Research Square

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Characterisation of Bedaquiline Resistance-Associated Mutations in Drug-Resistant Tuberculosis Phenotypes

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Research Article

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Abstract

Background

In Eswatini, the prevalence of bedaquiline resistance is at an alarming rate of 36%, and notably, there is no study done to profile mutations associated with bedaquiline resistance in Eswatini.

Methods

Sixty participants were prospectively enrolled from TB clinics based on predefined inclusion criteria. Demographic data were extracted from Medical files. DNA was then extracted using the CTAB method from *M. tuberculosis* subcultures. The extracted DNA was run on culture-based whole-genome sequencing (WGS), and our data were analysed using TB-profiler. Statistical analyses were conducted using Statistical Package for the Social Sciences version 25 software.

Results

The *Rv0678_p.Met146Thr* (n = 16) was the predominant mutation in the mutations associated with the BDQ resistance category. However, most strains harboured the *Rv1979c_c.-129A > G* (n = 50) and *Rv0676c_p.Ile948Val* (n = 55), which are mutations associated with bedaquiline resistance-interim. The *Rv0678_p.Met146Thr* (p = 0.011), *Rv1979c_c.129A > G* (p = 0.016), *Rv0676c_p.Ile948Val* (p = 0.016), *Rv3245c_p.Met517Leu* (p = 0.035), and particularly *Rv0676c_c.1065G_T* (p = < 0.001) were significantly associated with different TB first-line DR-TB phenotypes. In contrast, Rv0676c_p.Thr794Ile did not show a statistically significant association with first-line drug resistance status. The HR-TB; n = 15/60;25%, possessed pre-existing *Rv0678*/*mmpR5* mutations. The *Rv0678_p.Met146Thr* was the only mutation significantly associated with phenotypic BDQ resistance in this cohort.

Conclusion

The findings indicate that some mutation-related BDQ resistance was significantly associated with different DR-TB phenotypes, suggesting that these mutations may play important roles in the development or maintenance of first-line TB drug resistance. The pre-existing *Rv0678*/*mmpR5* mutations should be monitored.

Introduction

Tuberculosis (TB) remains a major global public health concern, causing millions of illnesses and deaths each year (1). An estimated 1.23 million fatalities worldwide were attributed to tuberculosis in 2024, including 150,000 among HIV-positive individuals. Eswatini is a Southern African country classified by the World Health Organisation (WHO) as one of the 30 high TB-burden countries globally. In 2024, TB killed 340 out of 100,000 people in Eswatini, a substantially high proportion (1). Although the incidence of tuberculosis

(TB) in Eswatini has declined substantially over the past decade, from a peak of 1,190 cases per 100,000 population in 2009 to 349 cases per 100,000 population in 2023, it remains unacceptably high. These findings underscore the need for ongoing monitoring of drug resistance and the emergence of clinically significant resistance-associated mutations, as well as more effective methods for their detection (2).

The global health community faces a significant challenge in combating drug-resistant TB, particularly with the emergence of resistance to crucial second-line TB drugs such as bedaquiline (BDQ) (3). In Eswatini, presumptive TB patients are diagnosed using the Ziehl-Neelsen microscopy method, molecular techniques (Line probe assay, GeneXpert® MTB/RIF assay (Cepheid, USA)), and/or TB culture methods (BACTEC™ MGIT™ 960 Mycobacteria Culture System (Becton Dickinson, USA)). The drug susceptibility testing on all culture-positive isolates is carried out on the same BACTEC MGIT™ 960 system platform. Rapid detection of resistance is the cornerstone of Eswatini's strategy for drug-resistant TB (DR-TB) management, facilitating timely case detection and initiation of treatment. However, standard diagnostic methods overlook certain clinically relevant resistance mutations, impeding the timely selection and implementation of optimal therapies.

Whole-genome sequencing (WGS) provides a high-resolution approach for characterising drug-resistant *M. tuberculosis*, which allows rapid and accurate detection of multidrug-resistant tuberculosis (MDR-TB) (4). This is particularly relevant as traditional culture-based and molecular assays often exhibit limitations in fully characterising DR-TB, potentially leading to misdiagnosis and inappropriate treatment initiation (5). Whole genome sequencing provides a powerful way to identify complex drug resistance mechanisms, including resistance to BDQ. This information is essential for guiding patient treatment and strengthening public health responses in high TB-burden settings (6). This is important for BDQ, where resistance-associated mutations are still emerging and may occur in genes such as *Rv0678/mmpR5*, *atpE*, and *pepQ*, where the relationship between genotype and phenotypic resistance may be complex (7, 8).

