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International Journal of Mycobacteriology
Prof. Ali Akbar Velayati,
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Dr. Parissa -Farnia,
Professor of Medical Microbiology
Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Tel: 00982126109505/00989122207382
E-mail: velayati@theaasm.org; farnia@theaasm.org

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Against All Odds: Reduction of Drug-resistant Tuberculosis Incidence in Niger – A Public Health Success Story

Alberto Piubello[1], Mahamadou Bassirou Souleymane[1], Ibrahim Maman Lawan[1], Soumaila Morou[1], Ibrahim Boukary[1], Cheikh Lawi Aboubacar[2], Kabirou Amoussa Abdoul Aziz[3], Issa Hamidou[4,5], Sala Attaher[6], Assiatou Gagara-Issoufou[7,8], Zelika Hamidou-Harouna[9], Alphazazi Soumana[2]

[1]Damien Foundation Niger, [2]National Tuberculosis Programme Niger, [3]Maradi Regional Hospital, CAT, Maradi, [4]André Salifou University, [5]Department of Pneumology, National Hospital, Zinder, [6]Regional Hospital, CAT, Tahoua, [7]Department of Pneumology, National Hospital Amirou Boubacar Diallo, [8]Abdou Moumouni University, [9]National Reference Laboratory, Niamey, Niger

Summary: Niger significantly reduced drug-resistant tuberculosis (DR-TB) incidence through strategic interventions. From 2015 to 2023, efforts included training healthcare workers, implementing rapid diagnostics and sample transportation, promoting patient-centered care, strengthening pharmacovigilance, ensuring close supervision, and strengthening the supply chains for medications. Niger's experience highlights the effectiveness of integrated public health strategies.

Background: Drug-resistant tuberculosis (DR-TB) remains a critical challenge in low-resource settings. Despite systemic constraints, including a fragile health system, a high malnutrition rate, and security threats, Niger implemented targeted strategic interventions through the National Tuberculosis Program (NTP) and partners, including Damien Foundation.

Methods: This study is a descriptive longitudinal analysis of routine surveillance, laboratory, and treatment cohort data collected by the NTP from 2015 to 2024 to assess the trends in DR-TB. The World Health Organization (WHO) estimates are used for contextual comparison and not for outcome measurement. No causal inference is claimed; associations between programmatic interventions and epidemiological trends are explored. Key interventions included health system strengthening through continuous training of healthcare workers involved in DR-TB management, focusing on diagnostic algorithms, patient management, and pharmacovigilance. A nationwide sputum sample transportation system was established with private courier companies to ensure timely delivery to reference laboratories and rapid results reporting. Diagnostic capacity was expanded through the scale-up of rapid molecular testing and decentralization of DR-TB services from a single referral center to four treatment facilities nationwide. Quarterly supportive supervision focused on clinical practice, data quality, and patient support mechanisms. An ambulatory model of care was implemented and reinforced, including transport reimbursement, nutritional support, peer-led home visits by former DR-TB patients, household contact screening, introduction of shorter regimens, and a strengthened pharmacovigilance system. In parallel, procurement and distribution systems for second-line anti-TB medicines were reinforced to ensure uninterrupted availability. Collectively, these interventions aimed to improve early detection, continuity of care, and overall management of DR-TB patients.

Results: Among 898 notified patients between 2015 and 2024, 88% (790/898) started treatment and 685 were evaluated (cohorts with final outcomes available, 2015–2023). Among them, 548 (80%) were cured bacteriologically confirmed, 89 (13%) died, 20 (3%) had treatment failure, and 28 (4%) were lost to follow-up. Notably, only three cases of acquired resistance to bedaquiline were recorded. In 2024, 151 DR-TB cases were notified among the 190 estimated according to routine national data, with a coverage of 79.5% with a substantial increase from 17.9% recorded in 2015. In 2024, 46% of the total new TB cases were tested, and the percentage of DR-TB was 1% (0.7–1.3). Among retreatment TB cases, 95% of them were tested, with 6.3% (4.5–8) being DR-TB. According to the routine data, the estimated number of DR-TB cases among the 14,135 new cases and the 772 retreatment cases notified in 2024 was 190 (134–246), which is lower than the WHO modeled estimate (680, 95% confidence interval [CI]: 110–1200) for the same year. According to the 2025 WHO Global TB report, the estimated percentage of DR-TB declined among new TB cases from 3.9% (1.3–9) in 2015 to 2.6% (1.4–4.4), and among previously treated patients, from 20% (16–24) in 2015 to 8.5% (7.1–10) in 2024. DR-TB incidence declined by 43% from 4.4 per 100,000 (95% CI: 0–9.5) in 2015 to 2.5 (0.41–4.6) in 2024.

