg/mL PNB (*para*-nitrobenzoic acid)-containing LJ medium [4]. The positive control was set up by inoculating the suspension on PNB free LJ medium. All the slants were incubated at 37 °C up to 4 weeks and observed for any growth on the medium. If no growth seen on PNB containing LJ

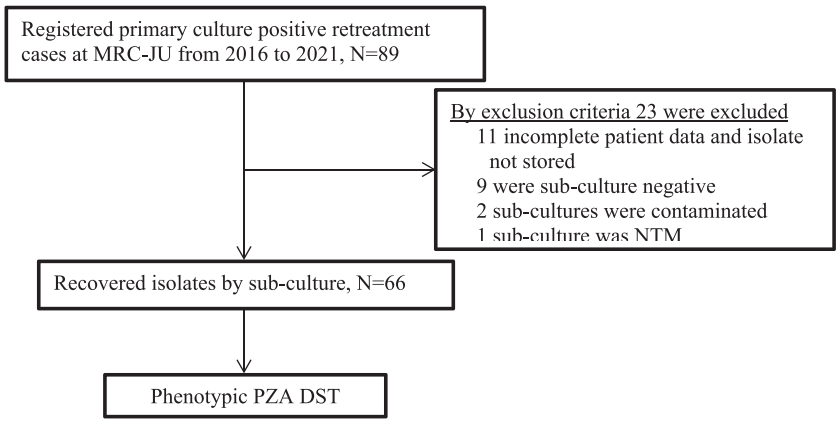

Fig. 1. Flow chart showing the data collection procedure used to determine phenotypic PZA resistance in previously treated TB cases in Southwest Oromia, Ethiopia. DST = Drug Susceptibility Test, PZA = Pyrazinamide, MRC-JU = Mycobacteriology Research Center of Jimma University, NTM = Non-tuberculosis Mycobacteria, TB = Tuberculosis.

media and growth seen on PNB free LJ media, the isolate was identified as MTBC.

Isolates identified as MTBC were sub-cultured in MGIT tube which contains 7 mL basic MGIT medium and 0.8 mL of BD BACTEC MGIT growth supplement. Then the tubes were incubated in BATEC MGIT instrument. For these tubes to be used as DST inoculum the time to positivity must be $\geq$ 4 days. If the tubes become positive in $<$ 4 days, the first step to prepare seed tube repeated and a new seed tube was prepared. Quality of staining reagents, LJ media, and every new lot of MGIT media, growth supplements, PZA kits and PZA supplements were controlled by using standard quality control strain H37RV of MTBC as positive control.

2.4.2.2. PZA DST using the BACTEC MGIT 960 system. Phenotypic DST to the PZA was done using BD BACTEC^{TM} MGIT^{TM} 960 system (Becton Dickinson, Franklin Lakes, NJ, USA) at the MRC-JU as described in its manual [21]. The MGIT 960 medium is a modified Middle brook 7H9 broth with a reduced PH of 5.9. The frozen MTBC isolates were sub-cultured as indicated above, and those seed tubes having time to positivity $\geq$ 4 days were used. Lyophilized preparations of the antibiotic drug of MGIT 960 PZA kit was reconstituted in sterile distilled water. Final concentration of PZA (100.0 μg/mL) was used. For the preparation of the test inoculum, a positive seed MGIT tube used the day after it first becomes positive on the BD BACTEC MGIT instrument (Day 1), up to and including the fifth day (Day 5) after instrument positivity. For Day 1 and Day 2 positive tubes, no dilution was made, and the tubes were used for the inoculation procedure. Using a sterile pipette, 0.5 mL of the culture suspension was inoculated into the PZA tube.

The inoculum is first diluted 1:10 for growth control inoculation by adding 0.5 mL of the culture suspension (the one used for the drug tube) to 4.5 mL of sterile saline. If the tube is day 3, day 4, or day 5 positive, then 1 mL of the positive broth was diluted in 4 mL of sterile saline (1:5 dilutions) and thoroughly mixed; this was used as the DST inoculum for proceeding to the inoculation procedure for the PZA susceptibility test.

A growth control (GC) tube without drug was included for each isolate. The relative growth ratio between the drug-containing tube and GC tube was determined by the BD BACTEC^{TM} MGIT^{TM} 960 system's software algorithm when 400 growth units for the GC tube were reached. PZA-DST results were reported qualitatively as resistant or sensitive. Quality control was maintained by testing the batch of MGIT medium PZA kit using *M. tuberculosis* H37Rv laboratory strain.

