




Drug Exposure Dictates Evolutionary Dynamics of Drug-Resistant Tuberculosis: A Longitudinal Cohort Study in Beijing, China

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Background: Despite global efforts to combat drug-resistant tuberculosis (DR-TB), key gaps remain in understanding how resistance amplifies during treatment and the role of collateral drug effects in this process. Resistance amplification complicates management and undermines outcomes. This study aimed to identify the risk factors and collateral drug effects contributing to resistance amplification.

Methods: We conducted a retrospective, longitudinal cohort study of 133 DR-TB patients with paired phenotypic drug susceptibility tests (pDSTs) at Beijing Chest Hospital in Beijing, China, from January 2018 to January 2023. Patients were classified into “progression” and “non-progression” groups based on acquisition of new drug resistance. Multivariable logistic regression identified risk factors, and a restrictive cohort analysis assessed collateral drug effects.

Results: The median number of resistances per isolate increased significantly from 7.0 (IQR 5–9) to 8.0 (IQR 6–10) ($P < 0.05$), with 76 patients (57.1%) exhibiting resistance progression. A dominant trajectory was identified from isoniazid monoresistance (INH-Mono) to multidrug-/rifampicin-resistant TB (MDR/RR-TB) (9/14, 64.3%). In univariate analysis, bedaquiline (BDQ) use was associated with reduced progression ($P=0.032$), while pyrazinamide (PZA) use correlated with increased progression ($P=0.035$), though neither remained significant in multivariable models. Importantly, collateral effect analysis revealed that PZA exposure was strongly associated with acquired rifabutin (Rfb) resistance (OR, 5.94; 95% CI, 1.90–18.53; $P=0.001$).

Conclusion: Resistance amplification during DR-TB treatment is frequent, with INH-Mono representing a high-risk transitional state. While BDQ may offer a protective effect, the strong collateral association between PZA and Rfb resistance highlights the need for careful regimen design. These findings underscore the importance of individualized, resistance-informed therapy to mitigate further resistance amplification in DR-TB patients.

Keywords: drug-resistant tuberculosis, acquired resistance, bedaquiline, pyrazinamide, collateral drug association

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*) complex, remains one of the most significant public health challenges worldwide. Globally, an estimated 10.8 million people fell ill with TB in 2023, resulting in approximately 1.25 million deaths.¹ The emergence and spread of drug-resistant TB (DR-TB) pose a major threat to global health security. China represents a critical region in this landscape, ranking third globally in estimated TB incidence and fourth in new cases of MDR/RR-TB, accounting for 7.3% of the global total.¹

The distribution and spread of DR-TB are influenced by a complex array of demographic and clinical determinants. Previous studies have identified factors such as male sex, older age, and socio-economic disparities as key variables affecting TB epidemiology. More importantly, clinical drivers such as a history of previous anti-TB treatment and poor treatment adherence represent primary risk factors for harboring resistant strains.² The prolonged, multi-drug regimens required for treatment further promote the emergence of drug-resistant TB (DR-TB).³ Currently, the global treatment success rate for MDR/RR-TB remains under 70%, a stark contrast to the nearly 90% success rate for drug-susceptible TB, highlighting the inadequacy of existing strategies in halting resistance evolution.¹

A key challenge in the management of MDR/RR-TB is not only the baseline resistance profile but also the dynamic resistance amplification that occurs during therapy. Isoniazid mono-resistance (INH-Mono) serves as a quintessential starting point on this dangerous trajectory. Although often misperceived as a “mild” form of resistance in clinical practice,^{3,4} extensive research has confirmed that without precise and intensified treatment, INH-Mono cases are at a significantly elevated risk of progressing to MDR-TB.^{5,6} This stepwise evolution from lower- to higher-level resistance rapidly erodes the limited arsenal of effective drugs, markedly increasing the risk of treatment failure and mortality.^{1,5}

While considerable attention has been paid to the mechanisms of resistance development and progression in DR-TB, the role of collateral drug effects, in which the use of one drug indirectly influences the susceptibility to others, has received limited exploration. Epidemiological studies have suggested that resistance to one drug (eg, INH) may predispose patients to resistance to other drugs (eg, RFP), but the specific interactions between anti-TB drugs and their collateral effects remain incompletely understood.^{7–14} This critical knowledge gap is particularly relevant in light of the increasingly complex regimens used to treat DR-TB, where drug interactions could play a significant role in resistance amplification.

Accordingly, the present study will commence with diverse initial resistance patterns, such as INH mono-resistance, to systematically elucidate the evolutionary trajectories toward higher-level resistance. It will precisely identify and quantify the key clinical and population-based risk factors contributing to resistance amplification, while analyzing the synergistic effects of core therapeutic agents including PZA and BDQ on the acquisition of resistance to other anti-TB drugs. Through these endeavors, this study aims to systematically investigate the dynamic mechanisms and key drivers underlying resistance amplification in *Mtb* during treatment, with a focused emphasis on drug synergistic effects.

Materials and Methods

Study Setting

This study was conducted at Beijing Chest Hospital, Capital Medical University, in Beijing, China. As a national tertiary referral center specializing in tuberculosis and thoracic diseases, the hospital serves a diverse patient population referred from across the country, providing a comprehensive database for studying drug-resistant tuberculosis (DR-TB) evolutionary dynamics.

Study Design and Participant Selection

This study employed a retrospective, longitudinal cohort design. We reviewed the electronic medical records of patients with drug-resistant tuberculosis (DR-TB) hospitalized at Beijing Chest Hospital, Capital Medical University, between January 2018 and January 2023. The study protocol was approved by the Ethics Committee of Beijing Chest Hospital, Capital Medical University (Approval No. 2024 Clinical Review–Research–32). Due to the retrospective nature of the study and the use of de-identified data, the requirement for individual informed consent was waived by the Ethics Committee.

Initially, 553 DR-TB patients were screened from the hospital database. Through a consecutive sampling approach, 133 patients were ultimately included based on the following inclusion criteria: (i) At least two phenotypic drug susceptibility tests (pDSTs) for 16 anti-tuberculosis drugs during a single treatment episode; (ii) Complete data on the interim anti-TB treatment regimen; (iii) A baseline pDST result showing resistance to at least one drug. Exclusion criteria were: (i) the time interval between the two pDSTs was less than 18 months; (ii) Patients with exclusively extrapulmonary TB. The final cohort size was primarily determined by the strict requirement for paired longitudinal pDST results with a sufficient follow-up interval.

Based on the evolution of their resistance profiles between T1 and T2, patients were stratified into two groups: (i) the Resistance Progression Group, defined as patients who acquired phenotypic resistance to at least one additional drug at T2 to which they were susceptible at T1; and (ii) the Non-progression Group, defined as patients who demonstrated no newly acquired drug resistance at T2, including those with stable or de-escalating resistance profiles.

To isolate true collateral effects from direct selective pressures, we implemented a restrictive cohort design. When evaluating whether exposure to drug A influenced the acquisition of resistance to drug B, we restricted our analysis to patients who: (1) were susceptible to drug B at baseline (T1), and (2) did not receive drug B during the treatment interval between T1 and T2. This methodological approach eliminated the confounding effect of direct selection pressure from drug B, allowing us to identify genuine collateral effects where use of one drug (A) might influence susceptibility to another drug (B) through indirect mechanisms, such as metabolic alterations or selective evolutionary pressures on shared resistance pathways.

For all enrolled patients, we systematically collected demographic, clinical, and baseline laboratory data, as well as the complete results of the two pDSTs and the corresponding interim treatment regimens from the electronic medical record system.