Bedaquiline-containing regimens have become a cornerstone of DR-TB treatment, including in Eswatini, where updated DR-TB treatment regimens were implemented in 2019 (9). Despite their effectiveness, resistance to the drugs used in these newer regimens remains poorly understood, particularly in Eswatini, where WGS data on the prevalence and transmission of resistance-associated mutations are limited. These knowledge gaps highlight the importance of expanding the use of WGS, which provides a powerful tool for identifying resistance-associated mutations, characterising the genetic basis of drug resistance, and guiding personalised treatment strategies (2, 10).

This study aimed to characterise the genetic variability and distribution of BDQ-resistant *M. tuberculosis* circulating in Eswatini. We used culture-based WGS to: (i) identify resistance-associated mutations in genes linked to BDQ resistance; (ii) determine the predominant BDQ resistance-associated mutations; and (iii) evaluate the association between these mutations and isoniazid mono-resistant (RH-TB), rifampicin mono-resistant (RR-TB) and MDR-TB and extremely drug-resistant (XDR-TB).

Materials and methods

Recruitment and enrolment of participants

The study prospectively recruited RH-TB, RR-TB, MDR-TB and XDR-TB patients between June 2022 and November 2023. Patients were recruited when seeking care at the Manzini TB Centre, Raleigh Fitkin Memorial Hospital (both in the Manzini region), Nhlangano Health Centre (Shiselweni region), and Piggs Peak Government Hospital (Hhohho region). These facilities are located in three of the four regions of Eswatini: Manzini, Shiselweni, and Hhohho (Figure 1). According to 2022 National TB Reference Laboratory Statistics, most RH-TB, RR-TB, MDR-TB and XDR-TB patients were isolated from Manzini TB Centre (15%), Raleigh Fitkin Memorial Hospital (17%), Piggs Peak Government Hospital (9%) and Nhlangano Health Centre (21%). As these facilities account for a substantial proportion of drug-resistant TB cases in Eswatini, they were considered representative sites for investigating bedaquiline (BDQ) resistance.

Patients were recruited when seeking care at the study sites, where the study was presented to them. Before the patient could consult with the clinician, the patients were given information about the study participation risks, benefits and the participants' rights during the study, which includes voluntary participation. Participants willing to be part of the study signed the consent forms. Baseline data were obtained through a review of participants' medical records, from which age, gender, and HIV status were extracted. Participants aged <18 years, those with incomplete medical records and or with drug-susceptible TB were excluded. The study enrolled 60 participants **(Figure 2)**.

Ethical approval

Ethical approval for this study was obtained from the Durban University of Technology Institutional Research Ethics Committee (IREC 129/22) as well as from the Eswatini National Human and Health Research Review Board (ENHHRRB103/2021). Written informed consent was received from all participants recruited in the study. All methods were performed in accordance with the relevant guidelines and regulations.

M. tuberculosis culture and phenotypic drug susceptibility testing

The participants' sputum specimen was decontaminated using MycoPrep (Becton Dickson) and cultured on BACTEC Mycobacteria growth indicator tube (MGIT^{TM}) TB system (Becton Dickson, Sparks, Maryland). After a positive MGIT culture, the Mycobacterium Protein 64 (MPT64) antigen assay was performed (SD Bioline) to confirm *M. tuberculosis* complex, followed by phenotypic culture and drug sensitivity methods as previously published (11, 12).

DNA extraction and whole-genome sequencing

The DNA was extracted from *M. tuberculosis* isolates using the cetyltrimethylammonium bromide (CTAB) method, followed by culture-based whole-genome sequencing on the Illumina NextSeq platform (Illumina, San Diego, CA, USA), generating 150-base-pair (bp) paired-end reads targeting a depth of at least 100X. Sequencing was done at Stellenbosch University, Centre for Epidemic Response and Innovation (CERI). The CTAB method was used because it is highly reliable for generating intact, long-fragment genomic DNA. It also yields highly concentrated DNA solutions that are perfectly suited for sensitive downstream molecular applications.

