BMC Infectious Diseases

RESEARCH Open Access

Genomic characterization of XDR *Mycobacterium tuberculosis* isolates in Argentina (2006–2015)

Florencia A. Castello[1,4], Ezequiel J. Sosa[2,4], Josefina Campos[3], Johana Monteserin[3], Tomás Poklepovich[3], Miranda C. Palumbo[1,4], Federico Serral[1,4], Joaquín Messano[1,2], L. Gabriel García[1,2], Norberto Simboli[3], Adrián Turjanski[2,5], Roxana Paul[3], Beatriz López[3], Mario Matteo[6], María M. Palomino[2,4,5], Marcelo Martí[2,4,5] and Darío Fernández Do Porto[1,2,4*]

Abstract

Background Tuberculosis (TB), caused by the intracellular bacterium Mycobacterium tuberculosis complex (Mtbc), remains a significant global health challenge, with Mtbc once again being the leading infectious killer worldwide. Despite over a century of research, the disease continues to pose a major threat, with an estimated one-fourth of the global population latently infected. According to the World Health Organization (WHO), approximately 1.3 million deaths were attributed to TB in 2024 alone. The emergence of multidrug-resistant (MDR) strains, resistant to isoniazid and rifampicin, and extensively drug-resistant (XDR) strains, resistant to rifampicin (and may also be resistant to isoniazid), to at least one fluoroquinolone (levofloxacin or moxifloxacin) and to at least one other Group A drug (bedaquiline or linezolid), further complicates the situation, posing significant challenges for healthcare systems. While the WHO definition of XDR-TB has recently been updated, in this study we applied the classification in effect during the 2006–2015 period, when the isolates were collected and characterized. In Argentina, TB burden is moderate compared to other countries, with approximately 10,500 new cases and 1,000 deaths reported annually. While standard therapy is generally effective, XDR Mtb infections require prolonged and costly treatment and are often associated with a guarded prognosis.

Methods In this work, we applied whole-genome sequencing analysis to characterise XDR-TB strains circulating in Argentina between 2006 and 2015. Genotypic variants of each isolate were compared against resistance-associated variant databases and subjected to local and global phylogenetic analyses.

Results The analysis revealed no common origins for the most frequently observed resistance mutations. Notable variants associated with resistance to first-line drugs included *katG* Ser315Thr and *fabG1* -15C > T for isoniazid, *rpoB* Ser450Leu and Asp435Val for rifampin, *embB* Gly406Ala, and Met306Ile for ethambutol, as well as multiple variants in the *pncA* gene linked to pyrazinamide resistance.

*Correspondence:
Darío Fernández Do Porto
dariofd@gmail.com

Full list of author information is available at the end of the article

Conclusions This study provides valuable insights into the molecular mechanisms of antibiotic resistance in *M. tuberculosis*, specifically focusing on XDR strains circulating in Argentina. The findings highlight the genetic diversity and complexity of resistance-associated variants, emphasizing the need for continued research and surveillance efforts to address this pressing global health threat.

Clinical trial number Not applicable.

Keywords Multiresistance, Genomics, Tuberculosis, Drugs, XDR

Background

Tuberculosis (TB) remains a significant global health challenge, placing a substantial burden on healthcare systems and communities worldwide [1]. Caused by the Mycobacterium *tuberculosis complex* (*Mtbc*), TB affects millions of individuals each year, leading to considerable morbidity and mortality [2]. Despite concerted efforts to control the disease, TB persists, exacerbated by factors such as drug resistance, co-infections with HIV/AIDS, and socioeconomic disparities [3].

Resistance to TB treatment is categorized based on first-line and second-line drugs. First-line drugs, including isoniazid, rifampicin, ethambutol, and pyrazinamide, form the backbone of standard TB treatment regimens due to their high efficacy and relatively low toxicity [4, 5]. When resistance to these key drugs develops, second-line drugs—such as fluoroquinolones (e.g. levofloxacin, moxifloxacin) and injectable agents (e.g. amikacin, capreomycin, kanamycin, and streptomycin)—have historically been used, despite being associated with higher toxicity and reduced efficacy [6, 7]. It is important to note that, according to the latest WHO guidelines, kanamycin and capreomycin are no longer recommended, and streptomycin is only used in cases of hepatotoxicity [8]. However, during the period covered by this study, these drugs were still part of the recommended treatment regimens for multidrug-resistant TB.

Streptomycin was historically part of the first-line TB treatment regimen as the first antibiotic discovered to be effective against *Mtbc* [9–11]. However, due to increasing resistance and more effective oral alternatives, the World Health Organization (WHO) has reclassified streptomycin as a second-line drug. It is now primarily used in cases of drug-resistant TB when other injectable agents are unavailable or contraindicated [12].

Multidrug-resistant tuberculosis (MDR-TB) is defined as TB caused by *Mycobacterium tuberculosis complex* strains resistant to at least isoniazid and rifampicin, the two most potent first-line drugs [11, 13]. Extensively drug-resistant tuberculosis (XDR-TB) was historically defined as tuberculosis with additional resistance to fluoroquinolones and at least one second-line injectable drug (amikacin, capreomycin, or kanamycin). However, this definition has been updated in recent WHO guidelines to reflect changes in second-line treatment recommendations. The current definition classifies XDR-TB as a form of MDR-TB that is resistant to any fluoroquinolone and at least one of the Group A drugs, which currently include bedaquiline (BDQ), and linezolid (LZD). In this context, pre-extensively drug-resistant tuberculosis (pre-XDR-TB) is defined as TB caused by *Mtbc* strains that fulfil the definition of multidrug-resistant and rifampicin-resistant TB (MDR/RR-TB) and are also resistant to any fluoroquinolone. The definition used in this study reflects the classification that applied to the period covered by the analysis. Nowadays, characterization of XDR-TB under both historical and updated definitions can be performed using phenotypic methods such as BACTEC or genotypic approaches such as whole-genome sequencing. The emergence and spread of MDR-TB and XDR-TB pose significant challenges to TB control programs worldwide, highlighting the need for continuous surveillance and the development of effective treatment strategies [2, 14–16].

In the 1990s, Argentina was identified by the WHO as a hotspot for MDR-TB. During this period, hospital outbreaks of MDR-TB associated with acquired immunodeficiency syndrome (AIDS) were documented in the country. Initially emerging in the capital city, the outbreak spread to nearby areas, reaching significant proportions. With over 800 diagnosed cases between 1992 and 2004, this outbreak met the criteria for an epidemic. In the early 2000s, an increase in MDR-TB cases was observed among patients who were neither HIV-positive nor had a history of prior tuberculosis treatment [17]. Among the MDR strains identified in Argentina, strain M (which originated in Buenos Aires and surrounding districts) and strain Ra (Rosario, Argentina) have been the most predominant [11, 18], contributing significantly to the persistence and transmission of MDR-TB in the country. In 2002, strain M was isolated from the country's first two patients diagnosed with XDR-TB.

The evolution of *Mtbc* is primarily influenced by TB control strategies, alongside socio-economic, environmental, and human migration patterns, all of which add complexity to efforts to combat the disease effectively. Notably, the pathogen's biology significantly impacts the global dissemination of the disease. Molecular studies have revealed a remarkable intra-species genetic diversity within *Mtbc*, enabling its classification into nine major

lineages, each displaying distinct affinities for specific geographic regions and human ethnic groups. These lineages are categorized as Indo-Oceanic (Lineage 1), East Asian (Lineage 2, including the Beijing sublineage), East African-Indian (Lineage 3), Euro-American (Lineage 4), West African (Lineage 5, *M. africanum* I), and West African (Lineage 6, *M. africanum* II). Recent phylogenomic analyses have identified additional lineages with more restricted distributions, including Lineage 7, found in the Horn of Africa; Lineage 8, recently described in Central Africa; and Lineage 9, a newly discovered lineage primarily located in East Africa [19, 20]. Lineages 1, 5, and 6 are considered "ancient," while lineages 2, 3, and 4 are classified as "modern" based on the presence or absence of the TbD1 genomic region, which is absent in modern lineages [21–23]. Currently, the predominant *Mtbc* strains circulating in the Americas were introduced by Europeans during colonization, with the Euro-American lineage (Lineage 4) being the most prevalent. Within Lineage 4, notable sublineages include the Ra strain (4.3, also known as LAM3) and the M strain (4.1.2.1), both of which have been associated with outbreaks and drug-resistant cases in South America.

Since different *Mtbc* lineages dominate various regions worldwide, drug resistance acquisition may be influenced by the strains' pre-existing genetic background. The heterogeneity observed worldwide can be explained by the variability of these mutations [7]. In other words, the pre-existing genetic profiles of certain *Mtbc* strains may be preferentially associated with specific resistance-causing mutations, and the effect of these associations could modulate the biological fitness of the strains [24].

The advent of advanced sequencing technologies, coupled with bioinformatics tools, has revolutionized our understanding of TB pathogenesis, drug resistance, and transmission patterns. High-throughput sequencing enables comprehensive genomic analysis of *Mtbc* isolates, providing unprecedented insights into the molecular basis of drug resistance and virulence. By leveraging these technologies, researchers can gain insights into the intricate interplay between genetic determinants, host immunity, and environmental factors that shape TB epidemiology [25–27].