2.5. Data analysis

Data were checked for completeness, cleaned and double entered into Epidata statistical package version 3.1 (Jens M. Lauritsen & Michael Bruus the EpiData Association, Odense Denmark) and exported to SPSS software package version 21 for analysis. The differences in the proportion of PZA resistance between groups were compared using the chi-square test. The four previous treatment sub-categories were categorized into two category-I (treatment after failure, defaulter and other) and category-II (relapse cases).

In this study, the participants' treatment outcomes were divided into two: favourable outcomes and unfavourable outcomes. For evaluating the association between MTBC isolates PZA resistance and patient treatment outcome binary logistic regression analysis was undertaken. A treatment outcome was interpreted as favourable when the patients' treatment outcome was registered as cured or treatment completed, and unfavourable when the patients' treatment outcome was registered as a failure, loss to follow-up or death. For purpose of logistic regression analysis, dummy variables were used for independent variables with more than two categories. Finally, multivariate logistic regression analysis was undertaken by including factors found significant ($P < 0.2$) in univariate logistic analysis. P-values less than 0.05 were considered as statistically significant.

3. Results

3.1. Patients' characteristics

In this study, a total of 66 MTBC isolates from previously treated TB cases were included. Out of these, 69.7 % (46/66) were from males. The median age of the TB cases was 28 years (Interquartile range: 24–35). More than half of the cases, 56.1 % (37/66), were from urban community (Table 1). The HIV sero status was known for 92.4 % (61/66) of the cases. Of these, 9.8 % (6/61) were HIV positive. In this study, four previous treatment sub-categories of TB were observed, 57.6 % (38/66) cases were relapse, 36.4 % (24/66) were treatment after failure, 4.5 % (3/66) were treatment after loss to follow-up and for one case the previous treatment outcome was not documented (Table 1). From the total cases, 74.2 % (49/66) of them were RIF-resistant and 25.8 % (17/66) were RIF-sensitive as determined by Xpert MTB/RIF assay and/or LPA.

3.2. Prevalence of pyrazinamide resistance

Phenotypic PZA DST was performed for all 66 isolates. Accordingly, the overall prevalence of PZA resistance was 31.8 % (21/66), [95 % CI, 21.8–43.8]. The proportion of PZA resistance among previous treatment sub-categories was 29 % (11/38) in relapse and 33 % (8/24) among treatment failure (Fig. 2.). There is no statistically significant association between the previous treatment category, category-I (treatment after failure, defaulter and other) or relapse (category II) and PZA

Table 1
Socio-demographic and clinical characteristics of previously treated 66 TB cases from which isolates were obtained at MRC-JU.

Variable	Category	Frequency n (%)
Age	15-24	19 (28.8)
	25-34	28 (42.4)
	35-44	10 (15.2)
	45-54	6 (9.1)
	55-64	2 (3)
	65 and above	1 (1.5)
	Total	66 (100)
Sex of patient	Male	46 (69.7)
	Female	20 (30.3)
	Total	66 (100)
Geographic area	Urban	37 (56.1)
	Rural	29 (43.9)
	Total	66 (100)
HIV-status	Positive	6 (9.1)
	Negative	55 (83.3)
	Unknown	5 (7.6)
	Total	66 (100)
Previous treatment sub-categories	Relapse	38 (57.6)
	Treatment failure	24 (36.4)
	loss to follow up	3 (4.5)
	Other[1]	1 (1.5)
	Total	66 (100)
Rifampicin susceptibility status	Resistant	49 (74.2)
	Sensitive	17 (25.8)
	Total	66 (100)
Patient treatment outcome	Favorable	50 (76.9)
	Unfavorable	15 (23.1)
	Total	65 (100)

HIV = Human Immunodeficiency Virus.
MRC-JU = Mycobacteriology Research Center of Jimma University.
[1] Outcome after most recent course of treatment is undocumented.

susceptibility status (P = 0.56).

3.3. PZA resistance among isolates resistant to rifampicin

Among all isolates, 74.2 % (49/66) were resistant to RIF (Table 2). Of those RIF-resistant isolates, 38.8 % (19/49) were resistant to PZA. This proportion exceeded the number of PZA resistance in the 17 patients without RIF-resistant TB, 11.8 % (2/17) [chi-square = 4.2, p = 0.039]. Among 17 RIF-sensitive isolates, 88.2 % (15/17) were sensitive to PZA. The two patients with PZA resistance and RIF-sensitive were in relapse category, and one patient received cat-1 (2HRZE/4RH) regimen and the other received cat-2 regimen (2HRZES/1HRZE/5HRE).