Sample Size and Statistical Assumptions

As this was a retrospective cohort study utilizing all available eligible cases from a national referral center over a five-year period, a-priori sample size calculation (predefined alpha, beta, and confidence interval assumptions) was not performed. Instead, we employed a convenience sampling method, including all consecutive patients who met the stringent inclusion criteria to maximize the statistical power for identifying risk factors and collateral drug effects within this specific clinical setting.

Data Collection and Quality Control

Demographic, clinical, and laboratory data were extracted from the electronic medical record system by two independent researchers to ensure accuracy. The dataset was cleaned to resolve any inconsistencies or duplicates. To protect patient privacy, all clinical data were anonymized and de-identified prior to analysis, and access to the database was restricted to the research team.

Mycobacterial Identification and Drug-Susceptibility Testing

Species Identification

Isolates that failed to grow on p-nitrobenzoic acid medium and were presumptively identified as the *Mtb* complex underwent gene sequencing of 16S rRNA, *rpoB*, the 16S–23S rRNA internal transcribed spacer (ITS), and *hsp65*, per published protocols.^{15–17} Sequences were compared to the NCBI database via BLAST to confirm species.

Drug-Susceptibility Testing

Phenotypic susceptibility was determined using a commercial mycobacterial microdilution plate (Zhuhai YinKe Medical Engineering Co., Ltd.) employing the broth microdilution method. The minimum inhibitory concentration (MIC) for 16 anti-tuberculosis drugs was read per the manufacturer's instructions. MIC was defined as the lowest drug concentration inhibiting 99% of bacterial growth, compared against drug-free negative controls and drug-containing positive controls of a reference susceptible strain. Susceptibility was interpreted against World Health Organization critical concentrations: MIC \leq CC as susceptible (S) and MIC $>$ CC as resistant (R). Drug panels and CCs are detailed in [Supplementary Table S1](#).

Classification of Resistance Profiles

To characterize resistance evolution, we applied the 2021 WHO classification¹⁸ to T1 and T2 results, assigning each isolate to one of four categories: isoniazid-monoresistant (INH-Mono), poly-drug resistant (Poly-DR), multidrug-resistant/rifampicin-resistant (MDR/RR-TB), and pre-extensively drug-resistant (Pre-XDR-TB) ([Supplementary Table S2](#)). As pDST for BDQ and LZD was not performed, XDR-TB classification was not included.

Statistical Analysis

All analyses were conducted in R version 4.2.1. Categorical variables are presented as counts and percentages; between-group comparisons used the χ^2 -test or Fisher's exact test. Continuous variables are reported as mean \pm SD or median (IQR) and compared via Student's *t*-test or Wilcoxon rank-sum test. Within-group changes in the number of resistant drugs were assessed by paired *t*-test, and shifts between S \rightarrow R versus R \rightarrow S by McNemar's test.

Multivariable logistic regression was used to identify independent predictors of resistance progression. Variables with $P < 0.10$ in univariate analyses were included in the multivariate model. Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were calculated using the profile likelihood method.¹⁹ Collateral resistance effects were quantified using Fisher's exact test to calculate odds ratios (ORs) and corresponding 95% CIs. For all analyses, a two-sided P value < 0.05 was considered statistically significant. Missing data ($< 5\%$ for all variables) were handled using complete case analysis.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki. All patient data were extracted from the hospital's electronic database and fully anonymized prior to analysis. Access to the study database was restricted to authorized personnel to ensure participant confidentiality. As previously mentioned, the study was approved by the Ethics Committee of Beijing Chest Hospital, and informed consent was waived.

Results

Baseline Characteristics of the Study Cohort

A total of 133 patients with drug-resistant pulmonary tuberculosis (median age 52.0 years; 76.7% male) were included and stratified by phenotype progression into a progression group ($n = 76$) and a non-progression group ($n = 57$) (Figure 1). Demographic and clinical characteristics at baseline were comparable between the two groups (all $P > 0.05$). However, the non-progression group exhibited a significantly higher burden of baseline resistance: at timepoint 1 (T1), the mean number of drugs to which isolates were resistant was greater in the non-progression group than in the progression group (8.2 vs. 6.2; $P = 0.005$). Moreover, the proportions of patients with MDR/RR-TB and Pre-XDR-TB were higher in the non-progression group (51.0% [29/57] and 40.4% [23/57], respectively) (Table 1).

Overall Increase in Drug-Resistance Burden During Treatment

Across all patients, the median number of resistant drugs increased from 7.0 at T1 to 8.0 at T2 ($P < 0.05$), indicating a significant rise in overall resistance during therapy (Figure 2A). Resistance rates for most individual drugs also increased post-treatment, with rifamycin-class antibiotics (rifampin, rifapentine, rifabutin) showing the steepest upward trends (Figure 2B). A longitudinal paired analysis of 16 drugs (Table 2) revealed that six agents, led by the rifamycins, demonstrated a strong unidirectional acquisition of resistance (Figure 2C). For example, the incidence of newly acquired rifampin resistance (S \rightarrow R) was more than five times that of reversion (R \rightarrow S) (16.5% vs. 3.0%, $P < 0.001$), and rifapentine and rifabutin showed similar patterns (both $P < 0.05$). Ethambutol, kanamycin, and levofloxacin also exhibited statistically significant rates of acquired resistance (all $P < 0.05$). In contrast, a minority of drugs (para-aminosalicylic acid, streptomycin, isoniazid) displayed higher reversion than acquisition rates, though none reached statistical significance.

Dynamic Evolution of Resistance Patterns

Overall, for all patients, Table 3 shows the evolution of drug resistance patterns from T1 to T2. Among INH-Mono patients, most transitioned to MDR/RR-TB (9/14, 64.3%) or Pre-XDR-TB (4/14, 28.6%). Poly-DR patients showed relatively stable resistance, with 43.8% remaining in the same category and 43.8% progressing to MDR/RR-TB. The majority of MDR/RR-TB patients progressed to Pre-XDR-TB (9/58, 15.5%) or remained MDR/RR-TB (46/58, 79.3%). Pre-XDR-TB patients largely maintained their resistance category, with 80% remaining pre-XDR-TB at follow-up. When stratified by outcome group, these overall patterns diverged markedly. The non-progression cohort universally maintained