Conclusion: The success in reducing DR-TB incidence in Niger underscores the importance of comprehensive and integrated public health strategies even in fragile and insecure settings.

Keywords: Drug-resistant tuberculosis, Niger, patient-centered care, public health, tuberculosis control

Conflicts of interest

There are no conflicts of interest

The Potential Role of Common Antibacterial Drugs in the Individualized Therapy of Patients with Severely Drug-resistant Tuberculosis

Sven Hoffner

Department of Global Public Health, Karolinska Institute, Stockholm, Sweden

Drug-resistant tuberculosis (TB) is an increasing problem and a challenge for the control and cure of the disease. Severely resistant TB is especially problematic since the effective treatment alternatives are limited. To avoid even more severe problems, it is crucially important to detect drug-resistant cases early and to ensure that effective drug combinations are used to avoid situations close to mono-therapy with a single effective drug, such as Bedaquiline. If not, there is a clear risk for both a continued and increased transmission of highly drug-resistant *Mycobacterium tuberculosis* strains such as fluoroquinolone-resistant multidrug and extensively drug-resistant tuberculosis, as well as further development of such strains.

Even though the pipeline of new potential TB drugs looks promising, the process to go from a substance with shown *in vitro* anti-TB activity to an approved drug ready for clinical use is very time-consuming and costly. To meet todays demandes, we must consider other alternatives.

Examining the possible anti-TB activity of existing antibacterial compounds, developed and used for the treatment of other bacterial infections, could be such an alternative. These compounds have the advantage of already being approved for human use. The side effects and pharmacokinetic characteristics are well known; only the information on possible anti-mycobacterial effects is missing. Since the mechanisms of action in most cases clearly differ from those of anti-TB drugs, the risk of cross-resistance between them and the different anti-TB drugs is unlikely. Possible synergistic or antagonistic effects of common antibacterial drugs and compounds used in TB therapy should, of course, also be considered.

Optimally, a TB drug should be active only against *M. tuberculosis* and other mycobacteria, not to disturb the microflora in the patient during the long period of drug therapy. This is a problem that must be recognised and limited as much as possible when introducing generally used antibacterial compounds in the treatment of TB.

In summary, the introduction of antibacterial compounds, with demonstrated activity also against TB, in combination with still active anti-TB drugs in patients with severely drug-resistant TB, is an option that should be further examined.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest.

Resistance of *Mycobacterium tuberculosis* to Fluoroquinolones among Newly Diagnosed Tuberculosis Cases in Russia

Valeria Guseva, Platon Eliseev, Irina Vasilyeva, Vadim Testov, Irina Lizinfeld

National Medical Research Center of Phthisiopulmonology and Infectious Diseases, Moscow, Russia

Introduction: Fluoroquinolones (FQ) are a key class of antituberculosis (TB) drugs used in treatment regimens for both drug-sensitive and drug-resistant TB, including short-term courses. However, resistance of *Mycobacterium tuberculosis* (MTB) to FQ can reduce the effectiveness of treatment.

Objective: To evaluate the prevalence and dynamics of MTB drug resistance to FQ among newly diagnosed TB cases in the Russian Federation between 2023 and 2024.

Methods: A retrospective analysis of reporting forms from regions of the Russian Federation was carried out for the period 2023–2024. The prevalence of resistance was calculated using the following formula: (number of resistance cases/total number of newly diagnosed TB cases tested) × 100. In addition, indicators of resistance to isoniazid and rifampicin, as well as combined resistance to rifampicin and FQ, were analyzed. To assess changes in the indicators over time, the absolute and relative growth/decline rates were calculated. The statistical significance of the differences was assessed using the Chi-squared test, with a significance level of $P < 0.05$.