3.4. PZA susceptibility status and treatment outcomes

Of the 66 patients, treatment outcomes were documented for 65 patients. One patient with undocumented treatment outcome was excluded from the outcome analysis. Accordingly, among all patients with recorded treatment outcome, 76.9 % (50/65) had favorable treatment outcome, and 23.1 % (15/65) of patients had unfavorable treatment outcomes. All patients who had favorable treatment outcome were cured. The treatment success rate was 82.4 % (14/17) among RIF-sensitive and 75 % (36/48) among RIF-resistant cases. Among 15 of the 65 patients with unfavorable treatment outcomes, four (6.2 %) were failure, five (7.7 %) were died, and six (9.2 %) were lost to follow-up. None of patients with treatment outcome died were found to be PZA-resistant, however, three were HIV positive and four patients had an MTBC isolate resistant to RIF. Treatment outcome among two patients

Table 2
PZA resistance characteristics in 66 previously treated TB cases compared to RIF-resistance, sex, geographic area, previous treatment category, and HIV status using chi-square test.

Characteristics		All	PZA (R) n (%)	PZA (S) n (%)	X^2	P-Value
RIF status	Resistant	49	19 (38.8)	30 (61.2)	4.25	0.039
	Sensitive	17	2 (11.8)	15 (88.2)		
Sex of patient	Male	46	12 (26.1)	34 (73.9)	2.29	0.13
	Female	20	9 (45)	11 (55)		
Age category	15–44	57	17 (29.8)	40 (70.2)	NA	NA
	≥45	9	4 (44.4)	5 (55.6)		
Geographic area	Urban	37	11 (29.7)	26 (70.3)	0.17	0.68
	Rural	29	10 (34.5)	19 (65.5)		
Retreatment category	Failure, defaulter and other	28	10 (35.7)	18 (64.3)	0.34	0.56
	Relapse	38	11 (28.9)	27 (71.1)		
HIV status	Positive	6	2 (33.3)	4 (66.7)	NA	NA
	Negative and unknown	60	19 (31.7)	41 (68.3)		

X^2 = chi square, PZA(R) = Pyrazinamide resistant, PZA(S) = Pyrazinamide sensitive, RIF = Rifampicin, NA = Not applicable, HIV = Human Immunodeficiency Virus.

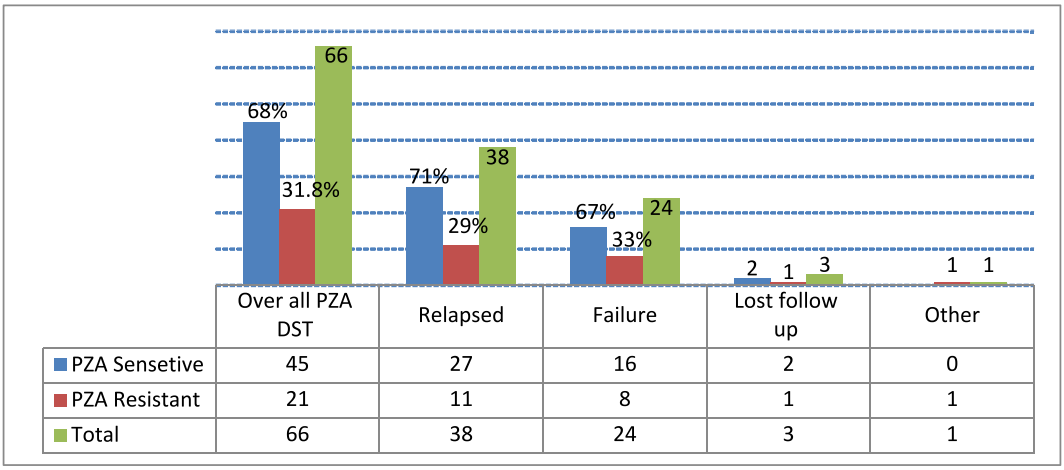

Fig. 2. Plot showing PZA susceptibility status of 66 MTBC isolates from previously treated cases at MRC-JU, categorized by different previous treatment categories (relapsed, failure, lost follow up and other). DST = Drug Susceptibility Test, PZA = Pyrazinamide, MRC-JU = Mycobacteriology Research Center of Jimma University, MTBC = Mycobacterium tuberculosis complex, Other = Patient whose outcome after his most recent course of treatment is undocumented.

with PZA-resistant but RIF-susceptible TB, one was cured and the other lost treatment follow-up.

