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ORIGINAL RESEARCH

Effect of Bdq-Containing Regimen and Molecular Detection of Bdq Resistance among Pre-XDR-TB Patients with Unfavorable Outcomes

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Purpose: The objective of this study was to evaluate the efficacy of bedaquiline (Bdq)-containing regimens in pre-extensively drug-resistant tuberculosis (pre-XDR-TB) patients in Shenzhen, China, and to investigate the association between Bdq resistance and unfavorable outcomes.

Methods: Data were collected from 84 pre-XDR-TB patients categorized into Bdq (n = 46) and non-Bdq (n = 38) groups. Individuals in the Bdq group were treated with Bdq alongside individualized background drugs. Commonly used drugs (>50% of patients) in both groups were linezolid (Lzd), clofazimine (Cfz), cycloserine (Cs) and pyrazinamide (Pza). Treatment outcomes were classified as cure, treatment completion, treatment failure, loss to follow-up, or death. Logistic regression analysis was conducted to determine independent predictors of treatment success using potential risk factors, including age, sex, body mass index (BMI), TB treatment history, and other factors. Whole-genome sequencing (WGS) was conducted on clinical isolates from 4 patients with unfavorable outcomes and 4 patients with favorable outcomes in the Bdq group.

Results: Favorable treatment outcomes were observed in 89.13% (41/46) of the Bdq group and 52.63% (20/38) of the non-Bdq group (P = 0.0005). Univariate and multivariate analyses identified Bdq was an independent factor associated with treatment success (odds ratio [OR] = 11.572, 95% CI: 2.183–61.343, P = 0.004). WGS identified an atpE_Ala63Pro mutation conferring Bdq resistance in one patient with an unfavorable outcome. Additional resistance mutations included Rv0678_Arg156fs (Bdq and Cfz resistance) and rplC_Cys154Arg (Lzd resistance).

Conclusion: Bdq-containing regimens significantly improved the treatment outcomes among pre-XDR-TB patients. The emergence of resistance mutations highlights the importance of routine drug resistance monitoring and rational drug use. Expanding access to Bdq and other novel drugs at affordable prices is vital for improving the success of pre-XDR-TB treatment.

Keywords: Bdq-containing regimen, pre-XDR-TB, treatment outcomes, resistance, WGS

Introduction

The emergence of drug-resistant tuberculosis presents a serious global threat and a substantial challenge to effective tuberculosis control efforts.[1] Encouragingly, the introduction of several novel or repurposed drugs, such as Linezolid (Lzd), Bedaquiline (Bdq), Clofazimine (Cfz) and Delamanid (Dlm), has led to marked improvements in the treatment outcomes of multidrug-resistant or rifampicin-resistant tuberculosis (MDR/RR-TB).[2] In 2022, World Health Organization (WHO) released new guidelines to recommend the 6-month BPaL/BPaLM regimen for most patients with MDR/RR-TB and the 9-month all-oral regimen for MDR/RR-TB patients with fluoroquinolones (FQ) susceptible.[3]

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The BPaLM regimen comprises Bdq, Lzd, Pretomanid and Moxifloxacin (Mfx). This regimen can be used without Mfx (BPaL) in patients with pre-extensively drug-resistant TB (pre-XDR-TB). BPaL/BPaLM has been reported to have approximately 90% efficacy against MDR/RR-TB and other highly drug-resistant TB cases.[4,5] In addition to favorable outcomes, the BPaL/BPaLM regimen resulted in better compliance, fewer adverse effects, and lower costs.[6–8] However, there are limitations to the global use of this regimen. First, the safety of pretomanid in children younger than 14 and in pregnant or lactating women remains unclear. Second, pretomanid and resistance testing for drugs in the BPaL/BPaLM regimen is currently unavailable in many countries and regions. Another multi-country Phase III trial (endTB-Q) aimed at assessing the effectiveness of a novel, all-oral, shortened regimen (Bdq-Dlm-Cfz-Lzd) in pre-XDR-TB patients has recently been completed.[9] This will offer new options for shorter, less toxic, and more effective regimens for pre-XDR-TB treatment. Based on these studies, the combination of Bdq with a fixed background regimen has been shown to effectively treat MDR-TB, particularly pre-XDR-TB. However, the Bdq-containing regimens used in real-world settings are often personalized with varying background anti-TB drugs and treatment durations. Given that the BPaL/BPaLM and Bdq-Dlm-Cfz-Lzd regimens are inaccessible to a considerable number of patients within a short period of time, further investigation into the efficacy of Bdq-containing regimens in real-world settings is essential.