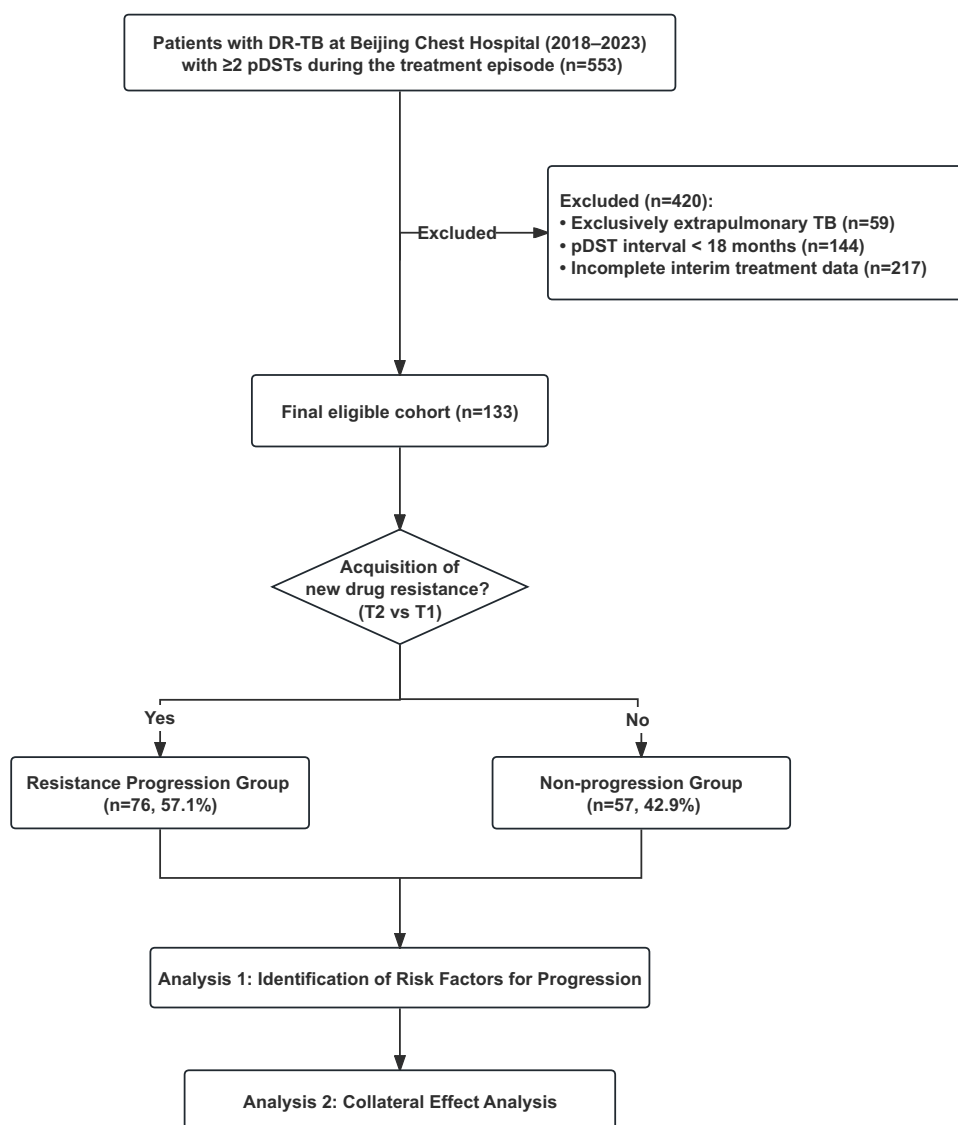


Figure 1 The workflow of this study. Analysis 1: Identification of Risk Factors for Progression; Analysis 2: Collateral Effect Analysis, Restrictive cohort analysis to assess the impact of specific drug exposures (e.g. BDQ, PZA) on resistance acquisition.

Abbreviations: TB, tuberculosis; drug-resistant pulmonary TB, DR-TB; Drug Susceptibility Tests, pDSTs.

stability or demonstrated resistance de-escalation (100%), whereas the progression cohort predominantly exhibited resistance spectrum amplification (76.3%). Stratified analysis further confirmed the distinct evolutionary trajectories between the two outcome groups (Figure 3).

Table 1 Baseline Characteristics of the Drug-Resistant Tuberculosis Cohort, Stratified by Resistance Progression Status

Characteristic	Total Cohort n= 133 ^a	Non-Progression Group n = 57 ^a	Progression Group n = 76 ^a	P-value ^b
Sex				0.59
Male	102 (76.7%)	45 (78.9%)	57 (75.0%)	
Female	31 (23.3%)	12 (21.1%)	19 (25.0%)	
Age (years), mean (±SD)	52.0 (±13.3)	51.5 (±14.3)	52.4 (±12.6)	0.87
BMI (kg/m ²), mean (±SD)	20.8 (±3.9)	20.6 (±4.3)	21.0 (±3.5)	0.43

(Continued)

Table 1 (Continued).

Characteristic	Total Cohort n= 133 ^a	Non-Progression Group n = 57 ^a	Progression Group n = 76 ^a	P-value ^b
Smoking History				0.58
No	69 (51.9%)	28 (49.1%)	41 (53.9%)	
Yes	64 (48.1%)	29 (50.9%)	35 (46.1%)	
Alcohol History				0.63
No	88 (66.2%)	39 (68.4%)	49 (64.5%)	
Yes	45 (33.8%)	18 (31.6%)	27 (35.5%)	
Treatment History				0.60
New case	110 (82.7%)	46 (80.7%)	64 (84.2%)	
Previously treated	23 (17.3%)	11 (19.3%)	12 (15.8%)	
Comorbidities				0.57
No	119 (89.5%)	52 (91.2%)	67 (88.2%)	
Yes	14 (10.5%)	5 (8.8%)	9 (11.8%)	
Extrapulmonary TB				0.50
No	124 (93.2%)	52 (91.2%)	72 (94.7%)	
Yes	9 (6.8%)	5 (8.8%)	4 (5.3%)	
Malignant Tumor				0.51
No	131 (98.5%)	57 (100.0%)	74 (97.4%)	
Yes	2 (1.5%)	0 (0.0%)	2 (2.6%)	
Viral Hepatitis				0.35
No	122 (91.7%)	54 (94.7%)	68 (89.5%)	
Yes	11 (8.3%)	3 (5.3%)	8 (10.5%)	
Diabetes Mellitus				>0.99
No	84 (63.2%)	36 (63.2%)	48 (63.2%)	
Yes	49 (36.8%)	21 (36.8%)	28 (36.8%)	
Rheumatic Disease				0.19
No	123 (92.5%)	55 (96.5%)	68 (89.5%)	
Yes	10 (7.5%)	2 (3.5%)	8 (10.5%)	
C-reactive protein (mg/L), mean (±SD)	32.2 (35.2)	34.0 (36.3)	30.8 (34.5)	0.47
Hemoglobin (g/L), mean (±SD)	124.1 (20.6)	122.5 (20.0)	125.3 (21.1)	0.28
Platelet count (10 ⁹ /L), median (IQR)	265.0 (195.0–314.0)	275.0 (195.0–347.0)	257.0 (195.0–292.0)	0.138
Lymphocyte (%), mean (±SD)	20.3 (8.9)	20.0 (10.0)	20.6 (8.1)	0.42
Monocyte (%), mean (±SD)	8.5 (2.9)	8.3 (2.8)	8.7 (3.0)	0.52
Eosinophil (%), mean (±SD)	2.4 (2.2)	2.0 (1.6)	2.6 (2.6)	0.12
Baseline Smear Status				0.26
Positive	106 (79.7%)	48 (84.2%)	58 (76.3%)	
Negative	27 (20.3%)	9 (15.8%)	18 (23.7%)	
Smear Bacterial Load				0.24
< 2+	80 (60.2%)	31 (54.4%)	49 (64.5%)	
≥ 2+	53 (39.8%)	26 (45.6%)	27 (35.5%)	
No. of Resistant Drugs at T1, mean (±SD)	7.0 (±3.7)	8.2 (±3.5)	6.2 (±3.7)	0.005
Resistance Pattern at T1				0.008
INH-Mono	14 (10.6%)	0 (0%)	14 (18.3%)	
Poly-DR	16 (12%)	5 (8.8%)	11 (14%)	
MDR/RR-TB	58 (44%)	29 (51%)	17 (38%)	
Pre-XDR-TB	45 (34%)	23 (40%)	22 (29%)	

Notes: ^aData are presented as n (%), mean ± SD, or median (IQR) as appropriate. n (%); Mean (SD), or median (IQR). ^bP-values were calculated using Pearson's Chi-squared test, Fisher's exact test, or Wilcoxon rank-sum test. Bold values indicate statistical significance ($P < 0.05$). Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test.