The favorable treatment outcome was 84.4 % (38/45) among PZA-sensitive and 60 % (12/20) among PZA-resistant cases (Table 3). To analyze the effect of PZA resistance on patient treatment outcomes, univariate and multivariate logistic regression analysis were performed. In univariate logistic regression analysis HIV status and PZA DST status had a P-value < 0.2 and these two variables were included in multivariate logistic regression. In multivariate logistic regression analysis, being PZA resistance and HIV positive were an independent predictors for unfavorable treatment outcome. The risk of having unfavorable treatment outcome for previously treated TB cases with PZA resistant isolate was four times that of previously treated TB cases with PZA sensitive isolate [AOR (95 %CI) = 4.2(1.13–15.3); P = 0.032]. The risk of having unfavorable outcome for previously treated patients with HIV positive test result is ten times than that of previously treated patients with HIV negative test result [AOR (95 %CI) = 10.5(1.5–72); P = 0.017] (Table 3).

4. Discussion

In this study, a significant proportion of MTBC isolates from previously treated TB cases were found to be PZA-resistant. PZA resistance was common in patients treated for MDR-TB. PZA resistance is associated with unfavorable TB treatment outcomes, with a four-time higher risk in TB cases harboring PZA-resistant MTBC than in cases with PZA-sensitive MTBC. This indicates the importance of determining PZA resistance when treating TB cases, particularly those with a history of prior TB treatment.

The current study found that 31.8 % of isolates from previously treated TB cases were phenotypically resistant to PZA. This is in agreement with study from Senegal (31 %) [22] and Addis Ababa, Ethiopia (35.2 %) [23] and Guizhou province of China (36.2 %) [24]. This result was higher than that of a multi-country survey in South Africa (Gauteng), Bangladesh, South Africa (KwaZulu-Natal) and Pakistan which found the prevalence of 4.7 %, 13.8 %, 10.5 % and 8.9 % respectively among previously treated TB cases [12]. Furthermore, a systematic review and *meta*-analysis of PZA resistance in a global perspective found a prevalence of 15 % in the African W.H.O region, which is lower than our study result [25]. This difference in findings might be due to the imperfect correlation between *pncA* gene mutations and phenotypic resistance [26], and molecular tests targeting only mutations in the *pncA* gene than other mechanisms of PZA resistance in MTBC could falsely diagnose drug resistant TB as susceptible [15].

In this study, we found a significant difference in the proportion of PZA resistance among isolates of previously treated TB cases with RIF-resistant (38.8 %) and RIF-sensitive (11.8 %), in which the percentage were significantly higher in RIF-resistant group than those of RIF-susceptible group. This finding supports the evidence from a previous study of twelve Sub-Saharan African countries, which found that the rate of PZA resistance in RIF-resistant TB cases varied significantly by country, ranging from 21 % in Togo to 80 % in Burkina Faso [22]. Additionally, reports from China and United States shows that the prevalence of PZA resistance among MDR-TB was 38.5 % [11] and 38.0 % [30] respectively. This finding extends prior observations of low prevalence of PZA resistance in pan-susceptible but high prevalence of PZA resistance in MDR-TB isolates [25,27,28]. One possible reason for this might be due to the continued focus of DST on RIF [29] and addition of PZA to treatment regimen in the absence of documented sensitivity, which allows amplification of the resistant strains and continued transmission due to ineffective regimens [30].

The proportion of PZA resistance among RIF-resistant cases observed in our investigation was below those reported from China's Ningbo province and Thailand, where the PZA resistance rate among MDR-TB was 59.1 % and 49 %, respectively [31–33]. The possible explanation for the high prevalence of PZA resistance among RIF-resistant cases could be because of drug pressure in MDR bacteria in the course of a long duration of anti-tuberculosis treatment which can be responsible for the potential cross resistance among PZA and other drugs [34]. Furthermore, study from China indicated that being RIF-resistant and previous treatment were risk factors for PZA resistance [11] which may explain the high prevalence of PZA resistance among RIF-resistant isolates obtained from previously treated cases in the current study.

In this study 90.5 % [19/21] of isolates that were phenotypically resistant to PZA were also resistant to RIF (P = 0.039). This finding is consistent with the study from South Africa, which found 91 % of phenotypically PZA resistant isolates were also resistant to INH and RIF [35]. Patients can be infected by a PZA-resistant MTB either through antibiotic selection, which may reflect the manner in which these patients were treated previously or through transmission of a PZA-resistant MTB strain [35,36]. Therefore, in RIF-resistant previously treated TB cases, the detection of RIF-resistance should draw attention to the possibility of simultaneous presence of PZA resistance [12]. Hence, it is necessary to perform PZA susceptibility testing for proper management of RIF-resistant-TB cases with regimen containing PZA.