Although Bdq has improved treatment outcomes in MDR/RR-TB, resistance to Bdq is emerging. Bdq resistance has been reported in various countries, suggesting that drug susceptibility testing (DST) and treatment regimens may be insufficient to prevent the accumulation of drug resistance. In China, Bdq was introduced for drug-resistant TB treatment in 2018 and Bdq resistance has been monitored since then.[10] As previously reported, the Bdq resistance rate among MDR-TB varied between 1% and 7.16% in China.[10–14] Bdq resistance has been reported to be associated with genetic mutations in *atpE, Rv0678, mmpL5, Rv1979c*, and *pepQ*.[15–17] However, our current understanding of Bdq resistance remains limited, hindering the development of rapid molecular assays. Further data are needed to elucidate the phenotypic–genotypic association, particularly in individuals who experience treatment failure.

In this study, we investigated the efficacy of Bdq-containing regimens in pre-XDR-TB patients in South China, in comparison with non-Bdq regimens. We focused on pre-XDR-TB patients who received Bdq-containing regimens but achieved unfavorable outcomes, and performed genomic analysis by whole-genome sequencing (WGS) on the clinical isolates of those patients.

Methods

Study Participants Enrollment

This study was conducted in Shenzhen, China. We enrolled 115 patients who were diagnosed with pre-XDR-TB at Shenzhen Third People's Hospital from February 2017 to November 2021. Pre-XDR-TB was confirmed using GeneXpert MTB/RIF testing and phenotypic DST. After excluding 31 patients who did not receive treatment at the hospital, 84 eligible pre-XDR-TB cases were further analyzed.

Treatment Regimens

Patients were divided into two groups based on the treatment regimens. One group (46 patients) received Bdq-containing regimens, which included Bdq and 2–5 other background drugs. The other group (38 patients) received non-Bdq regimens, typically comprising 4–5 anti-TB drugs. Except for pasiniazid and isoniazid (Inh), which were occasionally used in the non-Bdq group, the background regimens for both groups were selected from the following drugs: Lzd, Cfz, cycloserine (Cs), pyrazinamide (Pza), Mfx, levofloxacin (Lfx), protionamide (Pto), amikacin/capreomycin (Am/Cm), p-aminosalicylic acid (Pas) and ethambutol (Emb). The dose of each drug was in compliance with the local drug resistance guidelines. To determine the final treatment regimen, multiple factors were evaluated, including the patients' economic capacity, tolerance of skin hyperpigmentation, drug availability, and any contraindications to specific medications.

Bacteriological Study

Sputum specimens were decontaminated with the NALC-NaOH method for 15 minutes, followed by neutralization with PBS and centrifugation for another 15 minutes. The resulting pellet was then resuspended in 2 mL of Phosphate-Buffered Saline (PBS), and a 0.5 mL aliquot was inoculated into mycobacterial growth indicator tubes (MGIT) (BD, USA). Positive cultures were verified via Ziehl–Neelsen staining, and Mycobacterium species was identified with the MPB64

monoclonal antibody assay (Genesis, China). Subsequently, positive isolates were sub-cultured on Roche solid medium for conventional DST. Minimum inhibitory concentration (MIC) testing was performed for 12 anti-TB drugs (Sensititre® MYCOTBI; Thermo Scientific, USA),[18] including 4 first-line drugs and 8 second-line drugs (Inh, rifampicin (Rfp), rifabutin (Rfb), Emb, Lfx, Mfx, Cs, streptomycin (Sm), kanamycin (Km), amikacin (AK), ethionamide (Eto) and Pas). GeneXpert MTB/RIF was conducted per manufacturer's instructions.

Outcomes

Treatment outcomes were categorized as either favorable or unfavorable.[19] Patients classified as "cured" or "treatment completed" were defined to have favorable outcomes, with unfavorable outcomes encompassed treatment failure, loss to follow-up and death. Cure was defined as having at least three consecutive negative cultures, collected at intervals of at least 30 days following the intensive phase, with no evidence of failure. Treatment completion was defined as completing the treatment without any evidence of failure, yet not fully meeting the criteria for cure. Treatment failure was defined as treatment termination due to a positive culture status at the end of the intensive phase or replacement of two or more drugs from the initial anti-TB regimen due to adverse events (AEs). Death was defined as all-cause mortality occurring during the treatment or follow-up period. Loss to follow-up was defined as interruption of anti-TB treatment for 2 consecutive months or more.

Safety

AEs were defined by monthly safety evaluations and graded based on the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm).[20] AEs classified as grade 3 or higher were defined as severe.

Statistical Analysis

Statistical analyses were carried out in R (version 4.3.1). Continuous variables were expressed as mean ±standard deviation (SD), while categorical variables were summarized as numbers and percentages. Continuous variables were compared using the Wilcoxon test, and categorical variables were compared using the chi-square and Fisher's exact tests. Variables with a P value less that 0.2 in the univariate analysis were incorporated into a multivariate logistic regression model to determine independent predictors of unfavorable outcomes. A P value < 0.05 was considered statistically significant. It should be noted that BMI values were missing for 11 patients and these missing values were imputed using the mean imputation method. Sensitivity analysis was also conducted after excluding patients who were lost to follow-up.