Abbreviations: BMI, body mass index; T1, baseline; CRP, C-reactive protein; WBC, white blood cell count; SD, standard deviation; IQR, interquartile range; INH-Mono, isoniazid mono-resistance; Poly-DR, polydrug-resistant tuberculosis; MDR/RR-TB, multidrug-/rifampicin-resistant tuberculosis; Pre-XDR-TB, pre-extensively drug-resistant tuberculosis.

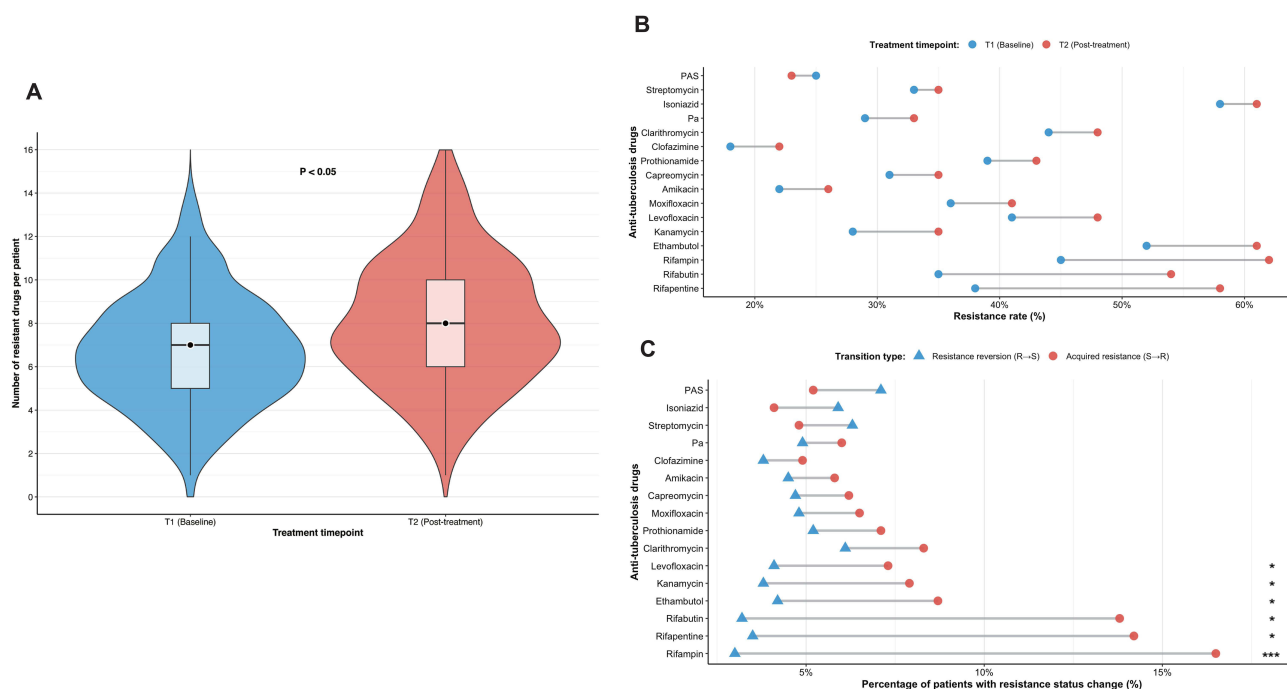


Figure 2 Dynamic Evolution of Drug-Resistance Burden and Profiles During Anti-Tuberculosis Treatment. **(A)** Distribution of the number of resistant drugs per isolate at baseline (T1) and post-treatment (T2). Median values increased significantly from 7.0 to 8.0 (Wilcoxon signed-rank test, $P < 0.05$). **(B)** Resistance rates for 16 anti-tuberculosis drugs at baseline (T1) and follow-up (T2). **(C)** The proportion of isolates showing resistance status changes for 16 drugs: acquired resistance (S→R, represented by red circles) and resistance reversion (R→S, represented by blue triangles). Statistical significance of transition patterns was determined using McNemar's test.

Notes: $P < 0.05$; * $P < 0.01$; ** $P < 0.001$.

Abbreviations: T1, baseline; T2, post-treatment.

In the non-progression resistance cohort (Figure 3A), resistance patterns demonstrated remarkable stability with partial “downgrading”. Among MDR/RR-TB patients ($n=29$), 28 cases (96.6%) maintained the same resistance classification at T2, while among Pre-XDR-TB patients ($n=23$), 18 cases (78.3%) preserved their original classification, and 5 cases (21.7%) translated to MDR/RR-TB. Overall, resistance pattern transitions in this group were characterized predominantly by maintenance of the initial resistance profile or evolution toward less severe patterns, with no observed progression toward more severe resistance profiles.

In contrast, the progression resistance cohort (Figure 3B) exhibited a pronounced tendency toward resistance spectrum expansion. All patients with INH-Mono resistance at baseline ($n=14$, 100%) progressed to more severe resistance patterns, with 9 cases (64.3%) advancing to MDR/RR-TB, 4 cases (28.6%) to Pre-XDR-TB, and 1 case (7.1%) to Poly-DR. Similarly, among patients with poly-drug resistance (Poly-DR) at baseline, 9 cases (81.8%) progressed to MDR/RR-TB or Pre-XDR-TB (7 and 2 cases, respectively). Among patients with baseline MDR/RR-TB, the principal evolutionary pathway was further development to Pre-XDR-TB (9/29, 31.0%), while 18 cases (62.1%) maintained their original resistance pattern, underscoring the heterogeneous nature of drug resistance evolution even within clinically progressing cases.

Risk Factors for Resistance Progression

To identify factors associated with resistance progression, we first performed univariate comparisons of 19 anti-TB drugs used during the treatment interval between the progression and non-progression groups (Table 4). The usage frequencies of most drugs showed no significant differences. However, BDQ and PZA did differ significantly, BDQ was used more frequently in the non-progression group (26.3% vs. 11.8%, $P = 0.032$), whereas PZA was more common in the progression group (55.3% vs. 36.8%, $P = 0.035$).

To assess the independence of these associations, we subsequently included these two drugs along with key baseline characteristics in a multivariable logistic regression model (Figure 4). After adjusting for potential confounders, neither

Table 2 Transitions in Drug Resistance Patterns Across Treatment in DR-TB Patients

Drug Name	S→S	R→R	S→R	R→S	S→R Proportion	R→S Proportion	P-value ^a
RFP	8	99	22	4	16.50%	3.00%	<0.001
Rft	2	107	19	5	14.30%	3.80%	0.008
Rfb	71	33	21	8	15.80%	6.00%	0.026
EMB	6	103	18	6	13.50%	4.50%	0.025
Km	90	22	16	5	12.00%	3.80%	0.029
Lfx	93	24	13	3	9.80%	2.30%	0.024
Pto	37	61	22	13	16.50%	9.80%	0.176
Clr	15	84	21	13	15.80%	9.80%	0.23
Cm	89	18	16	10	12.00%	7.50%	0.327
Cfz	86	12	20	15	15.00%	11.30%	0.499
AMK	85	19	16	13	12.00%	9.80%	0.71
Mfx	76	34	13	10	9.80%	7.50%	0.677
PAS	111	6	9	7	6.80%	5.30%	0.803
INH	20	100	6	7	4.50%	5.30%	>0.999
Sm	54	44	17	18	12.80%	13.50%	>0.999
Pa	89	18	10	16	7.50%	12.00%	0.327

Notes: ^aStatistical significance was determined using McNemar's test to compare the proportions of resistance acquisition (S→R) and reversion (R→S). Bold values indicate statistical significance ($P < 0.05$). Transitions are defined as follows: S→S, remained susceptible; R→R, remained resistant; S→R, acquired resistance (susceptible at baseline T1, resistant at follow-up T2); R→S, reverted to susceptible (resistant at T1, susceptible at T2). The S→R proportion is calculated as $S \rightarrow R / (S \rightarrow R + R \rightarrow S)$; the R→S proportion is calculated as $R \rightarrow S / (R \rightarrow R + R \rightarrow S)$.