Based on PZA phenotypic DST, previously treated TB cases with PZA resistant isolate was four times more likely to have unfavorable treatment outcome than that of previously treated TB cases with PZA

Table 3
Univariate and multivariate logistic regression analysis of patient characteristics, PZA and RIF status of isolate, and treatment outcome among previously treated TB cases at MRC-JU (N = 65).

Characteristics		Total (N = 65) n (%)	Treatment outcome		COR**95 % CI	P-value	aOR#95 % CI	P-value
			Unfavorable (N = 15) n (%)	Favorable (N = 50) n (%)				
Sex of patient	Female	20 (30.8)	5 (33.3)	15 (30)	Ref.			
	Male	45 (69.2)	10 (66.7)	35 (70)	0.86 (0.25–2.94)	0.81		
HIV status	Reactive	6 (9.2)	4 (26.7)	2 (4)	8.73 (1.4–53.8)	0.02*	10.5 (1.5–72)	0.017
	Negative & unknown	59 (90.8)	11 (73.3)	48 (96)	Ref.		Ref.	
Previous retreatment category	Category I	27 (41.5)	8 (53.3)	19 (38)	1.87 (0.58–5.97)	0.29		
	Category II	38 (58.5)	7 (46.7)	31 (62)	Ref.			
PZA DST	Resistant	20 (30.8)	8 (53.3)	12 (24)	3.6 (1.1–12.1)	0.036*	4.2 (1.13–15.3)	0.032
	Sensitive	45 (69.2)	7 (46.7)	38 (76)	Ref.		Ref.	
RIF status	Resistant	48 (73.8)	12 (80)	36 (72)	1.56 (0.38–6.36)	0.54		
	Sensitive	17 (26.2)	3 (20)	14 (28)	Ref.			
Geographic distribution	Urban	36 (55.4)	9 (60)	27 (54)	1.28 (0.39–4.13)	0.68		
	Rural	29 (44.6)	6 (40)	23 (46)	Ref.			

TB = tuberculosis, MRC-JU = Mycobacteriology Research Center of Jimma University, ** = Crude odds ratio, # = adjusted odds ratio, * = Fisher's Exact Test p-value, Ref. = reference category, 95 % CI = 95 % confidence interval, Category I = treatment after failure, defaulter and other, Category II = relapse, PZA = Pyrazinamide, RIF = Rifampicin, HIV = Human Immunodeficiency Virus, DST = Drug susceptibility test.

sensitive isolate. In line with this study, an individual patient data *meta*-analysis showed that use of PZA was significantly associated with unsuccessful treatment outcome if isolates were resistant to PZA drug [37]. This suggests that PZA resistance may contribute to unfavorable treatment outcome, similar to study from China which showed that previous treatment history and PZA resistance were negatively associated with final culture conversion [38]. Another study from China observed that the treatment outcomes were significantly better with PZA susceptible MDR patients; suggesting that the need for PZA resistance test to optimize treatment [33]. Therefore, along with other drugs, offering PZA DST before starting or adjusting treatment regimens may have a contribution in an improvement of the treatment outcomes for previously treated TB cases with PZA resistant isolates.

This study has some limitations. First, only phenotypic PZA DST performed in our study in which false-positive drug resistance may happen due to the intrinsic problems of phenotypic susceptibility testing methods [39]. Second, the current study is based on a retrospective collection of patient data in which misclassification of previous treatment sub-category may happen. In spite of these limitations, this is the first study in the study setting to investigate the burden of PZA resistance among isolates of previously treated TB cases. Moreover, this study provides important information on the effect of PZA resistance on the treatment outcome of previously treated TB cases. This study also provides an important evidence to diagnose PZA resistance and help guide the treatment with PZA for MDR-TB cases in this region and can also be used for better planning of TB management.

To conclude, the prevalence of PZA resistance among *M. tuberculosis* isolates from previously treated cases was higher than global PZA resistance estimates. The previously treated TB cases with rifampicin resistant isolates had a much higher percentage of PZA resistance than rifampicin susceptible isolates. Patients with PZA resistance were more likely to have unfavorable treatment outcome than that of patients with PZA sensitive. PZA susceptibility testing should be included in the MDR-TB diagnostic algorithm to improve management of these patients.

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

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