Whole Genome Sequencing

MTB isolates stored at −80°C were thawed and sub-cultured in MGIT. Genomic DNA was extracted from fresh cultures with the Magnetic Universal Genomic DNA Kit (TIANGEN, China). DNA libraries were prepared using the MGIEasy Universal DNA Library Prep Kit V1.0. Library quality was evaluated using the Qubit® 4.0 Fluorometer (Thermo Fisher, US), and fragment size distribution was checked with Qsep-100 (Hangzhou Houze, China). Quantification was performed using the KAPA Library Quant Kit (Roche, Switzerland). Qualified libraries were pooled at a concentration of 3 nmol, circularized and sequenced on MGISEQ-2000 platforms (MGI, China) using paired-end 150 bp reads.

Bioinformatic Analysis

Raw sequencing data were quality-filtered using Fastp.[21] Low-quality bases below quality 20 were trimmed. Reads shorter than 40 bases were also filtered. Clean reads were then mapped onto the *M. tuberculosis* H37Rv reference genome (NC_000962) by BWA.[22] SNPs calling were performed with the Genome Analysis Toolkit (GATK) pipeline.[23] Variants with a read depth less than 3, allelic depth less than 3, or allele frequency below 10% were excluded. Genotyping-based molecular DST (mDST) was performed on 20 anti-TB drugs, including 5 first-line drugs (Inh, Rfp, Rfb, Emb, and Pza) and 15 second-line drugs (Lfx, Mfx, Bdq, Lzd, Cfz, Cs, AK, Cm, Km, Sm, Eto, PAS, Delamanid (Dlm), pretomanid and protionamide (PTO)), based on known resistance associated mutations.[24] For Bdq resistance, mutations in five related genes (*atpE, Rv0678, mmpL5, Rv1979c* and *pepQ*) were screened for known and novel mutations.

Results

Participants and Treatment Regimens

Among 115 pre-XDR-TB patients diagnosed using GeneXpert MTB/RIF and phenotype DST, 84 patients were selected for further analysis (Figure 1). Among the 84 patients, 46 (55%) received Bdq-containing regimens, and 38 (45%) received non-Bdq regimens. Demographic and clinical characteristics of the study cohort were presented in Table 1. The