Bioinformatics classification of mutations

Bioinformatics analysis of the *M. tuberculosis* strains was performed using TB-Profiler version 6.6.6, a command line profiling tool for whole-genome sequencing data. Single-nucleotide polymorphism (SNP) calling and detection of resistance-associated variants were all determined by running the raw FASTQ files through TB-Profiler version 6.6.6 under its default settings. TB-Profiler version 6.6.6 report of detected mutation information based on its internal mutation database, which incorporates the WHO mutation catalogue second edition and expert curation, was generated. All identified SNPs that met the default coverage and quality criteria of B-Profiler version 6.6.6 were included in downstream interpretation (13, 14).

Statistical methods

The participants' DR-TB classification, treatment outcome, HIV status, and age data sets were entered into a Microsoft Excel program for descriptive analysis. For regression analysis, the Statistical Package for the Social Sciences (SPSS) version 25 software was used to determine the correlation between mutations associated with BDQ resistance and DR-TB type. The Chi-square test was used to measure the level of association.

Results

The study aimed to profile the genetic variability and distribution of BDQ-resistant *M. tuberculosis* circulating in Eswatini. Table 1 provides a breakdown of the study population, which was predominantly male (n = 32, 53%), with most coming from the Manzini region, and most participants were aged 38 (n=8/60, 13%), with 36 years being the mean age. Most participants had MDR-TB strains (n = 39, 65%), while pre-XDR-TB strains were identified in four participants (n = 4, 7%). Drug-resistant TB strains were isolated from both male and female participants.

Table 1: Demographic characteristics of participants

DR-TB type	Prevalence of DR-TB Type n (%)	Gender n (%)		Age group n (%) (mean age=36 years)			Region n (%)		
		Female	Male	15-30 years	31-40 years	41-65 years	Hhohho	Manzini	Shiselweni
HR-TB	15 (25%)	5 (8%)	10 (16%)	7 (12%)	4 (7%)	4 (7%)	5 (8%)	7 (12%)	3 (5%)
MDR-TB	39 (65%)	19 (32%)	20 (33%)	9 (15%)	18 (30%)	12 (20%)	1 (2%)	24 (40%)	14 (23%)
RR-TB	2 (3%)	1 (2%)	3 (5%)	2 (3%)	0 (0%)	0 (0%)	0 (0%)	2 (3%)	0 (0%)
Pre-XDR-TB	4 (7%)	3 (5%)	1 (2%)	2 (3%)	2 (3%)	0 (0%)	0 (0%)	2 (3%)	2 (3%)
Total	60 (100%)	28 (47%)	32 (53%)	20 (33%)	24 (40%)	16 (27%)	6 (10%)	35 (58%)	19 (32%)

The analysis of the mutations associated with BDQ resistance noted in the study shows that the Rv0676c_p.IIe948Val (n=55, 92%) and Rv1979c_c.-129A>G (n=50, 83%) were predominant in the study (Figure 3).

A total of n=21/60 (35%) isolates harboured at least one mutation associated with BDQ resistance (specific genetic changes in *M. tuberculosis* that reduce the bacteria's susceptibility to BDQ). The majority of isolates, n=34 (57%), contained mutations classified as interim (refers to specific genetic variants in *M. tuberculosis* linked to BDQ reduced susceptibility that currently lack sufficient clinical or laboratory data for a permanent resistance), and n=1 (2%) of mutations uncertain significance (referring Mutations of uncertain significance associated with BDQ mean genetic changes found in *M. tuberculosis* candidate genes where current scientific and clinical data are insufficient to prove whether they cause drug resistance or not), but did not possess any mutation currently associated with confirmed BDQ resistance (figure 4).

The findings indicate that *Rv0678_p.Met146Thr*, *Rv1979c_c.129A>G*, *Rv0676c_p.Ile948Val*, *Rv3245c_p.Met517Leu*, and particularly *Rv0676c_c.1065G_T,* were significantly associated with different DR-TB phenotypes (Table 2). The *Rv0678_p.Met146Thr* mutation was significantly associated with MDR-TB (n=15/39, 39%) and pre-XDR-TB (n=3/4, 75%) resistance profiles (p = 0.011). Similarly, the *Rv1979c_c.129A>G* mutation demonstrated a significant association with HR-TB (n=14/15, 73%), MDR-TB (n=31/39, 80%) and RR-TB (n=2/2, 100%) categories (p = 0.016). Whereas n=11/60 (18%) isolates did not harbour the mutation. An identical distribution was observed for the *Rv0676c_p.Ile948Val* mutation (p = 0.016).