Abbreviations: INH, isoniazid; RFP, rifampicin; EMB, ethambutol; Sm, streptomycin; Rft, rifapentine; Rfb, rifabutin; Lfx, levofloxacin; Mfx, moxifloxacin; Km, kanamycin; AMK, amikacin; Cm, capreomycin; Pto, prothionamide; PAS, para-aminosalicylic acid; Cfz, clofazimine; Clr, clarithromycin; Pa, isoniazid-p-aminosalicylic acid.

Table 3 Evolution of Drug Resistance Patterns

Baseline (T1) Resistance Pattern	Post-Treatment (T2)			
	Poly-DR	MDR/RR-TB	Pre-XDR-TB	Total
INH-Mono	1 (7.1%)	9 (64.3%)	4 (28.6%)	14 (100%)
Poly-DR	7 (43.8%)	7 (43.8%)	2 (12.5%)	16 (100%)
MDR/RR-TB	3 (5.2%)	46 (79.3%)	9 (15.5%)	58 (100%)
Pre-XDR-TB	0 (0%)	9 (20%)	36 (80%)	45 (100%)

Notes: Data are presented as n (%), representing the number (percentage) of patients within each baseline resistance category who transitioned to the respective follow-up pattern.

Abbreviations: T1, baseline; T2, post-treatment; INH-Mono, isoniazid monoresistance; Poly-DR, polydrug-resistant tuberculosis; MDR/RR-TB, multidrug-/rifampicin-resistant tuberculosis; Pre-XDR-TB, pre-extensively drug-resistant tuberculosis.

of the associations observed in the univariate analysis remained statistically significant. Although BDQ use still showed a protective trend (aOR=0.47, 95% CI: 0.17–1.23, $P=0.126$), and PZA use a risk trend (aOR=1.74, 95% CI: 0.83–3.68, $P=0.141$), no independent predictors were identified. In summary, this analysis did not identify any single therapeutic or baseline factor that could independently predict resistance progression.

Collateral Effects of BDQ and PZA

Given that the multivariable analysis failed to identify independent risk factors for overall resistance progression, we focus on the micro-level interactions between drugs. To this end, we conducted an exploratory analysis using a restrictive cohort method to precisely evaluate whether BDQ and PZA exhibit collateral effects on resistance to other drugs (Figure 5).

First, we assessed the impact of these two drugs on the final resistance status at T2. The analysis for BDQ revealed no statistically significant effects on resistance to any of the other 13 drugs (Figure 5A). In sharp contrast, the analysis for

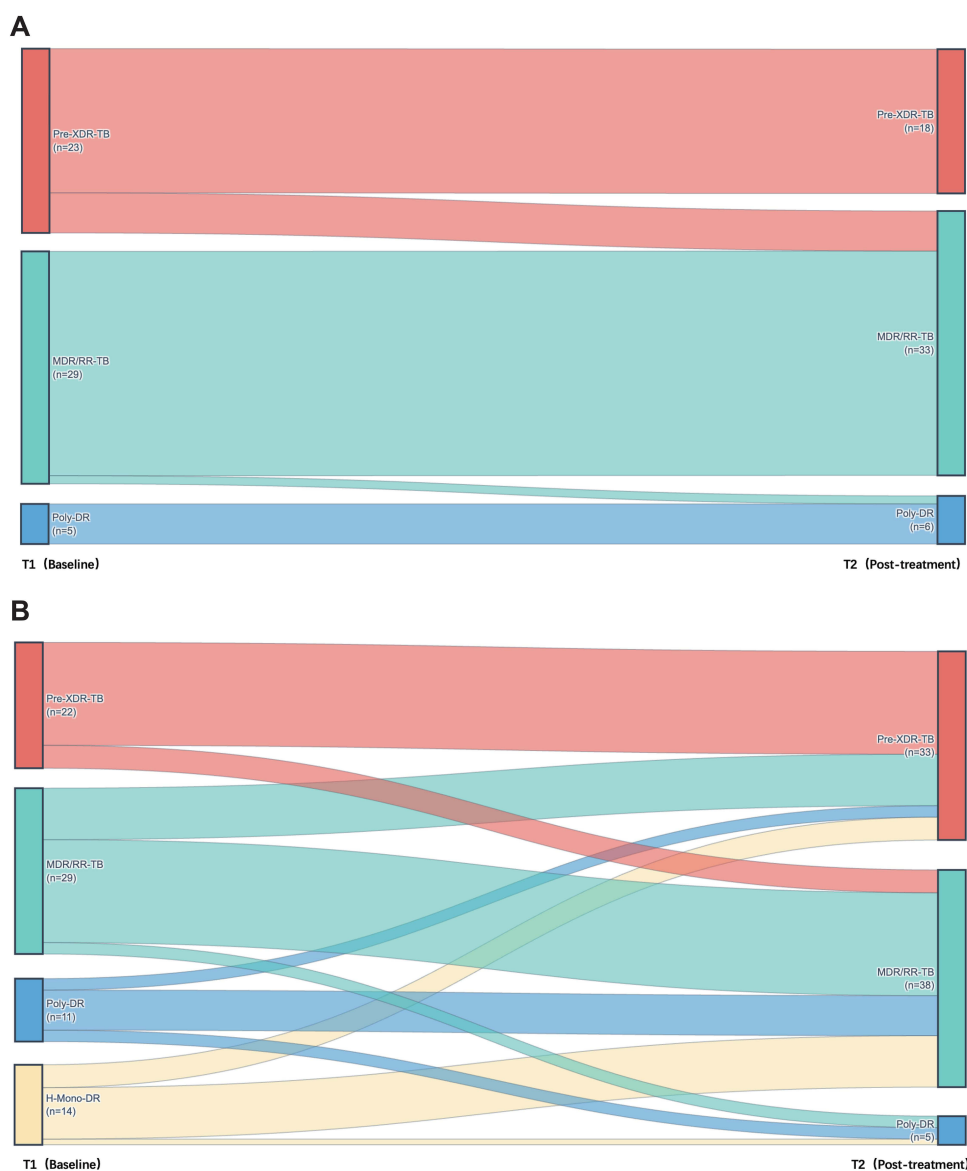


Figure 3 Evolution of drug resistance patterns from baseline (T1) to post-treatment (T2). **(A)** Sankey diagram illustrating resistance category transitions in the non-progression cohort, which demonstrated remarkable stability or partial resistance de-escalation. **(B)** Sankey diagram demonstrating the expansion of resistance spectrums in the progression cohort, characterized by a shift from lower-level resistance (e.g. INH mono-resistance) toward more severe categories such as MDR/RR-TB and Pre-XDR-TB. **Abbreviations:** T1, baseline; T2, post-treatment; Pre-XDR-TB, pre-extensively drug-resistant tuberculosis; MDR/RR-TB, multidrug-resistant/rifampicin-resistant tuberculosis; Poly-DR, polydrug-resistant tuberculosis; H-Mono-DR, isoniazid-mono-resistant tuberculosis.