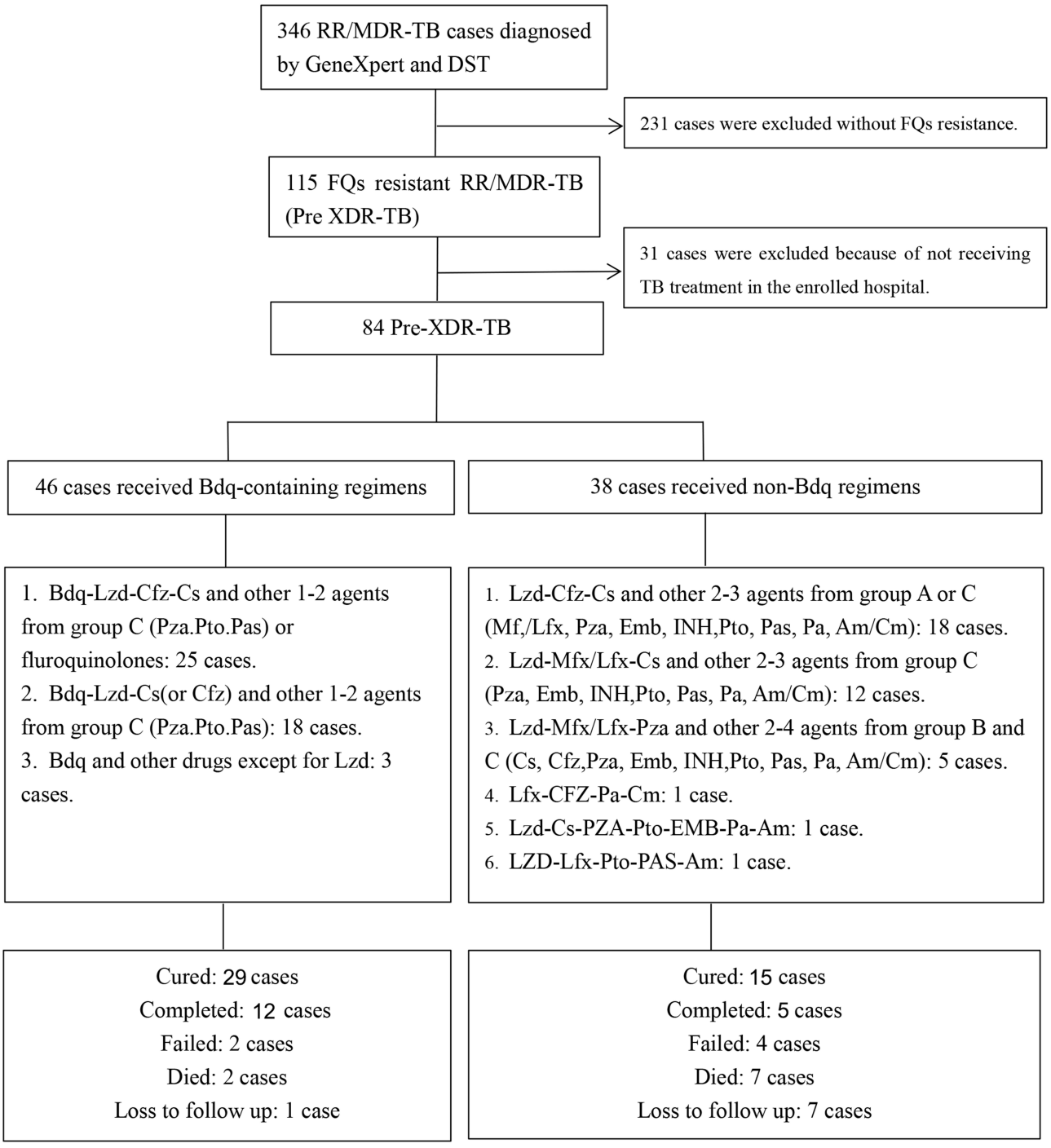

Figure 1 Procedure of the study.

Abbreviations: RR/MDR-TB, rifampin-resistant/multidrug-resistant tuberculosis; Pre-XDR-TB, resistant to fluoroquinolones, RIF, and (or) INH; FQs, fluroquinolones; Lfx, levofloxacin; Mfx, moxifloxacin; Pza, pyrazinamide; EMB, ethambutol; INH, isoniazid; Bdq, bedaquiline; Lzd, linezolid; Cs, cycloserine, Cfz: clofazimine; Pto, protionamide, Pas, p-aminosalicylic acid; Pa, Pasiniazid; Am, Amikacin; Cm, capreomycin.

Table 1 Demographic and Clinical Characteristics

Characteristics	Bdq-Containing Regimen (n=46)	Non-Bdq Regimen (n=38)	P Value
Age (mean)	40.61±1.20	47.17±2.50	0.0549
BMI index (mean)	20.71±0.58	18.64±0.56	0.0017*
Gender			0.0658
Male	32(69.57%)	18(47.37%)	
Female	14(30.43%)	20(52.63%)	
Married	36(78.26%)	30(78.95%)	1
Completed middle school	35(76.09%)	27(71.05%)	0.7848
Occupation			0.0194*
Employee	31(67.39%)	15(39.47%)	
Unemployed/Homeworker	15(33.61%)	23(60.53%)	
Shenzhen medical insurance			<0.0001*
Yes	34(73.91%)	9(23.68%)	
No	12(26.09%)	29(76.32%)	
Ever smoker	16(34.78%)	9(23.68%)	0.3856
Alcohol drinking	6(13.04%)	4(10.53%)	1
TB treatment history			0.6565
New cases	14(30.43%)	9(23.68%)	
Cases treated previously	32(69.57%)	29(76.32%)	
Exposure to FQ for ≧1 month previously	21(45.65%)	20(52.63%)	0.6762
Exposure to Lzd for≧1 month previously	16(34.78%)	3(7.89%)	0.0038*
Clinical features at baseline			
Cough	45(97.83%)	31(81.58%)	0.0204*
Expectoration	39(84.78%)	24(63.16%)	0.0429*
Hemoptysis	9(19.57%)	15(39.47%)	0.0771
Shortness of breath	15(32.61%)	12(31.58%)	1
Fever	2(4.35%)	5(13.16%)	0.2359
Bronchiectasis	4(8.70%)	2(5.26%)	0.6850
Comorbidities			
Type 2-diabetes	10(21.74%)	9(23.68%)	1
Viral hepatitis B or C	4(8.70%)	4(10.53%)	1
Hypertention	5(10.87%)	2(5.26%)	0.4487
Extent of TB in Chest CT			
Bilateral lungs	36(78.26%)	33(86.84%)	0.4618
Single cavity in one lung	7(15.22%)	3(7.89%)	0.5004
Multiple cavities in one lung	11(23.91%)	10(26.32%)	1
Multiple cavities in bilateral lungs	12(26.09%)	12(31.58%)	0.7551
Sputum smear at baseline			
Negative	20(43.48%)	20(52.63%)	0.5375
1+	7(15.22%)	3(7.89%)	0.5004
2+	4(8.70%)	5(13.16%)	0.7252
3+	11(23.91)	6(15.79%)	0.5160
4+	4(8.70%)	4(10.53%)	1

Notes: New cases: patients with no TB history. Cases treated previously: patients who have received TB-treatment for 1 month or more. * P value of <0.05 indicates a statistically significant difference.
Abbreviations: BMI, body mass index; FQs, fluoroquinolones; Lzd, linezolid; CT, computed tomography.