Table 2: Association between BDQ mutations and the type of DR-TB

Mutation		HR-TB (n)	MDR-TB (n)	RR-TB (n)	pre- XDR-TB (n)	Total (n)	p-Value
Rv0678_p.Met146Thr	Absent	15	24	2	1	44	0.011*
	Present	0	15	0	3	16	
Rv1979c_c.129A>G	Absent	1	8	0	2	11	0.016*
	Present	14	31	2	2	49	
Rv0676c_p.Ile948Val	Absent	1	8	0	2	11	0.016*
	Present	14	31	2	2	49	
Rv3245c_p.Met517Leu	Absent	3	8	0	2	13	0.035*
	Present	12	31	2	2	47	
Rv0676c_c.1065G_T	Absent	15	14	2	2	33	<0.001 *
	Present	0	**25**	0	2	27	
Rv0676c_p.Thr794Ile	Absent	13	37	1	4	55	0.199
	Present	2	2	1	0	5	

* Statistically significant ≤0.05

Each of the common mutations isolated were assessed for ability to confer BDQ resistance phenotypically in the Eswatini population (Table 3). The study observed that strains classified as HR-TB (n=15/60, 25%) possessed pre-existing *Rv0678/mmpR5* mutations. The *Rv0678 p.Met146Thr* was the only mutation significantly associated with phenotypic BDQ resistance in this cohort ($p < 0.001$).

Table 3: Association between mutations linked to BDQ resistance and bedaquiline phenotypic resistance

Mutation		BDQ-resistant		Total (n)	p-Value
		Absent (n)	Present (n)		
Rv0678_p.Met146Thr	Absent	40	4	44	<0.001*
	Present	0	16	16	
Rv1979c_c.129A>G	Absent	9	2	11	0.238
	Present	31	18	49	
Rc0676c_p.Ile948Val	Absent	8	3	11	0.637
	Present	32	17	49	
Rv3245c_p.Met517Leu	Absent	11	2	13	0.121
	Present	29	18	47	
Rv0676c_c.1065G_T	Absent	29	4	33	0.141
	Present	11	16	27	
Rv0676c_p.Thr794Ile	Absent	36	19	55	0.509
	Present	4	1	5	

* Statistically significant ≤0.05

Discussion

This study provides the first WGS characterisation of BDQ resistance-associated mutations among drug-resistant *M. tuberculosis* isolates in Eswatini. The principal findings were that 35% of isolates harboured confirmed BDQ resistance-associated mutations, 63% contained mutations of interim or uncertain significance, and *Rv0678_p.Met146Thr* was the predominant confirmed resistance-associated variant and the only mutation significantly associated with phenotypic BDQ resistance. These findings highlight the value of genomic surveillance for identifying circulating resistance-associated variants and informing molecular diagnostic strategies in high TB-burden settings.

The most prevalent variants identified in BDQ-associated genes were *Rv1979c_c.-129A>G* (n = 50, 83%) and *Rv0676c_p.Ile948Val* (n = 49, 82%). According to the WHO Catalogue of Mutations in Mycobacterium tuberculosis Complex and Their Association with Drug Resistance (2nd edition), both variants are classified as interim BDQ resistance-associated mutations (15). The predominance of these variants suggests that they are common among circulating *M. tuberculosis* strains in Eswatini and are likely to represent lineage-associated polymorphisms rather than primary drivers of BDQ resistance. Furthermore, most isolates (n = 38, 63%) harboured mutations classified as interim or of uncertain significance but lacked any mutation currently classified as being associated with confirmed BDQ resistance. Variants classified as interim BDQ resistance-associated mutations are often lineage-associated polymorphisms and generally remain phenotypically susceptible to bedaquiline (16, 17). In particular, *Rv1979c_c.-129A>G* and

Rv0676c_p.Ile948Val (together with *Rv0676c_p.Thr794Ile*) are frequently observed in Lineage 4 *M. tuberculosis* strains, which are common in Eswatini. This interpretation is consistent with the findings of Dlamini et al. (2023), who reported that Lineage 4 was the predominant lineage circulating in Eswatini (95/203, 46%). Rivière (2022) further suggested that these variants may represent ancestral fixed differences present in the H37Rv reference genome rather than true acquired BDQ resistance mutations (16). Collectively, these findings indicate that although many isolates carry genetic alterations in BDQ-related genes, the clinical significance of a substantial proportion of these variants remains uncertain and requires further phenotypic and functional investigation.