PZA uncovered a strong synergistic effect with rifabutin (Rfb). After rigorously excluding patients treated with any rifamycin-class drug (RFP, Rft, Rfb) to control for cross-resistance, the results showed that the proportion of Rfb resistance among PZA users was 37.8% (17/45), significantly higher than in non-users (8.5%, 4/47), corresponding to a robust odds ratio (OR) of 5.94 (95% CI: 1.90–18.53, $P=0.001$) (Figure 5B).

To further investigate whether this synergy was directly manifested in the process of acquiring new resistance, we then analyzed the proportion of acquired resistance (S→R) for key drug pairs (Figure 6). This analysis strongly corroborated the PZA-Rfb synergistic relationship: among patients susceptible to Rfb at baseline ($n=92$) and not treated with any rifamycins, the proportion who subsequently acquired Rfb resistance was significantly higher among PZA users (17/45, 37.8%) compared to non-users (4/47, 8.5%, $P=0.001$) (Figure 6A). Furthermore, this analysis revealed a non-significant trend of synergy between PZA and moxifloxacin (Mfx) (6/27, 22.2% vs. 1/25, 4.0%, $P=0.144$) (Figure 6B), a non-significant trend of collateral sensitivity between BDQ and amikacin (AMK) (0/14, 0.0% vs. 3/15, 20.0%, $P=0.191$) (Figure 6C).

Table 4 Anti-Tuberculosis Drugs Used in Treatment Regimens for the Non-Progression and Progression Groups

Names of Anti-Tuberculosis Drugs	Total Cohort n= 133 ^a	Non-Progression Group n = 57 ^a	Progression Group n = 76 ^a	P-value ^b
Ctz	1 (0.8%)	1 (1.8%)	0 (0.0%)	0.43
DLM	6 (4.5%)	3 (5.3%)	3 (3.9%)	>0.99
BDQ	24 (18.0%)	15 (26.3%)	9 (11.8%)	0.032
Lzd	79 (59.4%)	35 (61.4%)	44 (57.9%)	0.68
Cs	66 (49.6%)	27 (47.4%)	39 (51.3%)	0.65
PZA	63 (47.4%)	21 (36.8%)	42 (55.3%)	0.035
INH	18 (13.5%)	10 (17.5%)	8 (10.5%)	0.24
RFP	2 (1.5%)	0 (0.0%)	2 (2.6%)	0.51
EMB	41 (30.8%)	20 (35.1%)	21 (27.6%)	0.36
Rft	7 (5.3%)	4 (7.0%)	3 (3.9%)	0.46
Rfb	1 (0.8%)	0 (0.0%)	1 (1.3%)	>0.99
Lfx	26 (19.5%)	10 (17.5%)	16 (21.1%)	0.61
Mfx	66 (49.6%)	29 (50.9%)	37 (48.7%)	0.80
AMK	40 (30.1%)	15 (26.3%)	25 (32.9%)	0.41
Cm	28 (21.1%)	9 (15.8%)	19 (25.0%)	0.20
Pto	63 (47.4%)	28 (49.1%)	35 (46.1%)	0.73
PAS	5 (3.8%)	2 (3.5%)	3 (3.9%)	>0.99
Clr	5 (3.8%)	2 (3.5%)	3 (3.9%)	>0.99
Cfz	47 (35.3%)	21 (36.8%)	26 (34.2%)	0.75
Pa	35 (26.3%)	14 (24.6%)	21 (27.6%)	0.69

Notes: ^aData are presented as n (%), representing the number of patients exposed to the specific drug during the treatment interval. n (%): number of cases (percentage). ^bP-values were calculated using Pearson's Chi-squared test, Wilcoxon rank-sum test, or Fisher's exact test, as appropriate. Bold values indicate statistical significance ($P < 0.05$). P-values were derived from Pearson's Chi-squared, Wilcoxon rank-sum, or Fisher's exact tests.

Abbreviations: Ctz, contezolid; DLM, delamanid; BDQ, bedaquiline; Lzd, linezolid; Cs, cycloserine; PZA, pyrazinamide; INH, isoniazid; RFP, rifampicin; EMB, ethambutol; Rft, rifapentine; Rfb, rifabutin; Lfx, levofloxacin; Mfx, moxifloxacin; AMK, amikacin; Cm, capreomycin; Pto, prothionamide; PAS, para-aminosalicylic acid; Clr, clarithromycin; Cfz, clofazimine; Pa, isoniazid-p-aminosalicylate.

Discussion

Our study provides a comprehensive analysis of the complex, nonlinear evolution of drug-resistant tuberculosis (DR-TB), highlighting two distinct evolutionary paths. Consistent with the growing recognition that resistance evolution is multifaceted and non-linear,^{20,21} our longitudinal cohort analysis demonstrates that DR-TB often acquires additional resistances during treatment. However, these evolutionary trajectories are not uniform or strictly cumulative. Instead, they exhibit significant heterogeneity and bifurcation, which has important clinical implications. The expansion of resistance reduces therapeutic options, leading to higher risks of treatment failure, relapse, and increased mortality, while also enhancing community transmission of resistant strains.^{22–24}

Our data reveal two diverging outcomes for DR-TB strains. In the progression cohort, INH-Mono isolates were particularly unstable, with 100% evolving to more extensive resistance and 64.3% progressing to MDR/RR-TB. This rate significantly exceeds those reported in previous studies, such as a meta-analysis by Gegia et al, which found a progression rate of only 15%.⁵ These findings underscore that INH-Mono may represent a more significant threat than previously appreciated. They also support the World Health Organization's post-2018 focus on the "early identification and aggressive intervention for INH-Mono cases",^{18,25} suggesting that existing guidelines may not fully capture the urgency of intervention in these cases.

By contrast, the non-progression cohort, which had a higher baseline resistance burden, showed remarkable stability, with occasional reversion observed. Notably, 17.4% (4/23) of Pre-XDR-TB cases downgraded to MDR/RR-TB. This stabilization, or "downgrade", likely reflects the fitness cost associated with highly resistant strains, which limits their

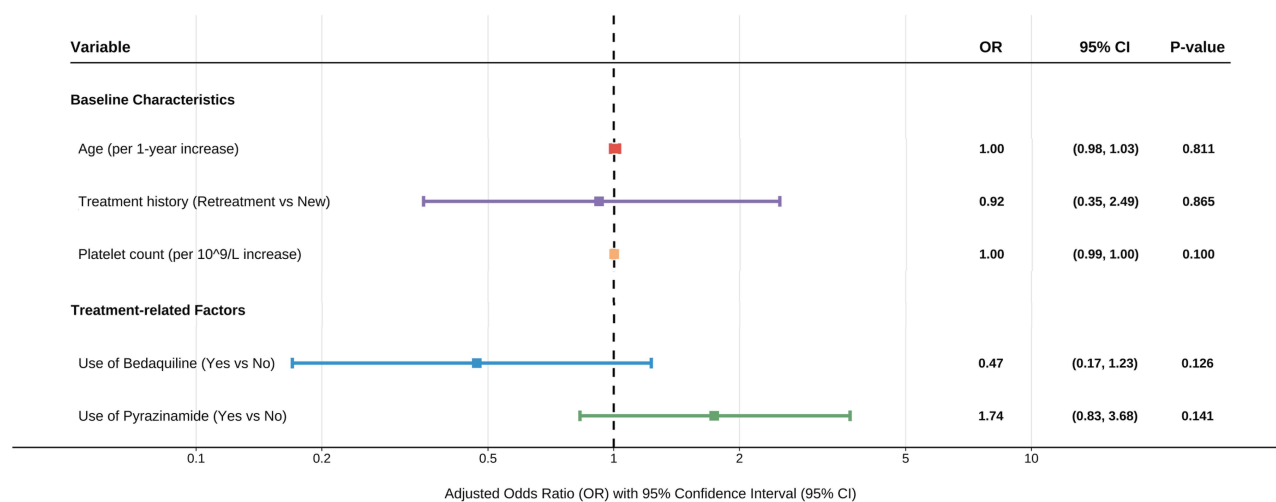


Figure 4 Multivariate logistic regression analysis of factors associated with resistance progression. The forest plot displays the adjusted odds ratios (aORs) for baseline clinical characteristics and specific drug exposures during treatment. Each square represents the point estimate of the aOR, and the horizontal bars represent the 95% confidence intervals (CIs). The dashed vertical line indicates the null effect (OR = 1). A P-value < 0.05 was considered statistically significant.