Understanding the molecular basis of antibiotic resistance in *Mtbc* is crucial for developing effective TB control strategies. Therefore, this work aimed to analyze the genetic basis of antibiotic resistance mechanisms in XDR clinical isolates obtained in Argentina between 2006 and 2015. By examining resistance mutations associated with different antibiotics—such as streptomycin, isoniazid, rifampicin, ethambutol, kanamycin, amikacin, capreomycin, pyrazinamide, ethionamide, and fluoroquinolones—we aimed to elucidate the molecular mechanisms driving drug resistance in *M. tuberculosis* strains circulating in Argentina [4, 16, 28, 29]. The findings from this study contribute to a better understanding of the genetic diversity and resistance patterns in *Mtbc.*

Methods

Clinical isolates and strain selection

Case selection was conducted with healthcare professionals from the "Servicio de Micobacterias at the Instituto Nacional de Enfermedades Infecciosas - Dr. Carlos G. Malbrán", ensuring representation of cases from diverse geographical regions of Argentina. Cultures were initiated for 120 isolates, corresponding to all reported cases of patients with XDR *Mtbc* between 2006 and 2015. Sufficient DNA was successfully extracted for sequencing from only 49 isolates.The remaining 71 isolates failed to grow in subcultures for confirmation and an additional 16 were excluded after sequencing due to their low average depth (< 15X), contamination and/or low horizontal coverage. Identification and DST for first-line anti-TB drugs were performed at Muñiz Hospital, Cetrángolo, and other regional centers, while second-line DST and confirmation of XDR status were conducted at the TB National Reference Laboratory (NRL) at the National Institute of Infectious Diseases Dr. Carlos G. Malbrán (ANLIS). In all cases, patient interaction was exclusively managed by the physician, and inclusion in the project was based on the physician's recommendation and clinical history analysis. All samples were anonymized. To complement the genomic analysis, limited sociodemographic and clinical metadata were available for a subset of the XDR-TB cases ($n = 33$), including age, sex, HIV serology, and geographic origin. Patients ranged in age from 20 to 58 years, with both male and female individuals represented. Most patients were HIV-negative, and a few were of foreign nationality (e.g., from Bolivia). All cases were managed at reference hospitals with experience in treating drug-resistant TB, such as Hospital Muñiz. A summary of the available metadata is provided in Supplementary Table S1.

Microbiological and molecular studies

All isolates were grown on Löwenstein-Jensen slants and identified as *M. tuberculosis* through biochemical and molecular tests. DST was performed using the reference standard proportion method in the Löwenstein-Jensen medium and/or BACTEC MGIT 960 system (Becton Dickinson, MD) under international standards [30]. A multiplex allele-specific PCR (MAS-PCR) was conducted on all isolates to detect mutations associated with INH and RIF resistance (codons katG315, inhA-15, rpoB450, 445, and 425) according to a modified protocol described elsewhere [31].

Genotyping was performed by spoligotyping and MIRU-VNTR according to standard procedures [32,

33], followed by a comparison with SITVITWEB [34] and MIRU-VNTRplus database [35]. In addition to traditional spoligotyping and MIRU-VNTR, phylogenetic lineage assignment was performed using phylogenetically consistent SNP markers derived from whole-genome sequencing data. These markers were identified following the robust SNP barcode approach for *Mtbc* strains [36], as implemented in TB Profiler 6.2.2. This allowed for lineage classification with higher phylogenetic consistency than spoligotyping, which was retained in this study for historical comparison with previous Argentine datasets.

Genome sequencing

For whole-genome sequencing (WGS), isolates were re-cultured on Löwenstein-Jensen slants. DNA was extracted following a standard protocol for mycobacteria [37]. Genomic libraries were prepared using the Nextera® XT DNA Sample Preparation Kit (Illumina) according to the manufacturer's instructions, with individual libraries indexed using the Nextera® XT Index Kit. Paired-end reads were generated for all isolates using the Illumina MiSeq platform at Unidad Operativa Centro Nacional de Genómica y Bioinformática, ANLIS. All sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA646920.

Resistance variants calling pipeline

The quality of the sequencing reads for each experiment was assessed using FastQC version 0.11.5 [38], and bases affected by biases or low quality were trimmed with Trimmomatic (reads shorter than 36 bp and mean Q < 20 were filtered out) [38, 39]. Subsequently, the reads from each strain were aligned to the *Mtb* H37Rv reference genome (NCBI NC_000962.3) using BWA v0.7.17 [40], and the alignment was processed in BAM format with Samtools v1.10 [41]. Next, variant calling was performed using GATK [42], and finally, the variants were annotated using SnpEff [43].

To determine the genotypic resistances of each strain, the variants obtained in the previous step were crossed with a custom database that integrates data from variants present in TBProfiler v. 6.2.2 [44], 2023 WHO "Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance - second edition", KvarQ v. 0.12.2 [45], TBDream [46], and CARD RGI 5.1.1 [47] with bibliographic data up to December 2023 (https://github.com/florenciacastello/tb_resistance/resistanceTB_2024.csv).

Positions with a sequencing depth of less than 10 reads were masked and excluded from downstream variant analysis. In cases where more than one allele was detected at a given position, the allele supported by the highest proportion of reads (at least 75% of the total coverage for that position) was selected, under the assumption that it most likely corresponds to the dominant population. Similar thresholds and conventions have been applied in the construction of SARS-CoV-2 consensus genomes [48] and in the detection of low-frequency SNPs in *Mtb* [49].

Phylogenetic analysis

For phylogenetic analysis, we first reconstructed a consensus genome for each isolate by applying the variants identified in the corresponding VCF files to the *M. tuberculosis* H37Rv reference genome using Samtools/bcftools (Samtools v1.10; bcftools v1.10). This step effectively converts the variant information into full-length genome sequences suitable for multiple sequence alignment and tree inference. Low-coverage areas and repetitive regions such as IS6110, PGRSs, CRISPRs, and VNTRs were masked to reduce potential sequencing and mapping artifacts. The resulting masked consensus sequences, containing 292 high-confidence SNPs, were aligned using MAFFT v.6.0 with default parameters. Prior to tree construction, the optimal nucleotide substitution model was determined using jModelTest v3.7 based on the Akaike Information Criterion (AIC). The selected model was the General Time Reversible model with a Gamma distribution of rate variation and a proportion of invariant sites (GTR + G + I). A Maximum Likelihood (ML) phylogenetic tree was then inferred using RAxML v.7.0.4, incorporating representative samples of the main *M. tuberculosis* lineages and sublineages downloaded from NCBI. Tree-building parameters were set according to the jModelTest output.

Processing pipelines

The pipelines described above were implemented using the Python language. The processing of isolates was divided into three scripts. All external programs are called through Docker, a platform for distributing and using programs transparently, which minimizes installation requirements: having Python and Docker installed. The code and usage instructions are available online in a GitHub repository (https://github.com/florenciacastello/tb_resistance).

No formal statistical analyses were performed due to the descriptive nature of the study and the limited number of high-quality XDR-TB isolates ($n = 33$), which would restrict the statistical power of any comparative assessments.

Results

Whole-genome sequencing data from 49 clinical isolates were initially mapped against the *Mtb* H37Rv reference genome. This collection represents approximately 30% of all XDR available isolates in Argentina from 2006 to 2015. After applying coverage and sequencing depth filters, 16

samples were discarded, leaving us with a final total of 33 samples. Following quality filtering and the exclusion of single-nucleotide polymorphisms (SNPs) in problematic genomic regions, 292 high-confidence SNPs were identified. These variants distinguished the 33 isolates, with an average pairwise SNP distance of 10.9 (Supplementary Table 1).

For a more detailed characterization of the samples, we performed spacer oligonucleotide typing (spoligotyping) and assigned lineages using TB-Profiler. Our analysis indicated that 6 out of 33 samples (18.1%) lacked a specific spoligotype. The results from spoligotyping were largely consistent with the lineage assignments from TB-Profiler. We classified the samples into four main groups based on the spoligotyping profile. The Haarlem (H) group (21.2%, H2 $n=7$), LAM group (27.2%, including LAM5 and LAM3, $n=9$), and T group (27.2%, including T, T1 and Tuscany, T2, and T3, $n=9$), collectively representing 75.6% of the samples. Among these groups, H2 was the most frequently identified spoligotype ($n=7$) (Fig. 1).

To contextualize our findings on a global scale, we constructed a phylogenetic tree using representative strains from each phylogenetic variant identified in our dataset [36, 50].

In addition to the 33 Argentine isolates, representative global genomes were included to evaluate whether the observed resistance mutations and phylogenetic profiles arose from a single clonal expansion or reflected multiple independent events. This strategy allowed us to explore potential diversification patterns and detect cases of convergent evolution in resistance acquisition.

For this analysis, we included a diverse selection of global *Mtb* strains characterized by Sekizuka et al. [51], allowing for a comparison between our isolates and major phylogenetic lineages worldwide. The resulting tree confirmed the presence of distinct sublineages among our samples and suggested that the strains in our collection may not originate from a single phylogenetic lineage, potentially indicating multiple independent introductions or diversification events. By integrating resistance genotype information with the phylogenetic topology, we evaluated whether resistance mutations arose from single or multiple independent events within the same phylogenetic branches. This approach revealed both lineage-specific resistance mechanisms, suggesting a common origin, and resistance variants distributed across unrelated branches, consistent with convergent evolution. These findings align with global trends, where *M. tuberculosis* lineages exhibit substantial geographic structuring [52, 53], underscoring the importance of detailed phylogenetic analysis in tracking transmission dynamics. Additionally, the phylogenetic tree further supports the assignment of isolates to specific sublineages, such as Haarlem, LAM, and T, in line with prior spoligotyping results, reinforcing the robustness of our characterization