In contrast to the interim and lineage-associated variants discussed above, 21 of the 60 isolates (35%) harboured *mmpR5/Rv0678* variants classified as being associated with BDQ resistance. The most frequent confirmed BDQ resistance-associated variant was *Rv0678_p.Met146Thr* (16/21, 76%). This variant is particularly important in the Eswatini context because it has previously been reported in local *M. tuberculosis* strains and may have circulated before the routine introduction of BDQ (18). The detection of this variant in a substantial proportion of isolates supports the value of WGS for DR-TB surveillance and highlights its utility in identifying clinically relevant resistance-associated mutations that may not be detected by targeted molecular assays.

In addition to the *Rv0678* mutations associated with confirmed BDQ resistance, the *Rv0676c_c.1065G>T* variant was significantly associated with MDR-TB isolates in this cohort ($p < 0.001$). However, unlike *Rv0678_p.Met146Thr*, this variant was not significantly associated with phenotypic BDQ resistance. This suggests that *Rv0676c_c.1065G>T* may represent a lineage-associated polymorphism or a marker linked to MDR-TB rather than a primary determinant of BDQ resistance, and its functional role warrants further investigation.

An important finding of this study was the comparison between resistance-associated mutations identified by WGS and phenotypic BDQ resistance. Among the mutations evaluated, *Rv0678_p.Met146Thr* was the only mutation significantly associated with phenotypic BDQ resistance and was consistently identified in phenotypically resistant isolates. In contrast, several mutations that were significantly associated with DR-TB phenotypes, including *Rv1979c_c.-129A>G*, *Rv0676c_p.Ile948Val*, *Rv3245c_p.Met517Leu*, and *Rv0676c_c.1065G>T*, showed no significant association with phenotypic BDQ resistance. This distinction indicates that not all variants identified in BDQ-related genes directly confer phenotypic resistance to BDQ. Some of these mutations may represent lineage-associated polymorphisms or genetic markers linked to drug-resistant backgrounds rather than causal resistance determinants. These findings are consistent with previous studies demonstrating that *Rv0678* mutations show stronger genotype-phenotype concordance than many noncanonical variants, reinforcing the importance of interpreting WGS findings alongside phenotypic susceptibility testing and WHO mutation catalogue classifications (15, 17, 18).

Notably, 15 of the 60 isolates (25%) harbouring BDQ resistance-associated mutations were identified in HR-TB cases without prior BDQ exposure, indicating that *Rv0678/mmpR5* resistance-associated variants are circulating within the Eswatini *M. tuberculosis* population independent of direct BDQ drug pressure. This finding suggests that reliance solely on treatment history to predict BDQ resistance may be inadequate and highlights the importance of proactive, population-level genomic surveillance to inform diagnostic

strategies and reduce the risk of initiating suboptimal treatment regimens in treatment-naïve patients. Previous studies have shown that HR-TB can progress to MDR-TB under selective drug pressure or inadequate treatment (19-21). Whether the pre-existing *Rv0678/mmpR5* mutations observed in this study contribute to subsequent progression from HR-TB to MDR-TB cannot be determined from the present cross-sectional study and should be investigated in future longitudinal studies.

Given the high burden of drug-resistant TB and frequent cross-border movement within the Southern African Development Community (SADC) region, integrating WGS into routine surveillance programmes could facilitate earlier detection of emerging BDQ resistance-associated variants and support more coordinated regional TB control strategies.

This study was limited by its relatively small sample size, which may not be representative of the broader Eswatini population. Additionally, socio-economic and treatment adherence factors that may influence resistance development were not assessed. Despite these limitations, the findings provide valuable insights into the distribution of BDQ resistance-associated mutations in Eswatini and underscore the importance of population-level genomic surveillance for the early detection and monitoring of emerging resistance-associated variants.

Conclusion

The findings of this study highlight the importance of genomic surveillance for detecting and monitoring BDQ resistance-associated mutations among drug-resistant *Mycobacterium tuberculosis* isolates in Eswatini. In particular, the significant association of *Rv0678_p.Met146Thr* with phenotypic BDQ resistance and its detection in HR-TB cases without prior BDQ exposure underscore the need for continued surveillance and early resistance detection. Integrating whole-genome sequencing into routine DR-TB surveillance programmes may strengthen resistance monitoring and support more informed treatment and public health decision-making in Eswatini.