Bdq group had a higher proportion of males (69.57%) than the non-Bdq group (47.37%). And individuals in the Bdq group were relatively younger (mean age: 40.61 vs 47.17) and had a higher body mass index (BMI) (20.71 vs 18.64) compared to the non-Bdq group. In addition, patients with medical insurance were more inclined to embrace a Bdq-containing regimen (73.91% in the Bdq group vs 23.68% in the non-Bdq group). At baseline, patients in the Bdq group

were more likely to experience expectoration (84.78% vs 63.16%) and cough (97.83% vs 81.58%) than those in the non-Bdq group. 32 patients (69.57%) in the Bdq group previously received TB treatment. Among them, 16 patients had been exposed to Lzd for more than one month, and 21 patients had been exposed to FQ for more than one month. The drug compositions of the two groups are summarized in Table S1.

Treatment Outcomes and Independent Predictor of Unfavorable Outcomes

A higher percentage of patients achieved favorable outcomes in the Bdq group compared to the non-Bdq group (Table 2). 41 of the 46 patients (89.13%) in the Bdq group achieved a cure or completed treatment. In contrast, only 20 of the 38 patients (52.63%) in the non-Bdq group achieved favorable outcomes. Five of the 46 patients in the Bdq group had unfavorable outcomes. Among them, two patients encountered treatment failure, one patient was lost to follow-up after receiving a 6-week Bdq treatment, and two patients died unfortunately. Unfavorable outcomes occurred in 18 patients within the non-Bdq group. Among them, 4 experienced treatment failure, 7 were lost to follow-up, and 7 died.

Univariate analysis of 12 possible independent factors associated with treatment outcomes was performed (Table S2). Risk factors including age, history of anti-TB therapy, possession of Shenzhen medical insurance, and use of Bdq were significantly associated with favorable outcomes ($P < 0.05$). In addition, sputum smear at baseline ($P < 0.1$) was also integrated into the multivariate logistic regression analysis (Table 3). In the multivariate analysis, the use of Bdq was identified as an independent factor associated with favorable outcomes (OR = 11.572, 95% CI: 2.183–61.343, P = 0.004) (Table 3). Logistic regression analysis was conducted after excluding patients lost to follow-up. The results of the sensitivity analysis were consistent with the main findings (Table S3).

Table 2 Efficacy Assessment Between the Two Treatment Regimens

Outcome	Bdq-Containing Regimen (n=46)		Non-Bdq Regimen (n=38)		P Value
	Number of Patients	Percentage (%)	Number of Patients	Percentage (%)	
Favorable outcomes	41	89.13	20	52.63	0.0005*
Cure	29	63.04	15	39.47	0.5319
Treatment Completion	12	26.09	5	13.16	0.232
Unfavorable outcomes	5	10.87	18	47.37	0.0005*
Treatment failure	2	4.35	4	10.53	0.4027
Loss to follow up	1	2.17	7	18.42	0.0204*
Death	2	4.35	7	18.42	0.0720

Notes: *P value of <0.05 indicates a statistically significant difference.

Table 3 Logistic Regression Model to Identify Determinants of Unfavorable Outcomes

Determinants	Favorable Outcomes (N=61)	Unfavorable Outcomes (N=23)	Univariate Analysis			Multivariate Analysis		
			OR	95% CI	P Value	OR	95% CI	P Value
Age (years)								
Age《35 (Ref)	27 (44.3%)	4(17.4%)	3.772	1.147–12.408	0.029*	3.725	0.779–17.818	0.1
Age>35	34 (55.7%)	19 (82.6%)						
Sex								
Male (Ref)	41 (67.2%)	19(82.6%)	0.432	0.130–1.438	0.171	0.557	0.125–2.478	0.442
Female	20(32.8%)	4(17.4%)						
Anti-TB therapy history								
No (Ref)	21 (34.4%)	2 (8.7%)	5.512	1.178–25.806	0.03*	4.477	0.783–25.599	0.092
Yes	40 (65.6%)	21 (91.3%)						

(*Continued*)

Table 3 (Continued).

Determinants	Favorable Outcomes (N=61)	Unfavorable Outcomes (N=23)	Univariate Analysis			Multivariate Analysis		
			OR	95% CI	P Value	OR	95% CI	P Value
Shenzhen medical insurance								
Yes (Ref)	38 (62.3%)	6 (26.1%)	4.681	1.614–13.580	0.005*	0.804	0.151–4.272	0.798
No	23 (37.7%)	17 (73.9%)						
Treatment regimen								
Bdq containing (Ref)	41 (67.2%)	5 (21.7%)	7.38	2.394–22.751	0.001*	11.572	2.183–61.343	0.004*
Non-Bdq	20 (32.8%)	18 (78.3%)						
Sputum smear at baseline								
Negative (Ref)	24(39.3%)	7(30.4%)	2.522	0.909–6.998	0.076	2.972	0.855–10.325	0.087
Positive	37(60.7%)	16(69.6%)						

Notes: *P value of <0.05 indicates a statistically significant difference.
Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.

Adverse Events

In both groups, the most frequent AEs were hyperuricemia, skin hyperpigmentation, peripheral neuritis, and anemia (Table S4). Severe QTc prolongation (> 500 ms) did not differ between the groups.