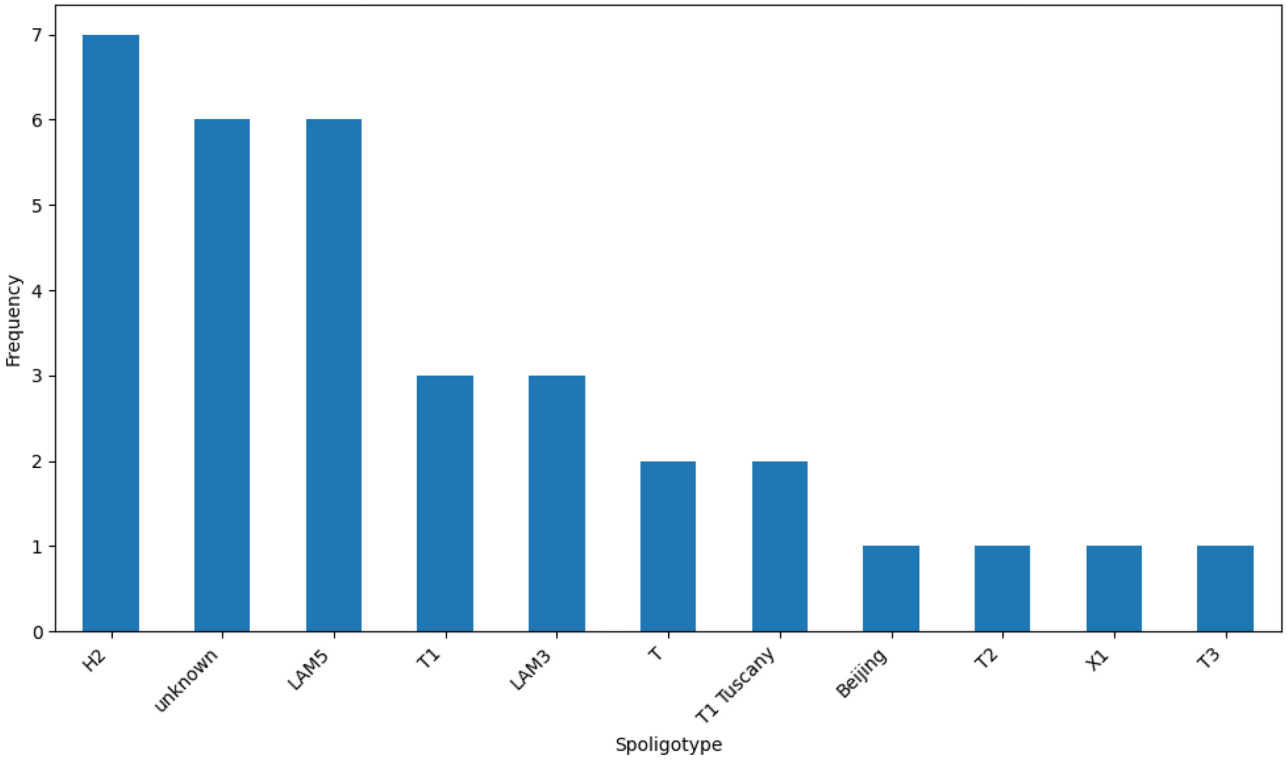


Fig. 1 Frequency distribution of XDR *M. tuberculosis* lineages identified in Argentina (2006–2015) using TB-Profiler

(Fig. 2). For completeness, the corresponding phylogram, which includes branch length information, is available as Supplementary Fig. 1.

Furthermore, spoligotyping analysis of the studied samples confirmed their assignment to Lineage 4 Euro-American, in agreement with existing genotyping studies conducted in Argentina [54]. Notably, approximately 80% of *Mtb* isolates in Buenos Aires belong to this lineage, predominantly comprising the T, LAM, and Haarlem families. This distribution reflects the historical influence of Hispanic colonization and recent immigration waves from the Mediterranean and neighboring countries.

Beyond lineage classification, understanding the genetic determinants of drug resistance is crucial for characterizing XDR *Mtb* isolates. Therefore, we analyzed resistance-associated variants, summarizing the most frequent mutations in Fig. 3. Overall, there was a strong correlation between phenotypic drug resistance and predictions based on the presence or absence of known resistance mutations for the four first-line drugs (isoniazid, rifampicin, ethambutol, and streptomycin), three second-line injectables (amikacin, kanamycin, and capreomycin), and fluoroquinolones. Since all isolates were phenotypically characterized as XDR, resistance to isoniazid and rifampicin was expected. The identified mutations further support this classification, reinforcing the reliability of our genetic resistance profiling.

> For the four first-line drugs, the predominantly identified mutations were (Fig. 3):
> Isoniazid: *katG* Ser315Thr (75.7% of samples), followed by *fabG1* -15 C > T (24.2%).
> Rifampicin: *rpoB* Ser450Leu (72.7%), followed by *rpoB* Asp435Val (21.2%).
> Ethambutol: Two predominant SNPs were detected in the *embB* gene: Gly406Ala (27.2%) and Met306Ile (42.4%).
> Pyrazinamide: *pncA* Gly10Pro (33,33%), followed by *pncA* Arg154Gly (25%).

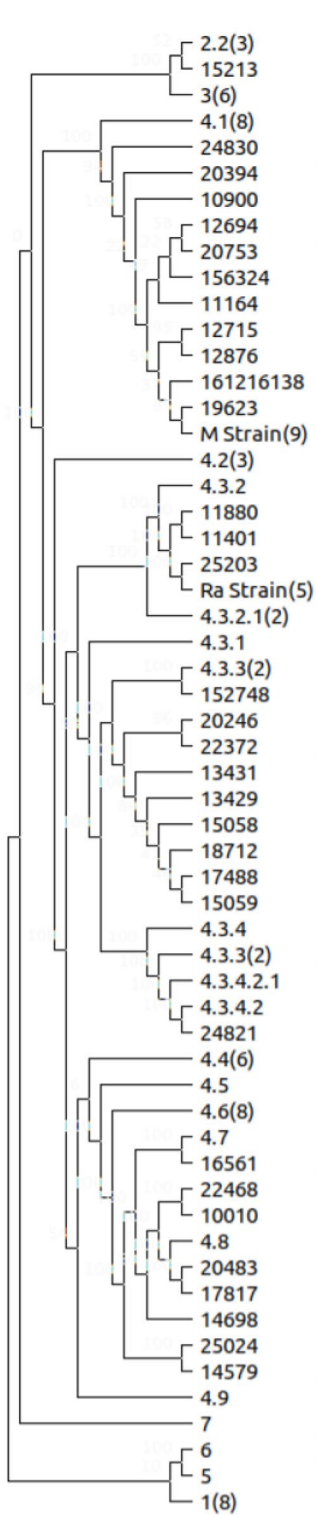
Regarding streptomycin, three predominant mutations were identified: a frameshift mutation at position 110 (24.2%) and a Leu16Arg substitution (39.4%) in the *gid* gene, and the 1401 A > G SNP in *rrs* (72.7%). Notably, since streptomycin shares its target with the injectable aminoglycosides studied (kanamycin, amikacin, and capreomycin), the *rrs* 1401 A > G mutation was also predominantly found in these drugs. For fluoroquinolones, four predominant SNPs were identified in the *gyrA* gene: Asp-94Gly (21.2%), Ala90Val (15.2%) and Asp94Ala (12.1%), Asp94His (12.1%). It is important to note that, for most drugs, the total percentage exceeded 100%, as multiple variations were found in most samples.

Figures 4 and 5 compare the groups defined by spoligotyping with the identified resistance profiles to explore the relationship between phylogenetic classification and drug resistance patterns. This analysis aimed to assess whether the clustering observed through spoligotyping correlates with distinct resistance signatures. The phylogenetic trees of the strains analyzed in this work (Figs. 4 and 5) confirm that all isolates belong to Lineage 4 (Euro-American), except for one Beijing isolate (Lineage 2). Five major groups were confirmed based on the spoligotyping profile: H, LAM3, LAM5, T, and T-Tuscany.

Group H includes all isolates molecularly characterized as H2 or H3. Within the group, the same profile of resistance mutations is observed. All samples harbor the *katG* Ser315Thr mutation, which confers resistance to isoniazid (INH). For rifampicin, ethambutol, pyrazinamide, and streptomycin resistance, the majority of samples share specific mutations: *rpoB* Ser450Leu (rifampicin), *embB* Gly406Ala (ethambutol), *pncA* Gln10Pro (pyrazinamide) and *rrs* 1401 A >G along with *gid* Val100fs (streptomycin). This pattern suggests a potential common phylogenetic origin for these resistance mutations. Notably, sample 20,394 carries a double mutation at codon 435 of the *rpoB* gene, resulting in Asp435Gly, previously associated with rifampicin resistance by Napier G. et al. [5]. All isolates in this group harbor the *rrs* 1401 A >G mutation, associated with resistance to second-line injectable drugs [55, 56]. In contrast, fluoroquinolone resistance exhibits greater variability, with distinct mutations identified across different isolates, including *gyrA* Asp94Gly, *gyrA* Asp94His, *gyrA* Asp94Ala, *gyrA* Ala90Val, *gyrB* Ala-504Val, and *gyrB* Arg446Cys [57–61].

The LAM5 group includes all isolates identified as belonging to this lineage through experimental and In Silico techniques. Previous studies have reported the presence of LAM5 among *M. tuberculosis* isolates in Argentina, as part of the broader Latin American–Mediterranean family [29]. However, sample 11,880 was classified as LAM5 based on the experimental spoligotyping, whereas In Silico spoligotyping assigned it to LAM3. Furthermore, its clustering with LAM3 isolates supports this classification; therefore, sample 11,880 was excluded from the LAM5 group.

All LAM5 group isolates share the same INH, RIF, PZA, and EMB resistance genotypes. Regarding second-line aminoglycosides, isolate 13,429 is the only one lacking the *rrs* 1401 A > G variant (STR). Additionally, isolates 13,429 and 13,431 are the only ones without a variant at codon 94 of *gyrA* gene for fluoroquinolones within the group. The phylogenetic tree is coherent with the hypothesis that mutation at gene *gyrA* codon 94 (Asp) may have arisen in a common ancestor of the LAM5 isolates in this group, while the *rrs* 1401 A > G mutation appears to have



In Silico Lin.	*In Vitro* Spol.	*In Silico* family	Group
-	-	-	
2.2.1	Beijing	Beijing	
-	-	-	
-	-	-	
4.1.1	X1	X1	
4.1.2	T3-T2*	T	
4.1.2.1	H3	T1	
4.1.2.1.1	H2	H2	H
4.1.2.1.1	H2	H2	
4.1.2.1.1	-	H2	
4.1.2.1.1	H2	H2	
4.1.2.1.1	H3	H2	
4.1.2.1.1	H2	-	
4.1.2.1.1	H2	H2	
4.1.2.1.1	H2	H2	
-	-	-	
-	-	-	
-	-	-	LAM3
4.3.2	LAM5	LAM3	
4.3.2	LAM3	LAM3	
4.3.2	-	LAM3	
-	-	-	
-	-	-	
-	-	-	
-	-	-	
4.3.3	-	-	
4.3.3	Tuscany	T-tuscany	T-tuscany
4.3.3	T	T-tuscany	
4.3.3	LAM5	LAM5	LAM5
4.3.3	LAM5	LAM5	
4.3.3	LAM5	LAM5	
4.3.3	LAM5	LAM5	
4.3.3	LAM5	LAM5	
4.3.3	LAM5	LAM5	
-	-	-	
-	-	-	
-	-	-	
-	-	-	
4.3.4.2	-	-	
-	-	-	
-	-	-	
-	-	-	
-	-	-	
4.7	T5 Madrid	-	T
4.8	T2	T2	
4.8	T1 Ghana	T1	
-	-	-	
4.8	-	T1	
4.8	T1 Ghana	-	
4.9	T1 Ghana	T1	
4	-	T3	
4	T3-T2	T	
-	-	-	
-	-	-	
-	-	-	
-	-	-	
-	-	-	