Abbreviations

BDQ
Bedaquiline
CERI
Centre for Epidemic Response and Innovation
CTAB
cetyltrimethylammonium bromide
DR-TB
Drug-resistant tuberculosis
pre-XDR-TB
pre-extensively drug-resistant TB
MDR-TB
Multidrug-resistant TB
M. tuberculosis

Mycobacterium tuberculosis
SNP
Single nucleotide polymorphism
TB
Tuberculosis
WGS
Whole-genome sequencing
XDR-TB
extensively drug-resistant TB

Declarations

Ethical approval and consent to participate

Ethical approval for this study was obtained from the Durban University of Technology Institutional Research Ethics Committee (IREC 129/22) as well as from the Eswatini National Human and Health Research Review Board (ENHHRRB103/2021). Written informed consent was received from all participants recruited in the study. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for Publication

Not Applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/1077072

Competing Interests

All authors declare that they have no competing interests.

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Author's contributions

TD collected the specimens and performed the laboratory investigations. TD, BM and YT contributed jointly to the analysis of data and writing the manuscript. BM and TD prepared the figures and tables. All authors read and approved the final manuscript.

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Figures



Figure 1

Eswatini Map showing the study sites in the Manzini, Hhohho and Shiselweni regions of Eswatini. The map was sourced and customized from https://zu.wikipedia.org/wiki/File:Eswatini,_administrative_divisions_-_en_-_colored.png (accessed on the 17 July 2026).