Molecular Detection of Bdq Resistance Among Patients with Unfavorable Outcomes

All patients with unfavorable outcomes in the Bdq group were male and aged 36–51 years (Table 4). Patient 1, who underwent 6-week Bdq treatment, was subsequently lost to follow-up. Patient 2 died after receiving 12-week Bdq treatment. Patient 3, complicated by a unilateral destroyed lung (UDL), received a 32-week regimen including Bdq and Lzd, but unfortunately died. Patient 4, also with UDL, underwent a 24-week regimen including Bdq and Lzd but experienced treatment failure. Patient 5, who manifested severe peripheral neuritis (PN), experienced treatment failure despite undergoing a 36-week Bdq-containing regimen. For patients with favorable outcomes in the Bdq group (Table S5), all but one who received 18 weeks of Bdq treatment were treated with Bdq for more than 24 weeks. Among them, 29 were cured and 12 completed their treatment.

Since the clinical isolate from patient 3 could not be successfully recovered, we performed WGS on clinical isolates from the other 4 patients with unfavorable outcomes and 4 randomly selected patients with favorable outcomes in the Bdq group (Tables 5 and S6). Three known resistance-associated mutations were identified in Patient 4: *atpE*, Ala63Pro (Bdq), *Rv0678*, Arg156fs (Bdq/Cfz), and *rplC*, Cys154Arg (Lzd). Patient 4 was first diagnosed with TB in September 2017. MDR-TB diagnosis was confirmed using phenotypic DST in November 2020. Treatment was initiated with a combination of Mfx, Lzd, Pza, Pto, and Cm. The culture remained positive after 8 months of treatment. In

Table 4 Patients with Unfavorable Outcome in Bdq Group

Patient	Sex	Age (Years)	Complication	Exposure to Lzd (More than 1 Month)	Regimen Composition	Duration of Bdq (Weeks)	Duration of Total Regimen (Months)	Outcome
1	Male	51	No	No	Bdq-Lfx-Cs-Pto-Pza	6	2	Loss to follow up
2	Male	56	No	No	Bdq-Lzd-Cfz-Pto	12	3	Death
3	Male	36	UDL	No	Bdq-Lzd-Cfz-Cs-Pza	32	8	Death
4	Male	43	UDL	Yes	Bdq-Lzd-Cfz-Cs	24	6	Failure
5	Male	41	PN	Yes	Bdq-Mfx-Cfz-Cs-Pas-Cm	36	36	Failure

Abbreviations: UDL, unilateral destroyed lung; PN, peripheral neuritis; Bdq, bedaquiline; Lzd, linezolid; Lfx, levofloxacin; Cs, cycloserine; Pto, protionamide; Pza, pyrazinamide; Cfz, clofazimine; Mfx, moxifloxacin; Pas, p-aminosalicylic acid; Cm, apreomycin.

Table 5 Mutational Analysis for Patients with Unfavorable Outcomes in Bdq Group

Patient	Type	Drug Resistance Profile	Bdq Mutation	Cfz Mutation	Lzd Mutation
1	Pre-XDR	FQs, INH, Lfx, Mfx, Rfp, Sm, Pto, Pza, Emb	Rv1979c:V426I	WT	WT
2	Pre-XDR	Emb, FQs, INH, Lfx, Mfx, Rfb, Rfp, Sm	WT	WT	WT
4	XDR	Bdq, Cfz, Emb, Eto, FQs, INH, Lfx, Lzd, Mfx, Pto, Pza, Rfb, Rfp, Sm	atpE:A63P	Rv0678:R156fs*	rplC:C154R
5	XDR	FQs, INH, Lfx, Lzd, Mfx, Rfb, Rfp, Pto	WT	WT	rplC:C154R

Notes: * conferring to both Bdq and Lzd resistance.
Abbreviations: Bdq, bedaquiline; Lzd, linezolid; Cfz, clofazimine; Pza, pyrazinamide; Pto, protionamide; Mfx, moxifloxacin; Lfx, levofloxacin; Emb, ethambutol; INH, isoniazid; Eto, ethionamide; Rfb, rifabutin; Rfp, rifampicin; Sm, streptomycin; FQs, fluoroquinolone.

February 2022, the treatment was modified and the patient received a Bdq-containing regimen (Bdq-Lzd-Cfz-Cs). However, no culture conversion was observed in the patient following 6 months of treatment. The treatment was then modified to a non-Bdq regimen. Clinical isolate from the patient in the fifth month of treatment with a Bdq-containing regimen was collected and sequenced. An Lzd resistance mutation (*rplC*, Cys154Arg) was also detected in Patient 5 (Table 5). None of the mentioned resistance mutations were identified in patients with favorable outcomes. One novel mutation (*Rv1979c*, Val426Ile), which may refer to Bdq resistance, was identified in patient 1 (Table S7).