Fig. 2 Global phylogenetic tree of XDR *Mycobacterium tuberculosis* isolates, including a subset of M strain and Ra strain samples, alongside isolates from the TGS-TB project [51]. Isolates from the TGS-TB project are labeled with the corresponding phylogenetic lineage followed by the number of representatives (in parenthesis) if multiple isolates belong to the same lineage. This labeling convention also applies to M (4.1.2.1) and Ra (4.3- LAM3) strain isolates. Three annotation columns accompany the project isolates: "In Silico Lin" (lineage determined computationally from phylogenetically consistent SNP markers using TB profiler), "In Vitro Spol" (experimentally determined spoligotype), and "In Silico family" (assigned by TB Profiler)). Missing values in the experimental spoligotype column indicate no laboratory experiment was performed, whereas missing values in the In Silico columns denote inconclusive results. The tree is shown as a cladogram for visual clarity. The corresponding phylogram, including branch lengths, is provided as Supplementary Figure S1

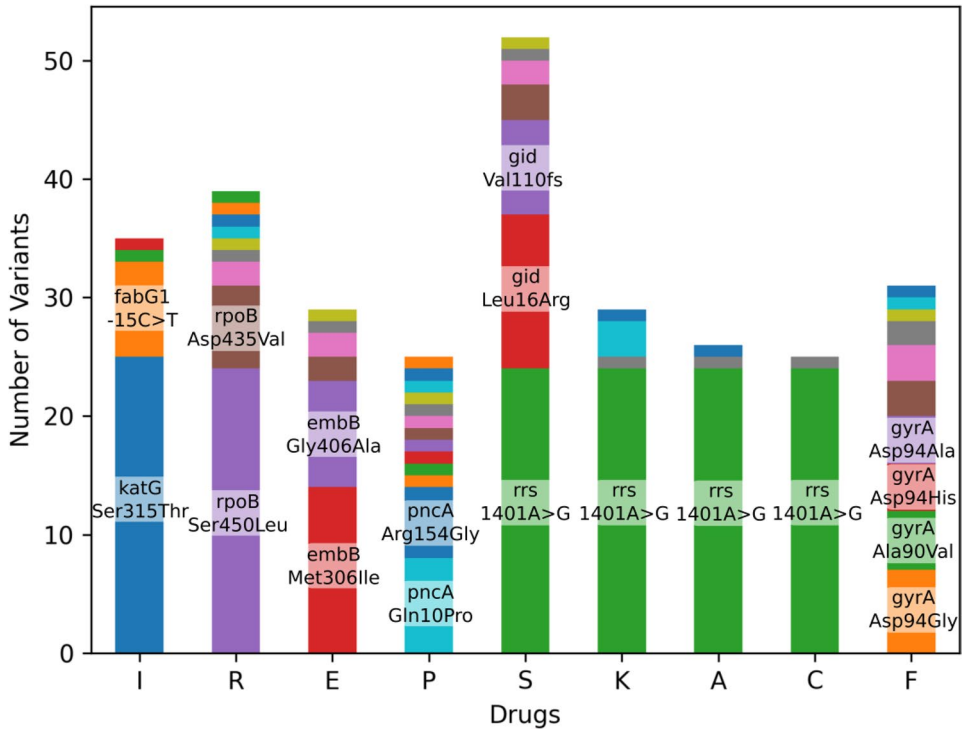


Fig. 3 Bar chart displaying the distribution of resistance-associated variants across nine drugs. The X-axis lists the drugs (isoniazid [I], rifampicin [R], ethambutol [E], pyrazinamide [P], streptomycin [S], kanamycin [K], amikacin [A], capreomycin [C], and fluoroquinolones [F]) while the Y-axis represents the number of identified variants. Each bar corresponds to a specific drug and is segmented by color to represent individual variants. Labels are shown only for variants present in at least 10% of resistant isolates for each drug, highlighting the most prevalent mutations associated with resistance. For some drugs, the number of variants exceeds the number of isolates because individual isolates can carry multiple mutations conferring resistance to the same antibiotic

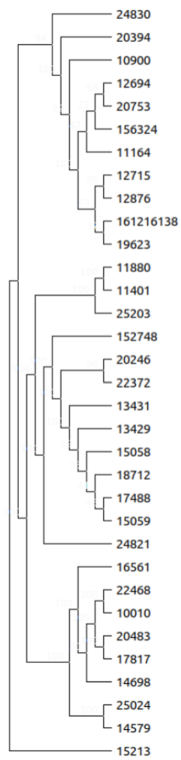
been lost in isolate 13,429, potentially as a result of a later evolutionary event.

The T-Tuscany group consists of two isolates, 22,372 and 20,246, which were experimentally characterized as belonging to the T lineage. Regarding resistance variants, these isolates do not harbor *katG* mutations associated with INH resistance but instead carry resistance mechanisms through *fabG1* promoter variants. Additionally, they share the *rrs* 1401 A >G mutation for aminoglycoside resistance, *embB* Met306Ile for ethambutol resistance [62], and *gyrA* Asp94Gly for fluoroquinolone resistance. Notably, neither isolate carries *pncA* mutations associated with PZA resistance.

In the global tree, Group LAM3 clusters with samples of sublineage 4.3.2, with isolate 25,203 positioned near the outbreak of the Ra strain, which has been characterized as sublineage 4.3 (LAM3 108) and exhibits an MDR resistance profile [16]. All LAM3 isolates share the same resistance variants for INH and RIF. Isolates 11,401 and 11,880 also share resistance mechanisms for STR (*rpsL* Lys88Arg) and *fabG1*-inhA – 15 C >T promoter variants, responsible for INH and ETH resistance. These two isolates share a KAN resistance variant in the *eis* – 12G >A promoter, differing from the *rrs* 1401 A >G variant associated with KAN in most studied isolates. While no FLQ resistance variants were found, these isolates are known to be phenotypically resistant. Notably, despite being experimentally classified as LAM5, isolate 11,880 exhibits an In Silico spoligotype and phylogenetic placement consistent with LAM3.

The T1 group includes nine isolates, all characterized as T spoligotypes both In Silico and experimentally. The group can be further subdivided into two subgroups, both defined by the *rpoB* Ser450Leu variant associated with RIF resistance. The first subgroup comprises isolates 22,468 and 10,010, in which INH resistance is mediated by the *katG* Ser315Thr mutation alongside genotypic variants associated with EMB resistance. The second subgroup includes isolates 17,817 and 20,483, where INH resistance is mediated by *fabG1* -15 C > T while no EMB resistance mutations were detected.

Adjacent to the T1 group in the phylogenetic tree, isolate 16,561 is classified as T5. It is one of the two isolates lacking an INH resistance mutation while exhibiting phenotypic resistance. Additionally, it harbors the *rpoB* Gln432Pro variant, which is associated with rifampicin resistance. Other isolates exhibit ambiguous classifications. Further analysis is needed to determine the underlying causes.



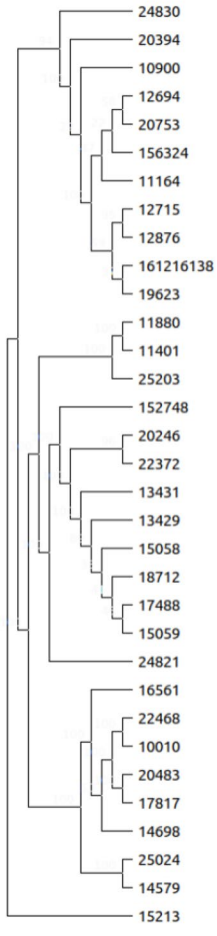
InVitro Spol.	*InSilico* family	I	R	E	P	S
X1	X1	katG Ser315Thr, fabG1 C-8T	rpoB Asp435His, rpoB His445Asp	embB Met306Ile, embA -11C>A	pncA Pro54Leu	rrs 1401A>G, rpsL Lys43Arg
T3-T2	T	katG Ser315Thr	rpoB Asp435Val, rpoB Asp435His	embB Met306Ile	pncA Asp86fs	-
H3	T1	fabG1 -15C>T	rpoB Ser450Leu	embB Met306Val	-	-
H2	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
H2	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
-	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
H2	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
H3	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
H2	-	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
H2	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
H2	H2	katG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	pncA Gln10Pro	rrs 1401A>G, gid Val110fs
LAM5	LAM3	katG Ser315Thr, fabG1 -15C>T	rpoB Ser450Leu	-	-	gid Leu16Arg, rpsL Lys88Arg
LAM3	LAM3	katG Ser315Thr, fabG1 -15C>T	rpoB Ser450Leu	-	-	gid Leu16Arg, rpsL Lys88Arg
-	LAM3	katG Ser315Thr	rpoB Ser450Leu	embB Gln497Arg	pncA Ser104Arg	gid Leu16Arg, rrs 1401A>G
-	-	fabG1 -15C>T	rpoB Ser450Leu	embB Gly406Asp	pncA His51Arg	gid Leu16Arg
Tuscany	T-Tuscany	fabG1 -15C>T	rpoB Ser450Leu	embB Met306Ile	-	gid Leu16Arg, rrs 1401A>G
T	T- Tuscany	fabG1 -15C>T	rpoB Ser450Leu	embB Met306Ile	-	gid Leu16Arg, rrs 1401A>G
LAM5	LAM5	katG Ser315Thr	rpoB Asp435Val	embB Met306Ile	pncA Arg154Gly	gid Leu16Arg, rrs 1401A>G
LAM5	LAM5	katG Ser315Thr	rpoB Asp435Val	embB Met306Ile	pncA Arg154Gly	gid Leu16Arg
LAM5	LAM5	katG Ser315Thr	rpoB Asp435Val	embB Met306Ile	pncA Arg154Gly	gid Leu16Arg, rrs 1401A>G
LAM5	LAM5	katG Ser315Thr	rpoB Asp435Val	embB Met306Ile	pncA Arg154Gly	gid Leu16Arg, rrs 1401A>G
LAM5	LAM5	katG Ser315Thr	rpoB Asp435Val	embB Met306Ile	pncA Arg154Gly	gid Leu16Arg, rrs 1401A>G
LAM5	LAM5	katG Ser315Thr	rpoB Asp435Val	embB Met306Ile	pncA Arg154Gly	gid Leu16Arg, rrs 1401A>G
-	-	katG Ser315Thr	rpoB Ser450Leu, rpoC Phe452Ser	embB Met306Ile	pncA Asp12Ala	gid Leu16Arg, rrs 1401A>G, rpsL Lys43Arg
T5 Madrid	-	-	rpoB Gln432Leu	-	pncA 331T>TCGTC	gid Arg39fs, rrs 1401A>G
T2	T2	KatG Ser315Thr	rpoB Ser450Leu	embB Gly406Ala	-	rrs 1484G>T
T1 Ghana	T1	KatG Ser315Thr	rpoB Ser450Leu	embA -11C>A, embB Asp328Tyr	pncA 320 T>TTCGAACCC	-
-	-	fabG1 -15C>T	rpoB Ser450Leu	-	pncA Val131fs, pncA Ala134Val	rrs 1401A>G
T1 Ghana	-	fabG1 -15C>T	rpoB Ser450Leu	-	pncA Val180Phe	rrs 1401A>G
T1 Ghana	T1	fabG1 Leu203Leu	rpoB Ser450Leu, rpoA Val183Ala	embB Met306Ile	pncA Leu172Pro	rrs 1401A>G, rpsL Lys43Arg
-	T3	katG Ser315Thr	rpoB Ser450Leu, rpoC Trp484Gly	embB Met306Ile	pncA Glu127*	rrs 1401A>G
T3-T2	T	katG Ser315Thr	rpoB Ser450Leu	embB Gln497Arg	pncA Trp68Ser	rrs 1401A>G
Beijing	Beijing	katG Ser315Thr	rpoB Ser450Leu, rpoC Val483Gly	embB Met306Ile	-	-