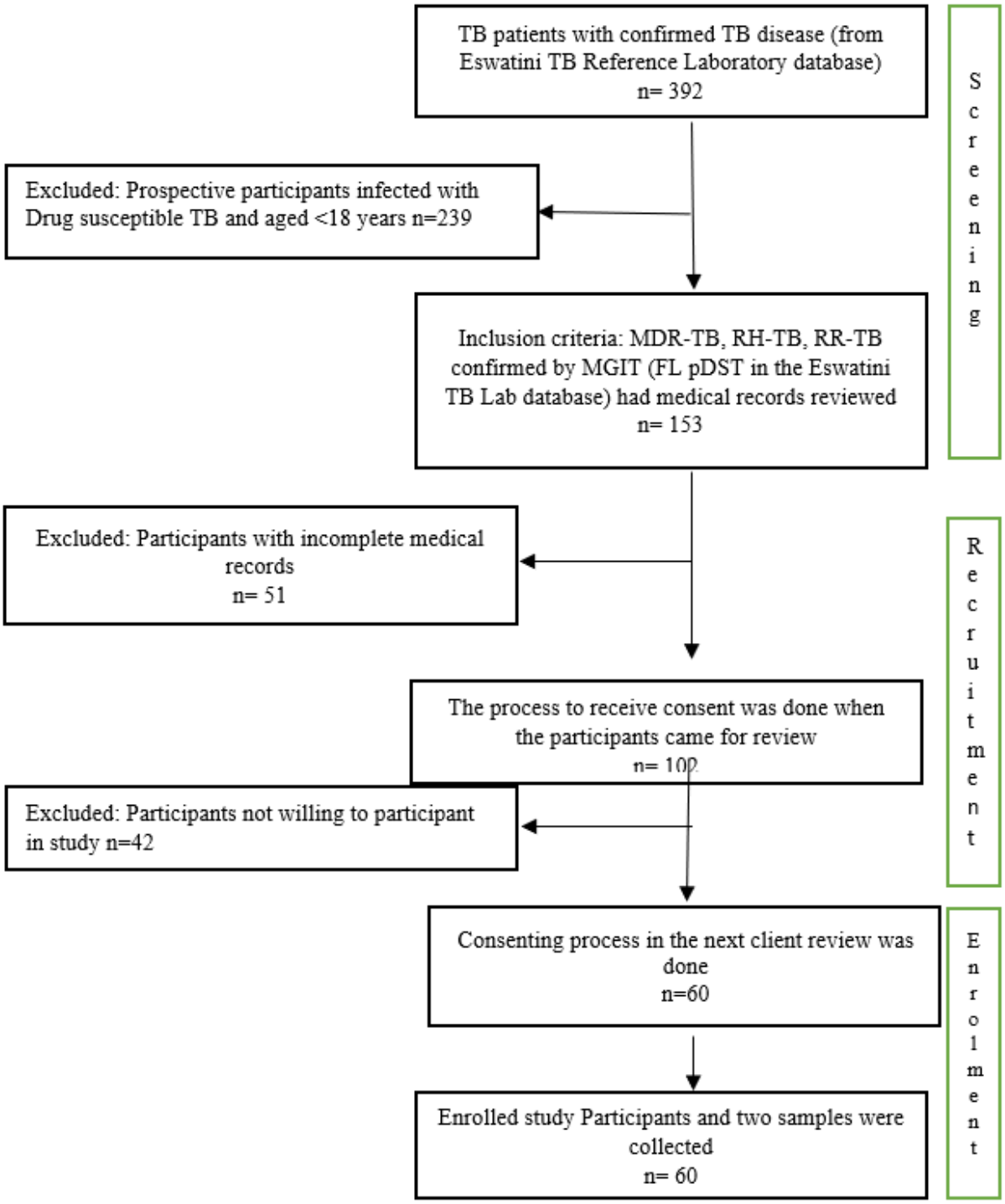

Figure 2

The study enrolled sixty participants who were RH-TB, RR-TB, MDR-TB and XDR-TB patients.