Discussion

This study evaluated treatment outcomes among pre-XDR-TB patients in South China. Our findings showed that regimens containing Bdq achieved a treatment success rate of 89.13%, compared to 52.63% in the non-Bdq group. Additionally, the incidence of AEs did not differ significantly between the two groups.

The study validated the efficacy of Bdq-containing regimens against MDR-TB and pre-XDR-TB, consistent with other published reports.[25–29] A multi-site study in India investigated the effectiveness of Bdq-Dlm-Cfz-Lzd for treating pre-XDR-TB, reporting favorable outcomes in 91% of 153 patients.[25] Another multi-center study in China assessed the efficacy of a 24-week Bdq regimen alongside individualized background drugs in 177 MDR-TB patients, with 85.3% achieving favorable outcomes.[26] A study by Zhang et al demonstrated that Bdq-containing regimens were more effective, showing a higher treatment success rate than non-Bdq regimens in refractory RR/MDR/XDR-TB (92.2% vs 63.0%).[27] Their findings further indicated that regimens combining Bdq with Lzd, Cfz, or Cs were independently associated with improved treatment outcomes among RR/MDR/XDR-TB. Similar outcomes were observed in the endTB-Q clinical trial, which used a shorter, personalized treatment strategy comprising Bdq, Cfz, Dlm, and Lzd (BCDL) to treat pre-XDR-TB. Preliminary findings presented at the Union World Conference on Lung Health in November 2024 showed that the 6-9-month BCDL regimen achieved high cure rates (87%), with particularly favorable outcomes (93% cure) in non-severe pre-XDR-TB cases. These findings suggested that Bdq-containing regimens are shorter, less toxic, and more effective than conventional therapies for pre-XDR-TB.

Some differences in baseline patient characteristics were observed between the two groups. BMI was higher in the Bdq group than in the non-Bdq group (P=0.0017). Recent studies have reported that patients with low BMI are at an increased risk of experiencing adverse events and unfavourable outcomes.[26,30] Researchers have hypothesized that a higher BMI may enable slow release of antibiotics from adipose tissue into the plasma, helping to maintain effective drug concentrations and thereby improve culture conversion rates.[26] However, association of low BMI with unfavorable outcomes was not verified in our study (OR = 1.382, 95% CI: 0.473–4.043, P=0.555). The observed inconsistency may be due to the relatively small number of unfavorable outcomes included in both studies, variations in anti-TB regimens, and other factors. The personal economic condition, especially whether the patient had medical insurance or not, significantly influenced the acceptance among patients towards the Bdq containing regimen. The cost for a regimen consisting of Bdq (6 months), Lzd (12 months), Cfz (12 months), Cs (12 months), and Pza (12 months) is estimated to be around 20,000 USD in China, with Bdq accounting for about half of the total cost. The treatment cost far exceeds the per capita disposable income in China (5,416 USD in 2023, released by National Bureau of Statistics of China). Furthermore, a significant number of pre-XDR-TB patients had additional comorbidities, including diabetes, hypertension and hepatitis, which added to the economic burden. Possession of local medical insurance covers 90% of inpatient and

over 60% of outpatient medical expenses in Shenzhen. Conversely, treatment regimens containing Bdq or other novel drugs proved financially inaccessible for the majority of the patients without medical insurance. Hence, we noted a significantly higher possession rate of Shenzhen medical insurance in the Bdq group (73.91%) compared to the non-Bdq group (23.68%) (P<0.0001). Using multivariate logistic regression analysis, we identified the use of Bdq was an independent determinant for treatment success (OR = 11.572, 95% CI: 2.183–61.343, P = 0.004). However, the high cost of Bdq significantly restricted the implementation of Bdq-containing regimens, particularly among low-income populations. Strategies to reduce the price of Bdq and other new drugs are urgently needed to enhance treatment accessibility.

In our cohort, adverse events were mild and well-tolerated, with no significant differences in common adverse events between the two groups. Given the retrospective nature of this study and the variability in the treatment regimens between the two groups, we were unable to accurately assessing the Bdq related adverse effects.

Despite advancements in new regimens, achieving a cure for Pre-XDR-TB remains a great challenge compared to FQ-sensitive MDR/RR-TB. Studies have reported that 24–66% of MDR/RR-TB cases exhibited high resistance to FQs,[13,31–33] likely due to the overuse of these antibiotics. In our study, approximately 49% (41/84) of the pre-XDR-TB patients had been prescribed FQs for more than 1 month. Additionally, resistance to Lzd and Bdq is emerging. In this study, 19 patients (22.6%) had prior Lzd exposure for more than 1 month, though none had been exposed to Bdq. Due to the limitation of laboratory technology, DST for Lzd and Bdq was not routinely performed, which prevented us from assessing the resistance rates of Lzd and Bdq in our cohort. Other published studies reported resistance rates in MDR-TB were 3.84–7.1% for Lzd and 2.2–7.16% for Bdq, respectively.[13,33,34]