Fig. 4 Phylogenetic tree of XDR *Mycobacterium tuberculosis* isolates aligned with drug resistance profiles. Each branch of the tree corresponds to a sample listed in the adjacent table, which contains six columns: "*Invitro* spol." (specifying the spoligotype classification), "InSilico family" (lineage family assigned by TB Profiler), and four drug resistance columns (representing the phenotypic resistance profile for isoniazid (I), rifampicin (R), ethambutol (E), and streptomycin (S)

The phylogenetic analysis, together with resistance profiles, highlights the prevalence of mutations *rpoB* Ser450Leu and *rpoB* Gln432Leu, which are strongly associated with RIF resistance, as well as *katG* Ser315Thr, the most common mutation conferring INH resistance, followed by mutations in the *fabG1* promoter. While *katG* Ser315Thr is strongly associated with high resistance levels, *fabG1* promoter mutations are also found in Argentinean lineages but confer lower levels of resistance [63]. Variants such as *rrs* 1401 A >G are also frequent, albeit to a lesser extent. Resistance mutations for EMB and PZA appear in specific phylogenetic groups, suggesting a shared evolutionary origin. In contrast, resistance mechanisms for FLQ and ETH seem to have emerged more recently and do not exhibit exclusive associations with particular phylogenetic branches.

Multiple mutations associated with resistance to the same drug were identified, as detailed in Figs. 4 and 5. However, some phenotypically resistant strains lacked known high-confidence resistance mutations reported in the literature (one for isoniazid, three for ethambutol, six for pyrazinamide, and two for streptomycin). Despite this, we identified undescribed mutations that might contribute to resistance to the antibiotics under study. For sample 16,561, a novel conservative in-frame insertion was found at position 1440 of the *katG* gene (protein position 404), an insertion not yet reported as associated with an AMR in the latest TBprofiler and WHO AMR 2023 databases [64] adds an extra alanine codon between *katG*'s two peroxidase domains (positions 404–405) according to InterPro analysis (Pfam ID: PF00141, Prosite ID: IPR002016), suggesting further research potential. Consistent with this observation, insertions in the *katG* gene (Rv1908c) are frequently observed and contribute to isoniazid resistance [19–22].

In the pyrazinamide false-negative analysis, a thorough manual analysis was performed using IGV program on the remaining samples (15,213, 10,900, 11,880, and 22,468), focusing on the genes *pncA*, *panD*, *rpsA*, *Rv1258c*, *Rv3236c*, and their respective promoters to identify poorly characterized mutations or other artifacts that could explain their phenotypic resistance. In sample 22,468, the complete absence of the *pncA* gene was observed. The complete absence of the *pncA* gene in sample 22,468 is a significant finding, as this gene is essential for the pyrazinamide conversion into its active form, pyrazinoic acid. The total loss or deletion of *pncA* has been identified as a mechanism of pyrazinamide resistance



InVitro Spol.	*InSilico* family	K	A	C	F
X1	X1	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Ala
T3-T2	T	-	-	-	gyrA Asp94Tyr
H3	T1	eis -10G>A	eis -10G>A	-	gyrA Asp94Gly
H2	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrB Ala504Val
H2	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	-
-	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	-
H2	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94His
H3	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp90Val, gyrB Arg466Cys
H2	-	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrB Arg466Cys
H2	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Ala
H2	H2	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Ala90Val
LAM5	LAM3	eis -12C>T	-	-	-
LAM3	LAM3	eis -12C>T	-	-	-
-	LAM3	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Asn
-	-	-	-	-	gyrA Ala90Val
Tuscany	T-Tuscany	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Gly
T	T- Tuscany	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Gly
LAM5	LAM5	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	-
LAM5	LAM5	-	-	-	-
LAM5	LAM5	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94His
LAM5	LAM5	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Ala, gyrB Arg466Cys
LAM5	LAM5	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Ala
LAM5	LAM5	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94His
-	-	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Gly
T5 Madid	-	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Gly
T2	T2	rrs 1484G>T	rrs 1484G>T	rrs 1484G>T	gyrA Asp94Tyr
T1 Ghana	T1	-	-	-	gyrA Asp94Tyr
-	-	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94His
T1 Ghana	-	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Gly, gyrA Ala74Ser
T1 Ghana	T1	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Ala90Val
-	T3	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Gly, gyrA Ala90Val
T3-T2	T	rrs 1401A>G	rrs 1401A>G	rrs 1401A>G	gyrA Asp94Asn
Beijing	Beijing	eis -12C>T	-	-	gyrA Gly88Cys

Fig. 5 Phylogenetic tree of XDR *Mycobacterium tuberculosis* isolates aligned with drug resistance profiles. Each branch of the tree corresponds to a sample listed in the adjacent table, which contains six columns: "*Invitro* spol." (specifying the spoligotype classification), "*InSilico* family" (lineage family assigned by TB Profiler), and four drug resistance columns (representing the phenotypic resistance profile for kanamycin (K), amikacin (A), capreomycin (C), and fluoroquinolones (F))

in *Mtb* [65, 66]. For sample 15,213, two SNPs of interest were identified: *Rv3236c* Ala370Thr and a synonymous variant in the *rpsA* gene (636 A >C; Arg340Arg). The first mutation has consistently been reported alongside a secondary mutation [67], the latter is mentioned in the "Catalogue of Mutations in *Mycobacterium tuberculosis* Complex and their Association with Drug Resistance – Second Edition" [64] as a rare mutation for PZA with low PPV. For samples 10,900 and 11,880, no mutations or regions with low coverage were found that could account for their resistance. Further investigation is required.

Identifying resistance-associated mutations in EMB-resistant organisms is one of the greatest challenges when diagnosing antibiotic resistance through variant analysis. It is believed that the resistance mechanism to this antibiotic in tuberculosis is not solely attributed to the *embABC* cassette but may result from a combination of different variants across multiple genes, making accurate detection challenging in some cases [64, 68–72]. In our samples, the three false negatives (11401, 11880, and 17817) shared the synonymous SNP *embC* Arg927Arg which has been widely reported for all isolates (resistant and sensitives) in the PolyTB database (http://pathogenseq.lshtm.ac.uk/polytb). Brossier et al. suggest that this SNP could have originated from a sequencing artifact in the *Mtb* reference strain H37Rv, as recorded in GenBank. (accession number AL123456.3) [73]. On the other hand, sample 17,817 presented multiple unreported variants, including four missenses (*embR* Phe283Leu, *embR* Cys294Gly, *embB* Phe642Ser, *embB* Asn675Thr), one disruptive inframe insertion (*embA* 3347 C < CCG) and two synonymous variants (*embR* Cys288Cys and the one described above). For samples 11,401 and 11,880, no variants were found in any reported gene most commonly associated with ethambutol resistance (seven genes were manually reviewed in IGV to verify the information: *embABC*, *iniABC*, and *embR*). In addition, both samples show optimal vertical coverage (< 30X), and no insertion

sequences (IS) were detected that could explain the phenotypic resistance. Further investigation will be essential to unravel these findings.

Additionally, resistance-associated variants were detected for Delamanid, Linezolid, and other second-line drugs, even though these drugs were not administered at the time of the study. For Delamanid, we identified one high-confidence mutation (fbiC Ala855fs) and seven low-confidence mutations (three in *fbiC* -Ile406Val, *V*al410Gly, Val415Gly- and four in fbiA -Ala30Thr, Gln120Arg, Ile208Val, and one synonymous variant). Regarding Linezolid, we detected two low-confidence synonymous mutations in *rplC* and one high-confidence mutation (*rplC* Cys154Arg). No resistance-associated mutations were found for Bedaquiline or Fosfomycin.

In conclusion, these findings reveal a diverse landscape of resistance mutations among XDR *M. tuberculosis* isolates in Argentina, with notable lineage-specific and convergent mutations. Our findings offer a comprehensive view of both established and lesser-known mutations, enriching the understanding of resistance patterns and evolutionary pathways in these isolates.

Discussion and conclusions

The study of extensively drug-resistant (XDR) tuberculosis (TB) in Argentina provides critical insights into the evolution, genetic diversity, and complexity of antibiotic resistance in *Mycobacterium tuberculosis complex* (*Mtbc*). Despite substantial progress in TB research and the development of drug resistance profiling techniques, XDR-TB remains a significant public health threat worldwide. In Argentina, where TB burden is moderate but concerning, especially with rising drug-resistant strains, our findings contribute to a better understanding of the genetic determinants of XDR-TB in the region and highlight the importance of genomic surveillance in guiding TB control strategies.

This study characterized the genetic basis of antibiotic resistance to first- and second-line drugs used in tuberculosis treatment, analyzing clinical XDR *Mtb* isolates from Argentina collected between 2006 and 2015.