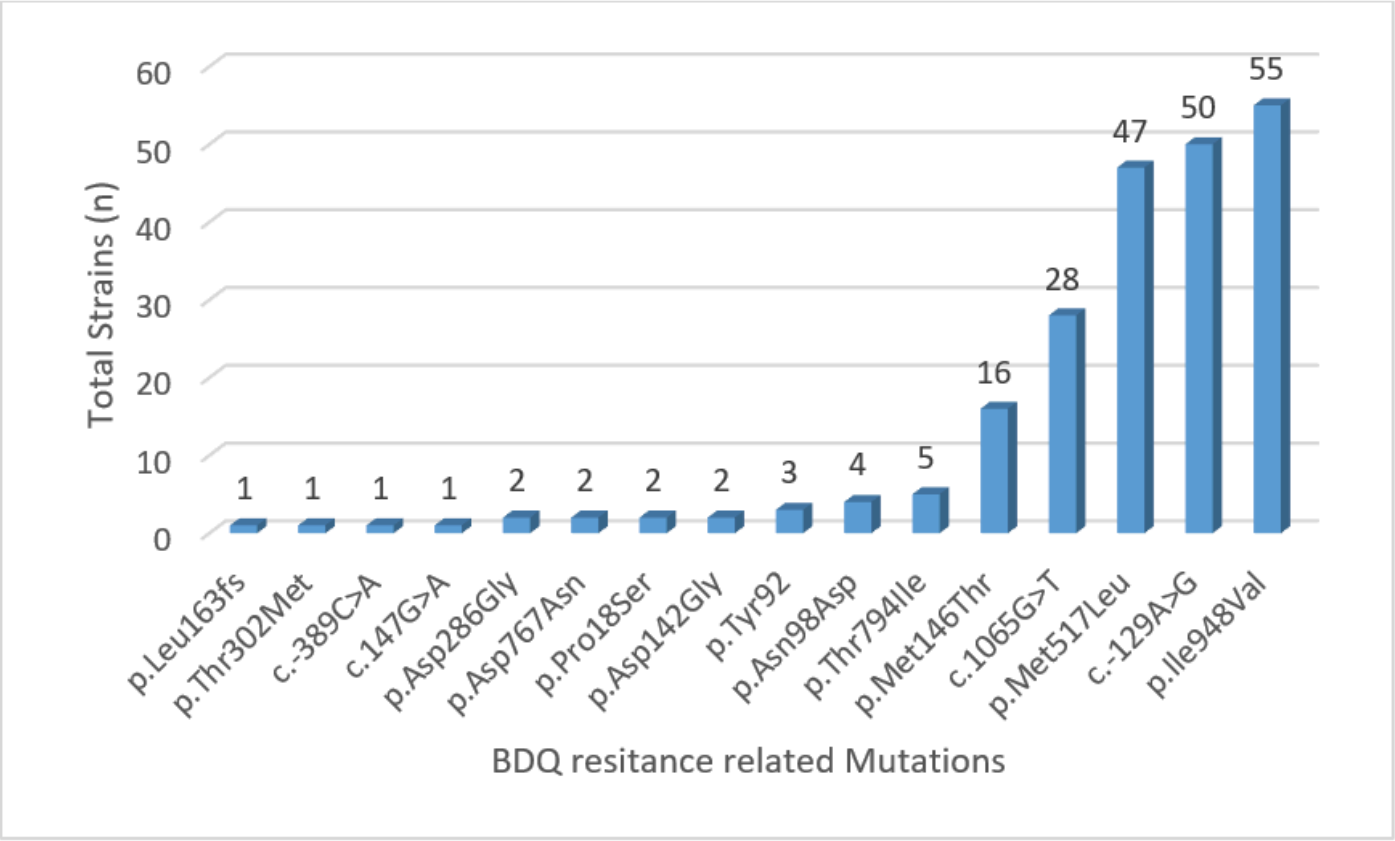

Figure 3

Mutations associated with BDQ-resistance

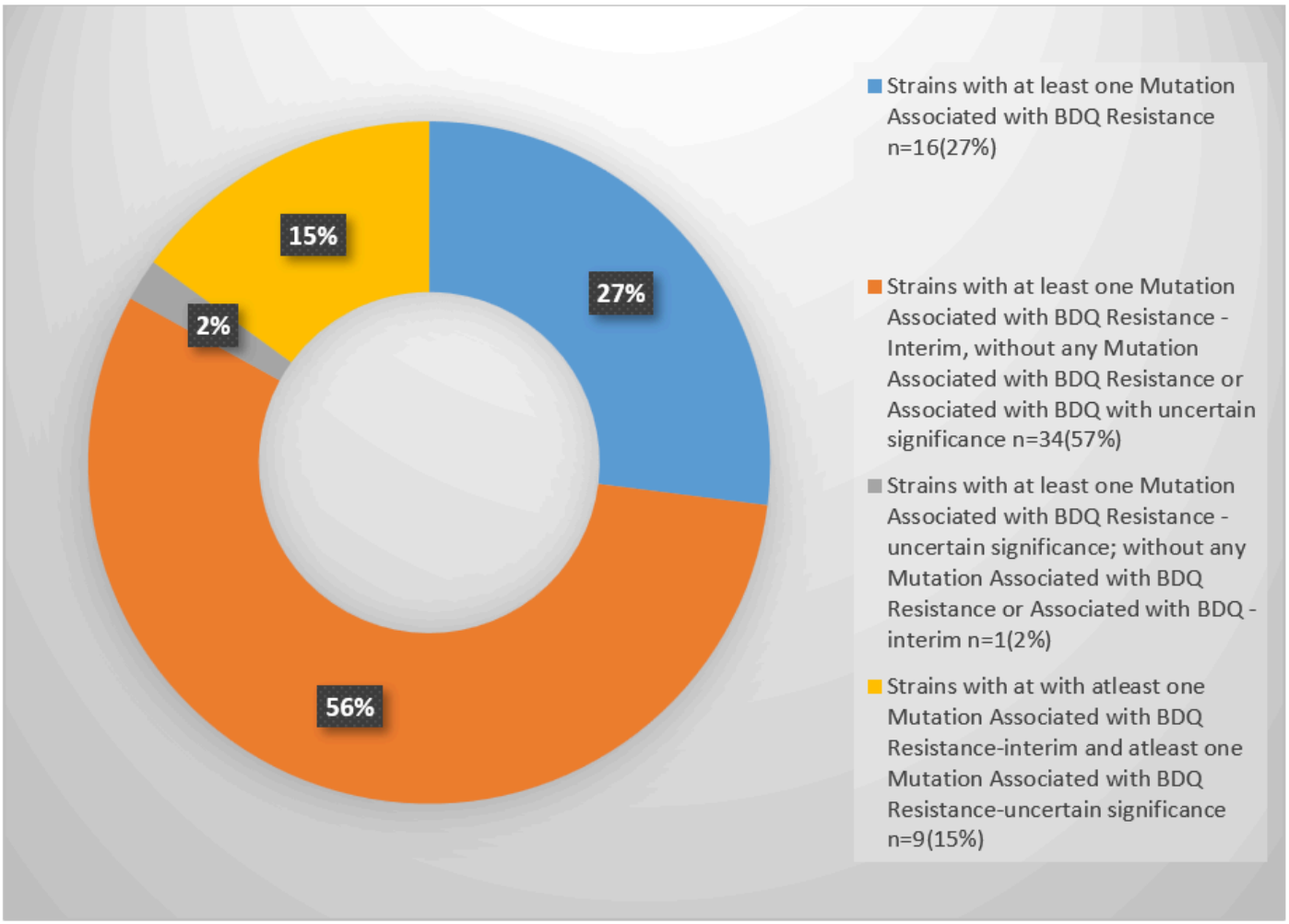

Figure 4

Distribution of *M. tuberculosis* strains with BDQ-associated mutations according to TB-Profiler (v6.6.6)