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval.

ability to acquire additional mutations while maintaining viability.^{26–31} These findings point to distinct drivers of resistance evolution, which we explore further in the context of collateral drug effects.

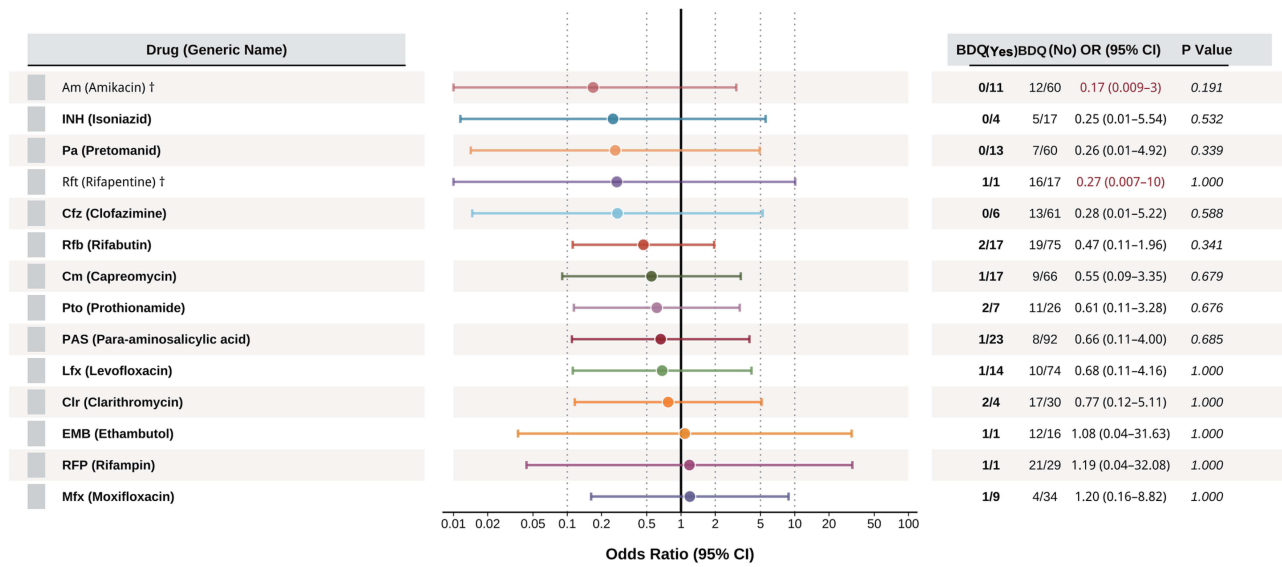
Our multivariable regression analysis did not identify any specific treatment or baseline factor that independently predicted overall resistance progression. This suggests that the underlying drivers of resistance evolution may involve subtler drug–drug interactions. Inspired by the known phenomenon of INH resistance promoting rifampin resistance,^{24,25} we focused on collateral effects across different drug regimens. Contrary to our initial hypothesis of a broad synergistic effect of PZA on rifamycin resistance, we observed a highly specific association. PZA exposure did not correlate with resistance to RFP or Rft, but it was strongly linked to newly acquired Rfb resistance. After excluding cross-resistant cases, PZA exposure increased the odds of Rfb resistance nearly six-fold (37.8% vs. 8.5%, OR = 5.94; $P = 0.001$).

This PZA-Rfb association appears to arise from the distinct pharmacodynamics of PZA and rifabutin within mycobacterial cells. While all rifamycins target RNA polymerase, rifabutin has unique structural properties that enhance its intracellular penetration and accumulation.^{32,33} As a prodrug, PZA is converted to pyrazinoic acid, which acidifies the intracellular environment.³⁴ We hypothesize that this acidification selectively alters efflux pump activity or modulates specific rpoB gene mutations responsible for Rfb resistance, while sparing other rifamycins.^{35–37} The clinical implications of this PZA-Rfb association are substantial. Rifabutin has become a critical treatment option for TB-HIV co-infected patients due to its reduced interaction with antiretroviral medications.^{38–42} Our findings suggest that TB patients with extensive PZA exposure, particularly those treated for MDR-TB with PZA-containing regimens and later developing recurrent disease, should undergo careful rifabutin susceptibility testing prior to retreatment. This is especially important in settings where routine rifabutin susceptibility testing is not performed, as failure to detect resistance could lead to ineffective treatment and further resistance amplification.

In contrast to PZA's collateral effect, BDQ exhibited a potential protective trend against resistance progression. Although not statistically significant (aOR = 0.47, 95% CI: 0.17–1.23, $P = 0.126$), BDQ use was associated with no new amikacin resistance, compared to 20.0% in non-users ($P = 0.191$). This “protective signal” supports BDQ's established role as a cornerstone in MDR-TB therapy.^{43–49} BDQ targets ATP synthase subunit c, encoded by the *atpE* gene,⁵⁰ disrupting energy metabolism by inhibiting ATP synthesis.⁵¹ Unlike most anti-TB drugs that affect cell wall synthesis or nucleic acids, BDQ's unique mechanism likely reduces cross-resistance with other drug classes. Furthermore, BDQ's long half-life (5.5 months) ensures sustained bactericidal activity, creating an “immunological window” that may allow the host immune system to clear remaining pathogens before resistance can emerge.^{52–54}

This study has several strengths, including its longitudinal cohort design and novel exploration of drug collateral effects. However, it also has limitations. As a single-center retrospective analysis with a modest sample size, the