Previous studies conducted in Argentina have documented the predominance of the katG S315T mutation in INH-resistant and MDR-TB strains, particularly in highly transmissible lineages such as LAM and the M outbreak strain, which has contributed significantly to local transmission clusters since the 1990s [54, 63]. While inhA promoter mutations are also frequent, they appear more often in low-resistance or non-clustered isolates. Genomic data from recent cohorts suggest a gradual accumulation of additional resistance-conferring mutations in MDR strains, including those targeting second-line drugs, indicating an evolutionary path toward pre-XDR and XDR phenotypes [28]. A striking case of within-host evolution was reported in an Argentinian patient over a five-year period of inadequate treatment, where an MDR strain sequentially acquired fluoroquinolone, pyrazinamide, and aminoglycoside resistance mutations, including novel mutations in whiB7, ultimately progressing toward an XDR phenotype [74]. These findings underscore the importance of ongoing genomic surveillance to detect and characterize evolving resistance patterns in M. tuberculosis.

The resistance-associated variants identified in this study remained consistent with those previously reported for drug-resistant isolates in Argentina. For isoniazid, *katG* Ser315Thr and *fabG1* -15 C >T were the most frequent mutations, aligning with published studies [15, 28, 75]. For rifampicin resistance, *rpoB* Ser450Leu, a variant documented locally, remained prevalent, while Asp435Val, one of the most frequently identified mutations in other studies, was also commonly detected [28, 54].

For pyrazinamide (PZA), *pncA* mutations exhibited expected variability but were mostly contained within specific monophyletic branches, such as the H and LAM5 groups, suggesting phylogenetic constraints on PZA resistance evolution. Ethambutol resistance was primarily associated with the *embB* Gly406Ala and Met306Ile variants, compatible with local reports [16, 76–78], with the former being exclusive to the H group.

For second-line aminoglycosides, the *rrs* 1401 A > G mutation remained predominant, although it was not conserved throughout the phylogeny. Fluoroquinolone resistance-associated mutations in *gyrA* exhibited a more varied distribution in the tree compared to other antibiotics. However, the most frequent variant locations, Ala90 and Asp94, known to cause high MIC and found locally in FQL-resistant isolates, were present.

Ethionamide resistance variants were found predominantly in the fabG1 promoter (also associated with INH resistance), with only one strain having a mutation in its target gene, inhA. This is consistent with the operonic organization of fabG1 and inhA, where promoter variants modulate inhA expression levels, leading to resistance through regulatory rather than structural mechanisms. The *fabG1* -15 C > T mutation seemed to be fixed in several groups but without a clear common origin.

More than one resistance mutation per drug was not uncommon, and many isolates harbored strain-specific mutations. Some displayed novel or unique combinations of variants, highlighting the genetic heterogeneity of XDR-TB and possible adaptation to local selective pressures.

We also identified less characterized mutations, such as novel insertions in *pncA* and *katG*, which warrant further investigation as they could contribute to pyrazinamide and isoniazid resistance, respectively. Some isolates

exhibited phenotypic resistance despite lacking known resistance mutations, suggesting the presence of alternative resistance mechanisms [79], epigenetic factors influencing drug susceptibility [80], or technical limitations such as low sequencing coverage [80, 81]. These undetected cases underscore the importance of comprehensive genetic analysis and the need for updated resistance databases that include less commonly reported mutations, which could enhance diagnostic [81] accuracy for XDR-TB.

The finding that XDR isolates were not derived from a single transmission event but rather multiple independent resistance acquisitions highlights the complexity of TB control efforts in Argentina. This suggests that strengthening infection control measures and targeted interventions are essential to prevent further spread. Future studies integrating genomic data with epidemiological and clinical data will be critical for designing more effective containment strategies.

Our results suggest that resistance mutations arose independently across different *M. tuberculosis* lineages, reflecting convergent evolution likely driven by shared selective pressures, while others are restricted to specific phylogenetic groups, suggesting a single acquisition event in a common ancestor. This highlights the importance of integrating high-resolution phylogenetic analysis with comprehensive resistance databases to enhance the accuracy of molecular diagnostics. Together, these approaches will improve our ability to detect emerging resistance patterns and inform tailored treatment strategies.

Although this study sheds important light on the mechanisms underlying drug resistance in *M. tuberculosis*, it is important to acknowledge certain limitations. One of them is the relatively small number of isolates that were sequenced and analyzed, which may constrain the broader applicability of some conclusions. In addition, the recent update of WHO definitions for pre-XDR and XDR-TB — which exclude resistance to second-line injectable drugs such as amikacin, kanamycin, and capreomycin — may affect the present-day clinical relevance of findings related to those drugs. Nevertheless, given that TB classifications and treatment guidelines evolve over time, reporting resistance data for these agents remains relevant. It enhances our understanding of the historical and evolutionary context of drug resistance in *M. tuberculosis*, which could ultimately support the development of improved treatment strategies. These considerations should be kept in mind when interpreting the results and reinforce the importance of ongoing surveillance aligned with current resistance definitions.

Recent national data provide further insight into XDR and pre-XDR-TB trends in Argentina following the study period. Between 2016 and 2020, an average of 5 XDR-TB cases per year (range: 3–9) were reported under the former WHO definition, based on resistance to fluoroquinolones and second-line injectable agents. After the adoption of the revised WHO definitions, no XDR-TB cases were identified in 2021–2022; however, pre-XDR-TB cases increased to an average of 20 per year, primarily involving MDR strains that acquired fluoroquinolone resistance. More recently, in 2023–2024, with broader implementation of treatment regimens containing bedaquiline and linezolid, 5 XDR-TB and 8 pre-XDR-TB cases were reported annually. This shift may reflect both expanded use of new core drugs and emerging resistance to them. These data, compiled by the Argentine Ministry of Health through national drug-resistant TB reports and tuberculosis and leprosy bulletins (2023–2024, issues No. 7 and 8) [82], underscore the need to closely monitor resistance evolution in response to therapeutic changes.

In this context, it is worth noting that the isolates analyzed in our study represent the full set of phenotypically confirmed XDR-TB cases in Argentina between 2006 and 2015 for which high-quality DNA could be successfully sequenced. These samples were not selected based on geographic criteria but originated from multiple regions—including CABA (Ciudad Autónoma de Buenos Aires), Buenos Aires Province, Rosario, Córdoba, and Jujuy—offering a geographically diverse snapshot of circulating XDR-TB strains during that period. Given that our dataset already encompassed all available Argentine XDR-TB isolates from the study period, additional inclusion of only-Argentine genomes was not possible. Therefore, representative global genomes were incorporated to provide broader phylogenetic context and to assess whether the Argentine isolates arose from a single clonal expansion or from multiple independent introduction events. While the absence of detailed spatial metadata limited formal geographic analyses, the breadth of sample origins suggests that resistance did not emerge from a single local outbreak. Future studies combining expanded genomic datasets with precise epidemiological and geographic information will be essential to elucidate transmission dynamics and regional patterns of spread.

While our analysis focused on a curated set of phenotypically confirmed XDR-TB isolates, we recognize the potential value of situating these genomes within the broader landscape of Argentine M. tuberculosis isolates available in public repositories such as TB Profiler. Incorporating such datasets in future studies may help identify circulating outbreak strains, trace transmission networks, and elucidate evolutionary dynamics at a national scale. However, many of the publicly available genomes lack essential metadata (e.g., phenotypic resistance profile, sampling year, geographic origin), limiting their immediate utility for our focused investigation. Moreover, our goal was to provide a detailed characterization of

confirmed XDR isolates using high-quality sequencing and curated clinical data, complemented by a global phylogenetic context to infer lineage distribution and potential convergent evolution events. Future work expanding on this framework will be essential to enhance national genomic surveillance and epidemiological tracking of resistant TB strains in Argentina.

In line with recent updates from the WHO, the definitions of pre-XDR and XDR-TB have been revised, with resistance to injectable drugs such as kanamycin, amikacin, and capreomycin no longer included in the criteria. Although this change may affect the present clinical categorization of some isolates, the analysis of resistance to these drugs in our dataset offers valuable historical insight into the evolution of drug resistance in Argentina and complements our genomic characterization of other key drug classes.

Finally, our work provides a detailed genomic characterization of XDR-TB isolates in Argentina, identifying both well-established and lesser-known resistance mutations. Our results remark the complexity of resistance evolution in *Mtb* and underscore the importance of integrating WGS into routine TB surveillance and diagnosis in developing countries such as Argentina.

Abbreviations

A	Amikacin
AIDS	Acquired Immunodeficiency Syndrome
AMK	Amikacin
ANLIS	Administración Nacional de Laboratorios e Institutos de Salud
BACTEC MGIT	Mycobacteria Growth Indicator Tube (BD system for culture and DST)
BD	Becton Dickinson
BDQ	Bedaquiline
C	Capreomycin
CAP	Capreomycin
CARD RGI	Comprehensive Antibiotic Resistance Database – Resistance Gene Identifier
CONICET	Consejo Nacional de Investigaciones Científicas y Técnicas
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	Deoxyribonucleic Acid
DST	Drug Susceptibility Testing
E	Ethambutol
EMB	Ethambutol
ETH	Ethionamide
F	Fluoroquinolones
FLQ	Fluoroquinolones
H	Haarlem (a spoligotype family)
HIV	Human Immunodeficiency Virus
I	Isoniazid
IGV	Integrative Genomics Viewer
INH	Isoniazid
IQR	Interquartile Range
IS	Insertion Sequence
KAN	Kanamycin
L	LAM (Latin American-Mediterranean) lineage
LAM	Latin American-Mediterranean (a spoligotype family)
LJ	Löwenstein-Jensen medium
LZD	Linezolid
M	M strain of Mycobacterium tuberculosis
MAS-PCR	Multiplex Allele-Specific Polymerase Chain Reaction
MAFFT	Multiple Alignment using Fast Fourier Transform
MDR	Multidrug-Resistant
MDR-TB	Multidrug-Resistant Tuberculosis
MIC	Minimum Inhibitory Concentration
MIRU-VNTR	Mycobacterial Interspersed Repetitive Unit - Variable Number Tandem Repeat
ML	Maximum Likelihood
Mtb	Mycobacterium tuberculosis
Mtbc	Mycobacterium tuberculosis complex
NCBI	National Center for Biotechnology Information
NRL	National Reference Laboratory
P	Pyrazinamide
PCR	Polymerase Chain Reaction
Pfam	Protein Family database
PZA	Pyrazinamide
RAxML	Randomized Axelerated Maximum Likelihood
R	Rifampicin
RIF	Rifampicin
rrs	Ribosomal RNA gene commonly associated with aminoglycoside resistance
S	Streptomycin
SNP	Single-Nucleotide Polymorphism
SRA	Sequence Read Archive
STR	Streptomycin
SITVITWEB	International database for M. tuberculosis spoligotypes
TB	Tuberculosis
TBProfiler	A bioinformatics tool for predicting TB resistance from WGS data
TGS-TB	Total Genotyping Solution for Tuberculosis
T	T lineage (a spoligotype family)
VNTR	Variable Number Tandem Repeat
VCF	Variant Call Format
WGS	Whole-Genome Sequencing
WHO	World Health Organization
XDR	Extensively Drug-Resistant
XDR-TB	Extensively Drug-Resistant Tuberculosis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11913-3.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
D.F.D.P., M.M., A.T., B.L., J.C. and N.S. conceived and designed the study. F.A.C. and D.F.D.P. wrote the main manuscript text. F.A.C. and E.J.S. prepared the figures with input from the other authors. J.C., J.Mo., T.P., N.S., R.P., B.L., M.M.M., and M.M.P. collected the samples and performed the wet lab experimental work. D.F.D.P., E.J.S., J.Me., L.G.G., M.C.P. and F.A.C. carried out the bioinformatic analyses. All authors reviewed and approved the final manuscript.