In this study, 3 mutations potentially conferring Bdq resistance were identified among patients with unfavorable outcomes in the Bdq group (Table 5). These mutations, harbored in *atpE, Rv0678* and *Rv1979c*, were previously reported to be linked to Bdq resistance.[15,35] All 3 mutations were listed in the latest WHO Catalogue of resistance-related genetic variants in Mycobacterium tuberculosis complex.[36] The *atpE* Ala63Pro mutation and the *Rv0678* Arg156fs mutation detected in patient 4 were both classified as associated with resistance–interim, with the former infers resistance to Bdq and the latter infers cross resistance to both Bdq and Cfz. The *Rv1979c* Val426Ile identified in patient 1 was classified as uncertain significance. More information will be needed on the sensitivity of this mutation. Cross resistance to Bdq and Cfz should be taken in caution although mutations in *Rv1979c* were rare.[37]

AtpE encodes the F1F0-adenosine triphosphate (ATP) synthase, which is the primary target of Bdq.[15] Mutations in *atpE* confer high-level Bdq resistance but are rarely described in clinical strains.[15,16,35,38–40] There is controversy on whether the Ala63Pro mutation is predictive of treatment failure for patients receiving Bdq treatment.[16,40,41] In this study, it was challenging to determine whether the Ala63Pro mutation identified in patient 4 caused treatment failure, as the mutation was detected five months after he received Bdq treatment. Since the strains before and during the early phase of treatment did not successfully recover, we could not confirm whether the patient had baseline Bdq resistance or developed resistance during treatment. *Rv0678* encodes a repressor of the MmpL5-MmpS5 efflux pump. Mutations in *Rv0678* caused Bdq MICs to increase 2- to 8-fold and caused low-level cross-resistance to Cfz.[15] Most variants of *Rv0678* were widely distributed across the gene and occurred primarily in phenotypically susceptible clinical isolates.[16] Recent studies from Chongqing, Shanxi, and Shenzhen, China, have reported that BDQ resistance in MDR-TB in these settings was predominantly associated with mutations in *Rv0678*.[11,14,42] The *Rv0678* Arg156fs mutation was previously reported to confer resistance to both Bdq and Cfz, leading to a 2–8 fold MIC increase for Bdq and a 2–4 fold MIC increase for Cfz in vitro.[43] It remained us when using regimens containing both Cfz and Bdq, caution is warranted due to the potential for cross-resistance between the two drugs. Moreover, an Lzd resistance mutation (*rplC*, C154R) was detected in Patient 4 and 5, respectively. Patient 5 received a Bdq-containing regimen, which did not include Lzd. However, he had been previously treated with Lzd for one year, likely acquired the *rplC* mutation during that time. The detection of resistance mutations in patients with unfavorable outcomes highlights the importance of performing DST (either culture-based or genotype-based) before initiating treatment.

This study has some limitations. First, the participants were not randomly selected, which may have introduced selection bias. For instance, patients without local medical insurance are more likely to decline Bdq-containing regimens because of the high cost of Bdq. The results may have been affected by differences in baseline characteristics. Second, the use of individualized treatment regimens may have introduced confounding factors, complicating the direct comparison of treatment outcomes. Third, the limited number of strains subjected to WGS restricted the generalizability of findings regarding the molecular characteristics

of drug resistance. Nevertheless, the available WGS data offered valuable insights into genetic variations that may contribute to unfavorable outcomes. A larger sample size in future studies will be necessary to thoroughly investigate the genetic basis of Bdq resistance. Furthermore, due to limitations in clinical laboratory technology in Chinese hospitals, DST could not be performed for all drugs included in the treatment regimens (eg, Bdq, Lzd, and Cfz). Functional validation assays were also not conducted to confirm the causal role of detected mutations in conferring resistance. Addressing these limitations in future research will be essential to deepen our understanding of Bdq resistance and improve treatment strategies.

Conclusion

This study confirmed that Bdq-containing regimens achieved better outcomes than non-Bdq regimens in pre-XDR-TB patients. We found that Bdq was an independent predictor associated with favorable outcomes. However, the high cost of Bdq hinders the widespread adoption of Bdq-containing regimens, especially among low-income populations. To improve treatment outcomes for MDR-TB, there is an urgent need to lower the cost of Bdq and enhance access to other new drugs. Additionally, we detected Bdq resistance mutations that may be associated with treatment failure. This emphasizes the urgency of implementing rapid and reliable DST for Bdq resistance monitoring.

Data Sharing Statement

The WGS data of 8 patients has been submitted to the Sequence Read Archive of the National Center for Biotechnology Information (BioProject ID: PRJNA1010475).

Ethical Statement

The study was approved by the Ethics Committee of Shenzhen Third People's Hospital (No. 2022-196-02; December 19, 2022). The requirement for informed consent was waived by the Ethics Committee of Shenzhen Third People's Hospital, owing to the retrospective nature of this study. The patients' data were confidential and used for research purposes only and in compliance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest.

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