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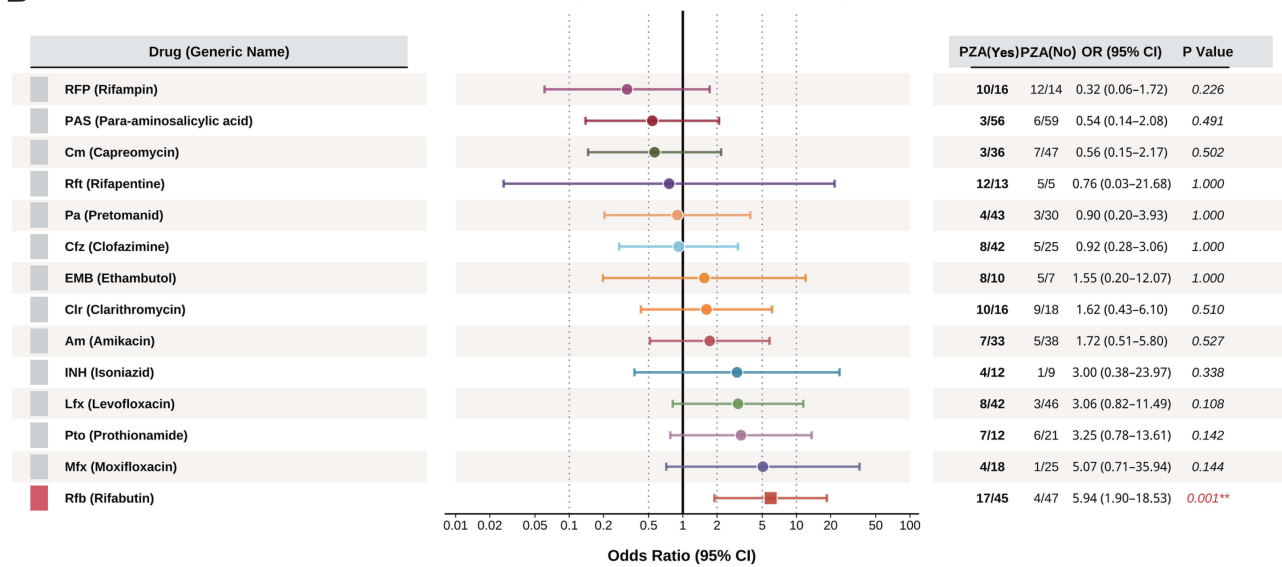


Figure 5 Collateral drug effects of bedaquiline (BDQ) and pyrazinamide (PZA) on resistance development. **(A)** Forest plot depicting the association between BDQ exposure and the subsequent acquisition of resistance to 13 companion drugs. **(B)** Forest plot showing the association between PZA exposure and acquired drug resistance. A significant collateral association was identified between PZA exposure and acquired rifabutin (Rfb) resistance (OR: 5.94; 95% CI: 1.90–18.53; P = 0.001).

Notes: Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using a restrictive cohort approach. The dagger (†) indicates variables with extremely wide 95% CIs that were capped to fit the visual scale of the plot. Square symbols represent the point estimates of the OR. Statistical significance is indicated as follows: P < 0.05, *P < 0.01, **P < 0.001.

Abbreviations: BDQ, bedaquiline; PZA, pyrazinamide; Rfb, rifabutin; Am, amikacin; INH, isoniazid; Pa, pretomanid; Rft, rifapentine; Cfz, clofazimine; Cm, capreomycin; Pto, prothionamide; PAS, para-aminosalicylic acid; Lfx, levofloxacin; Clr, clarithromycin; EMB, ethambutol; RFP, rifampicin; Mfx, moxifloxacin.

generalizability of our findings may be limited, and certain comparisons may be underpowered. Moreover, the reliance on phenotypic susceptibility testing for only 16 drugs, excluding newer agents such as BDQ and linezolid, restricts our ability to fully assess resistance evolution under contemporary all-oral regimens. Future studies should validate these findings in larger multicenter cohorts and integrate whole-genome sequencing to elucidate the molecular basis of the PZA-Rfb synergy and BDQ’s protective effect, ultimately refining treatment regimens.

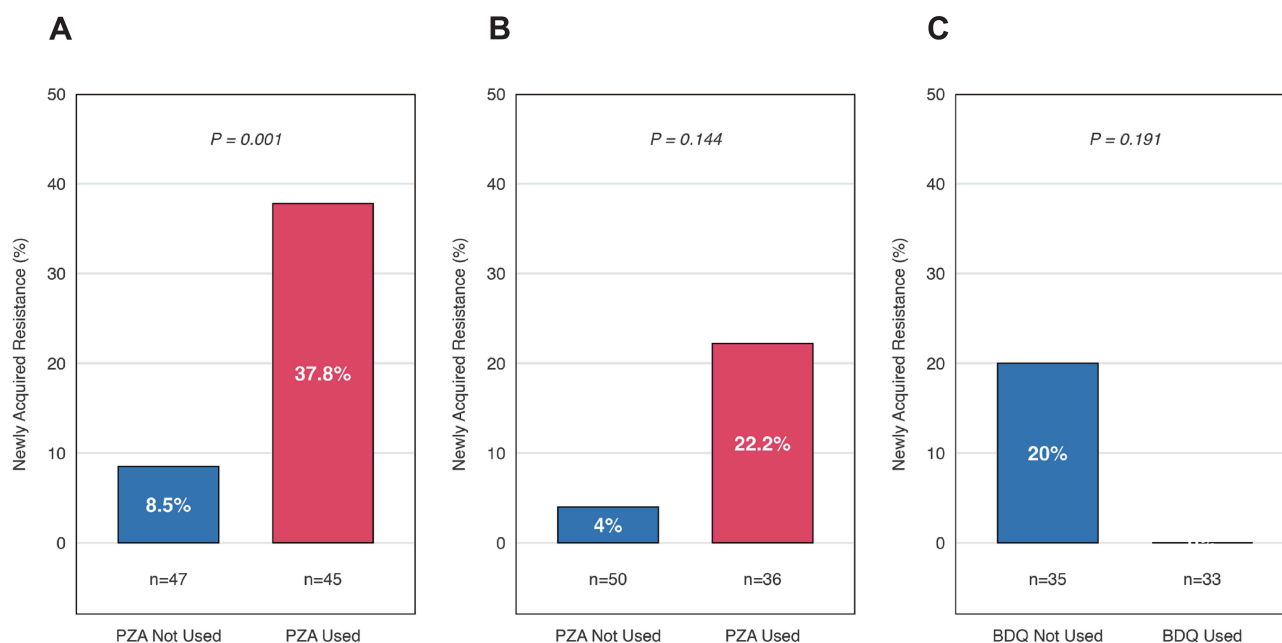


Figure 6 Impact of anti-tuberculosis drug usage on the acquisition of resistance to companion agents. **(A)** Effect of PZA usage on newly acquired Rfb resistance. **(B)** Effect of PZA usage on newly acquired Mfx resistance. **(C)** Effect of BDQ usage on newly acquired AMK resistance.

Notes: Data are presented as the percentage of patients who acquired resistance during treatment. The analysis was restricted to patients who were phenotypically susceptible to the respective companion drugs at baseline (T1). P-values were calculated using Fisher's exact test.

Abbreviations: PZA, pyrazinamide; Rfb, rifabutin; Mfx, moxifloxacin; BDQ, bedaquiline; AMK, amikacin.

In summary, our study systematically characterizes the diverse evolutionary paths of DR-TB during treatment, emphasizing the high-risk nature of INH-Mono cases. We also present the first clinical evidence of a specific collateral effect between PZA and rifabutin. These insights contribute to the precision-medicine framework, highlighting that individualized TB care must go beyond static susceptibility profiles and consider the dynamic risk of resistance evolution and inter-drug interactions. This approach will more effectively curb spectrum expansion and improve patient outcomes.

AI Usage Statement

Generative artificial intelligence tools (e.g., GPT-4) were used to assist in [e.g., language refinement, grammatical correction, suggesting stylistic improvements, or drafting parts of the cover letter and submission guidance based on the scientific content provided by the authors]. The authors thoroughly reviewed, edited, and verified all AI-generated content to ensure accuracy and scientific integrity, and take full responsibility for the final content of the manuscript.

Ethical Statement

The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Beijing Chest Hospital, Capital Medical University (Approval No. 2024 Clinical Review–Research–32). The requirement for informed consent was waived by the Ethics Committee due to the retrospective nature of the study and the anonymity of the data. All patient data were treated with strict confidentiality and were anonymized prior to analysis.

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

All authors declare no competing interests.

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