Funding
Agencia Nacional de Promoción Científica y Tecnológica [ANPCyT, PICT START UP: PICT-2018-04663 to D.F.D.P.]. CONICET membership of the research career [D.F.D.P., M.M., A.T.], CONICET doctoral fellowship and support staff [M.C.P., F.S., F.A.C. and E.J.S.]. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data availability
All data generated or analysed during this study are included in this published article. Additional datasets are available from the corresponding author on reasonable request. Sequence data have been deposited in the European Nucleotide Archive (ENA) under accession number [ERP171849] (https://www.ebi.ac.uk/ena/browser/view/ERP171849).

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the INEI ANLIS research review board.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
[3]Instituto Nacional de Enfermedades Infecciosas-ANLIS Carlos Malbrán, Buenos Aires, Argentina
[4]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[5]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales, IQUIBICEN, CONICET, Buenos Aires, Argentina
[6]Instituto de Tisioneumonología Raúl F. Vaccarezza, Hospital de Infecciosas Dr. F. J. Muñiz, Buenos Aires, Argentina

Received: 15 April 2025 / Accepted: 7 October 2025
Published online: 17 November 2025

References

1. Global Tuberculosis Report. 2024 [Internet]. [cited 2025 Jul 4]. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2024
2. Harding E. WHO global progress report on tuberculosis elimination. Lancet Respir Med. 2020;8(1):19.
3. Duc PT, Nhat BS, Luyen LT. Establishing population pharmacokinetic model for pyrazinamide in pulmonary tuberculosis patients. VNU J Sci Med Pharm Sci [Internet]. 2021; Available from: https://js.vnu.edu.vn/MPS/article/view/4326
4. Palmero D, González Montaner P, Cufré M, García A, Vescovo M, Poggi S. First series of patients with XDR and pre-XDR TB treated with regimens that included meropenen-clavulanate in Argentina. Arch Bronconeumol. 2015;51(10):e49–52.
5. Napier G, Campino S, Phelan JE, Clark TG. Large-scale genomic analysis of Mycobacterium tuberculosis reveals extent of target and compensatory mutations linked to multi-drug resistant tuberculosis. Sci Rep. 2023;13(1):1–9.
6. [cited 2025 Feb 12]. Available from: https://www.who.int/es/news/item/29-01-2018-high-levels-of-antibiotic-resistance-found-worldwide-new-data-shows
7. Trauner A, Borrell S, Reither K, Gagneux S. Evolution of drug resistance in tuberculosis: recent progress and implications for diagnosis and therapy. Drugs. 2014;74(10):1063–72.
8. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-susceptible tuberculosis treatment. World Health Organization; 2022. p. 72.
9. Finken M, Kirschner P, Meier A, Wrede A, Böttger EC. Molecular basis of streptomycin resistance in Mycobacterium tuberculosis: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. Mol Microbiol. 1993;9(6):1239–46.
10. Zade A, Shah S, Hirani N, Kondabagil K, Joshi A, Chatterjee A. Whole-genome sequencing of presumptive MDR-TB isolates from a tertiary healthcare setting in Mumbai. J Glob Antimicrob Resist. 2022;31:256–62.
11. Eldholm V, Monteserin J, Rieux A, Lopez B, Sobkowiak B, Ritacco V, et al. Four decades of transmission of a multidrug-resistant Mycobacterium tuberculosis outbreak strain. Nat Commun. 2015;6(1):7119.
12. Antibacterial agents in clinical development: An analysis of the antibacterial clinical development pipeline, including tuberculosis. 2017.
13. Coll F, Phelan J, Hill-Cawthorne GA, Nair MB, Mallard K, Ali S, et al. Author correction: Genome-wide analysis of multi- and extensively drug-resistant Mycobacterium tuberculosis. Nat Genet. 2018;50(5):764–764.
14. Niemann S, Supply P. Diversity and evolution of Mycobacterium tuberculosis: moving to whole-genome-based approaches. Cold Spring Harb Perspect Med. 2014;4(12):a021188.
15. Alame Emane AK, Guo X, Takiff HE, Liu S. Highly transmitted M. tuberculosis strains are more likely to evolve MDR/XDR and cause outbreaks, but what makes them highly transmitted? Tuberculosis (Edinb). 2021;129(102092):102092.
16. Monteserin J, Pérez-Lago L, Yokobori N, Paul R, Rodríguez Maus S, Simboli N, et al. Trends of two epidemic multidrug-resistant strains of Mycobacterium tuberculosis in Argentina disclosed by tailored molecular strategy. Am J Trop Med Hyg. 2019;101(6):1308–11.
17. Eldholm V, Rieux A, Monteserin J, Lopez JM, Palmero D, Lopez B, et al. Impact of HIV co-infection on the evolution and transmission of multidrug-resistant tuberculosis. Elife. 2016;5:e16644.
18. [cited 2025 Feb 12]. Available from: https://www.argentina.gob.ar/sites/default/files/informe_tb-mr_2023.pdf
19. Coscolla M, Gagneux S, Menardo F, Loiseau C, Ruiz-Rodriguez P, Borrell S, et al. Phylogenomics of reveals a new lineage and a complex evolutionary history. Microb Genom [Internet]. 2021;7(2). Available from: https://doi.org/10.1099/mgen.0.000477
20. Bespiatykh D, Bespyatykh J, Mokrousov I, Shitikov E. A comprehensive map of Mycobacterium tuberculosis complex regions of difference. mSphere. 2021;6(4):e0053521.
21. Hirsh AE, Tsolaki AG, DeRiemer K, Feldman MW, Small PM. Stable association between strains of Mycobacterium tuberculosis and their human host populations. Proc Natl Acad Sci U S A. 2004;101(14):4871–6.
22. Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, et al. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2006;103(8):2869–73.
23. Reed MB, Pichler VK, McIntosh F, Mattia A, Fallow A, Masala S, et al. Major Mycobacterium tuberculosis lineages associate with patient country of origin. J Clin Microbiol. 2009;47(4):1119–28.
24. Borrell S, Gagneux S. Strain diversity, epistasis and the evolution of drug resistance in Mycobacterium tuberculosis. Clin Microbiol Infect. 2011;17(6):815–20.
25. Phelan JE, O'Sullivan DM, Machado D, Ramos J, Oppong YEA, Campino S, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. Genome Med. 2019;11(1):41.
26. Serral F, Castello FA, Sosa EJ, Pardo AM, Palumbo MC, Modenutti C, et al. From genome to drugs: new approaches in antimicrobial discovery. Front Pharmacol. 2021;12:647060.
27. Sosa EJ, Burguener G, Lanzarotti E, Defelipe L, Radusky L, Pardo AM, et al. Target-pathogen: a structural bioinformatic approach to prioritize drug targets in pathogens. Nucleic Acids Res. 2018;46(D1):D413–8.
28. Hurtado J, Coitinho C, Nin N, Buroni M, Hurtado FJ, Robello C, et al. Clinical and epidemiological features of tuberculosis isolated from critically ill patients [Internet]. Revista Argentina de Microbiología. 2022;54:43–7. Available from: https://doi.org/10.1016/j.ram.2021.02.011
29. Díaz Acosta CC, Russomando G, Candia N, Ritacco V, Vasconcellos SEG, de Berrêdo Pinho Moreira M, et al. Exploring the Latin American mediterranean family and the RDRio lineage in Mycobacterium tuberculosis isolates from Paraguay, Argentina and Venezuela. BMC Microbiol. 2019;19(1):131.
30. [cited 2024 Nov 12]. Available from: https://apps.who.int/iris/bitstream/handle/10665/260470/WHO-CDS-TB-2018.5-eng.pdf
31. Paul R, Masciotra N, Lafuente G, Avendaño D, Monteserin J, Belen OM, et al. Field validation of multiplex allele-specific PCR(MAS-PCR) for rapid detection of multidrug-resistant tubercubarcodelosis (MDR_TB) in Argentina [Internet]. 2015 May 18. Available from: https://f1000research.com/posters/1097888
32. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol. 2006;44(12):4498–510.
33. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol. 1997;35(4):907–14.
34. Demay C, Liens B, Burguière T, Hill V, Couvin D, Millet J, et al. SITVITWEB – A publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology. Infect Genet Evol. 2012;12(4):755–66.

35. Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res. 2010;38(Web Server issue):W326–31.
36. Coll F, McNerney R, Guerra-Assunção JA, Glynn JR, Perdigão J, Viveiros M, et al. A robust SNP barcode for typing Mycobacterium tuberculosis complex strains. Nat Commun. 2014;5:4812.
37. van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol. 1991;29(11):2578–86.
38. Wingett SW, Andrews S. FastQ screen: A tool for multi-genome mapping and quality control. F1000Res. 2018;7:1338.
39. Cantu VA, Sadural J, Edwards R. PRINSEQ++, a multi-threaded tool for fast and efficient quality control and preprocessing of sequencing datasets [Internet]. PeerJ. 2019. Available from: https://doi.org/10.7287/peerj.preprints.27553
40. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–60.
41. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and samtools. Bioinformatics. 2009;25(16):2078–9.
42. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a mapreduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20(9):1297–303.
43. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, snpeff: SNPs in the genome of drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 2012;6(2):80–92.
44. Coll F, McNerney R, Preston MD, Guerra-Assunção JA, Warry A, Hill-Cawthorne G, et al. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. Genome Med. 2015;7(1):51.
45. Martí-Carreras J, Maes P. VANIR—NextFlow pipeline for viral variant calling and de Novo assembly of nanopore and illumina reads for high-quality DsDNA viral genomes. Viruses 2020—Novel concepts in virology. Basel Switzerland: MDPI; 2020. p. 117.
46. Flandrois JP, Lina G, Dumitrescu O. MUBII-TB-DB: a database of mutations associated with antibiotic resistance in Mycobacterium tuberculosis. BMC Bioinformatics. 2014;15(1):107.
47. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, et al. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 2017;45(D1):D566–73.
48. Carolina T, Laura M, Dolores A, Sofía A, Ariel A, Paula A, et al. Surveillance of SARS-CoV-2 variants in Argentina: detection of Alpha, Gamma, Lambda, Epsilon and Zeta in locally transmitted and imported cases [Internet]. bioRxiv. medRxiv; 2021. Available from: https://doi.org/10.1101/2021.07.19.21260779
49. Eldholm V, Norheim G, von der Lippe B, Kinander W, Dahle UR, Caugant DA, et al. Evolution of extensively drug-resistant Mycobacterium tuberculosis from a susceptible ancestor in a single patient. Genome Biol. 2014;15(11):490.
50. Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Żaczek A, et al. Methodological and clinical aspects of the molecular epidemiology of Mycobacterium tuberculosis and other mycobacteria. Clin Microbiol Rev. 2016;29(2):239–90.
51. Sekizuka T, Yamashita A, Murase Y, Iwamoto T, Mitarai S, Kato S, et al. TGS-TB: total genotyping solution for Mycobacterium tuberculosis using short-read whole-genome sequencing. PLoS ONE. 2015;10(11):e0142951.
52. Senghore M, Diarra B, Gehre F, Otu J, Worwui A, Muhammad AK, et al. Evolution of Mycobacterium tuberculosis complex lineages and their role in an emerging threat of multidrug resistant tuberculosis in Bamako. Mali Sci Rep. 2020;10(1):327.
53. Orgeur M, Sous C, Madacki J, Brosch R. Evolution and emergence of Mycobacterium tuberculosis. FEMS Microbiol Rev [Internet]. 2024;48(2). Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC10906988/
54. Monteserin J, Paul R, Gravina E, Reniero A, Hernandez T, Mazzeo E, et al. Genotypic diversity of Mycobacterium tuberculosis in Buenos Aires, Argentina. Infect Genet Evol. 2018;62:1–7.
55. Ahmad K, Ahmad Z, Somayya R, Ali A, Rahat S. Analysis of Rrs gene mutations in Amikacin resistant clinical isolates of Mycobacterium tuberculosis from Khyber Pakhtunkhwa, Pakistan. Microb Pathog. 2017;108:66–70.
56. Sarwer MI, Khan MT, Khurshid S. Novel Rrs mutations in second-line injectable drug-resistant clinical isolates of Mycobacterium tuberculosis from the Punjab Province of Pakistan. J Infect Chemother. 2022;28(8):1119–24.
57. Lau RWT, Ho PL, Kao RYT, Yew WW, Lau TCK, Cheng VCC, et al. Molecular characterization of fluoroquinolone resistance in Mycobacterium tuberculosis: functional analysis of GyrA mutation at position 74. Antimicrob Agents Chemother. 2011;55(2):608–14.
58. Pitaksajjakul P, Wongwit W, Punprasit W, Eampokalap B, Peacock S, Ramasoota P. Mutations in the GyrA and GyrB genes of fluoroquinolone-resistant Mycobacterium tuberculosis from TB patients in Thailand. Southeast Asian J Trop Med Public Health. 2005;36(Suppl 4):228–37.
59. Singh P, Jain A, Dixit P, Prakash S, Jaiswal I, Venkatesh V, et al. Prevalence of GyrA and B gene mutations in fluoroquinolone-resistant and -sensitive clinical isolates of Mycobacterium tuberculosis and their relationship with MIC of Ofloxacin. J Antibiot (Tokyo). 2015;68(1):63–6.
60. [cited 2025 Feb 13]. Available from: https://www.researchgate.net/figure/Multiple-substitutions-in-GyrA-GyrB-or-both-GyrA-and-GyrB-reported-in_tbl4_221779213
61. Finci I, Albertini A, Merker M, Andres S, Bablishvili N, Barilar I, et al. Investigating resistance in clinical Mycobacterium tuberculosis complex isolates with genomic and phenotypic antimicrobial susceptibility testing: a multicentre observational study. Lancet Microbe. 2022;3(9):e672–82.
62. Bwalya P, Solo ES, Chizimu JY, Shrestha D, Mbulo G, Thapa J, et al. Characterization of embb mutations involved in ethambutol resistance in multi-drug resistant Mycobacterium tuberculosis isolates in Zambia. Tuberculosis (Edinb). 2022;133(102184):102184.
63. Monteserin J, Paul R, Latini C, Simboli N, Yokobori N, Delfederico L, et al. Relation of Mycobacterium tuberculosis mutations at katG315 and inhA-15 with drug resistance profile, genetic background, and clustering in Argentina. Diagn Microbiol Infect Dis. 2017;89(3):197–201.
64. World Health Organization. Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance, second edition. 2023;2.
65. Kobayashi M, Aoki S, Saito T, Aono A, Mitarai S. 1696Total-deletion mutation of PncA as a new mechanism of Pyrazinamide resistance in Mycobacterium tuberculosis - The first report from Japan. Open Forum Infect Dis. 2014;1(suppl1):S453–453.
66. Gomes LC, Campino S, Marinho CRF, Clark TG, Phelan JE. Whole genome sequencing reveals large deletions and other loss of function mutations in Mycobacterium tuberculosis drug resistance genes. Microb Genom [Internet]. 2021;7(12). Available from: https://doi.org/10.1099/mgen.0.000724
67. Alexander DC, Ma JH, Guthrie JL, Blair J, Chedore P, Jamieson FB. Gene sequencing for routine verification of Pyrazinamide resistance in Mycobacterium tuberculosis: a role for PncA but not RpsA. J Clin Microbiol. 2012;50(11):3726–8.
68. Ramaswamy SV, Amin AG, Göksel S, Stager CE, Dou SJ, El Sahly H, et al. Molecular genetic analysis of nucleotide polymorphisms associated with ethambutol resistance in human isolates of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2000;44(2):326–36.
69. Alcaide F, Pfyffer GE, Telenti A. Role of embb in natural and acquired resistance to ethambutol in mycobacteria. Antimicrob Agents Chemother. 1997;41(10):2270–3.
70. Deng L, Mikusová K, Robuck KG, Scherman M, Brennan PJ, McNeil MR. Recognition of multiple effects of ethambutol on metabolism of mycobacterial cell envelope. Antimicrob Agents Chemother. 1995;39(3):694–701.
71. Iwainsky H. EMB: mode of action, biotransformation and pharmacokinetics. In: Bartman K, editor. Antituberculosis drugs. Berlin, Germany: Springer-; 1988. pp. 533–40.
72. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuber Lung Dis. 1998;79(1):3–29.
73. Brossier F, Sougakoff W, Bernard C, Petrou M, Adeyema K, Pham A, et al. Molecular analysis of the EmbCAB locus and embr gene involved in ethambutol resistance in clinical isolates of Mycobacterium tuberculosis in France. Antimicrob Agents Chemother. 2015;59(8):4800–8.
74. Fernandez Do Porto DA, Monteserin J, Campos J, Sosa EJ, Matteo M, Serral F, et al. Five-year microevolution of a multidrug-resistant Mycobacterium tuberculosis strain within a patient with inadequate compliance to treatment. BMC Infect Dis. 2021;21(1):394.
75. Luo D, Chen Q, Xiong G, Peng Y, Liu T, Chen X, et al. Prevalence and molecular characterization of multidrug-resistant M. tuberculosis in Jiangxi province, China. Sci Rep. 2019;9(1):7315.
76. Imperiale B, Di Giulio Á, Mancino M, Zumárraga M, Morcillo N. Surveillance and characterization of drug-resistant Mycobacterium tuberculosis isolated in a reference hospital from Argentina during 8 years' period. Int J Mycobacteriol. 2019;8(3):223.

77. Imperiale BR, Zumárraga MJ, Di Giulio AB, Cataldi AA, Morcillo NS. Molecular and phenotypic characterisation of Mycobacterium tuberculosis resistant to anti-tuberculosis drugs. Int J Tuberc Lung Dis. 2013;17(8):1088–93.
78. Hiebert M, Sharma MK, Rabb M, Karlowsky L, Bergman K, Soualhine H. Mutations in embB406 are associated with low-level ethambutol resistance in Canadian Mycobacterium tuberculosis isolates. Antibiot (Basel). 2024;13(7):624.
79. Cancino-Muñoz I, Moreno-Molina M, Furió V, Goig GA, Torres-Puente M, Chiner-Oms Á, et al. Cryptic resistance mutations associated with misdiagnoses of Multidrug-Resistant tuberculosis. J Infect Dis. 2019;220(2):316–20.
80. Marimani M, Ahmad A, Duse A. The role of epigenetics, bacterial and host factors in progression of Mycobacterium tuberculosis infection. Tuberculosis (Edinb). 2018;113:200–14.
81. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM. Deciphering drug resistance in Mycobacterium tuberculosis using whole-genome sequencing: progress, promise, and challenges. Genome Med. 2019;11(1):45.
82. Argentina.gob.ar [Internet]. 2024 [cited 2025 Aug 4]. Boletines VIH, ITS, Hepatitis virales y Tuberculosis. Available from: https://www.argentina.gob.ar/salud/vih-its/boletines-vih-its-hepatitis-virales-y-tuberculosis

Publisher's note