Results: Between 2023 and 2024, the number of newly diagnosed TB cases in the Russian Federation decreased by 9.4%, from 39,856 to 36,104. The proportion of bacteriologically confirmed TB cases remained stable, at 52.5% (20,920 cases) in 2023 and 53.0% (19,124 cases) in 2024 (a difference of +0.5 percentage points; $P > 0.05$). Meanwhile, the coverage of isoniazid and rifampicin sensitivity testing increased, from 98.2% (20,542 cases) to 98.6% (18,861 cases) (an increase of +0.4%; $P < 0.05$). In 2024, there was a slight increase in the proportion of cases of isoniazid-resistant TB, rising from 10.7% (2195 cases) in 2023 to 11.8% (2222 cases), an increase of 1.1% ($P < 0.05$). Against the backdrop of a decline in the overall number of new TB cases, the number of patients with multidrug-resistant (MDR) TB has also decreased. At the same time, the proportion of patients in this category increased by 0.7% (from 34.9% to 35.6%) between 2023 and 2024, but this increase was not statistically significant ($P > 0.05$). Among this group, drug sensitivity testing for FQ was performed in 95.4% of cases in 2023 (6837 cases) and 95.7% in 2024 (6421 cases), an increase of 0.3% ($P > 0.05$). The prevalence of FQ resistance was 24.1% in 2023 (1646 cases) and 24.7% in 2024 (1589 cases) (an increase of 0.6%; $P > 0.05$). Conversely, 75.9% of patients were sensitive to FQ in 2023, a figure which decreased to 72.3% in 2024 (−3.6%), reflecting a slight clinical decrease in sensitivity to FQ.

Conclusions: The level of FQ resistance among newly diagnosed MDR-TB cases in the Russian Federation has remained stable at 24%–25%, which highlights the need to strengthen molecular genetic monitoring. The combination of rifampicin resistance and FQ complicates therapy selection, lengthens treatment duration, and negatively affects its effectiveness.

Keywords: Drug-resistance, fluoroquinolones, Russia, tuberculosis

Conflicts of interest

There are no conflicts of interest

Tracking the Blood Trail: Do Firstline Anti-tuberculosis Drug Levels Predict Adverse Reactions?

Bijoy Kumar Panda

Department of Pharmacy Practice, Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth (Deemed to be University), Satara, Maharashtra, India

Background: Fixed-dose combination (FDC) antitubercular therapy (ATT) has improved treatment adherence; however, adverse drug reactions (ADRs) remain a major concern. Limited evidence exists on the correlation between plasma levels of first-line antitubercular drugs and the occurrence of ADRs.

Objectives: The objective of this study was to evaluate the association between plasma concentrations of first-line antitubercular drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) and the development of ADRs.

Methods: A prospective observational study was conducted among 129 newly diagnosed tuberculosis patients receiving daily FDC ATT. Patients were categorized into cases (with ADRs) and controls (without ADRs). Plasma drug concentrations (C2h) were estimated after 2 weeks of therapy. Clinical evaluation and laboratory investigations including liver function tests and serum uric acid were performed at baseline and during follow-up. ADRs were classified based on organ systems. Statistical analysis included comparison of plasma drug levels, Chi-square test, and odds ratio (OR) estimation.

Results: Out of 129 patients, 61 (46.3%) developed ADRs. The most common ADRs were gastrointestinal disturbances (67.2%), followed by hepatobiliary reactions (11.4%), cutaneous reactions (8.2%), and musculoskeletal symptoms due to hyperuricemia (6.5%). Patients aged >60 years showed a significantly higher risk of ADRs (P = 0.0024). Median plasma concentrations of isoniazid, pyrazinamide, and ethambutol were slightly higher in cases compared to controls but remained largely within reference ranges. Elevated pyrazinamide levels (>50 μg/mL) were significantly associated with ADRs (OR = 1.8). Symptomatic mild hepatitis occurred in 7 (5.4%) patients and hyperuricemia-related arthralgia in 4 (3.1%) patients. No treatment interruption was required, and all reactions were managed symptomatically.

Conclusion: ADRs are common among patients receiving daily FDC ATT, with gastrointestinal reactions being the most frequent. Advanced age and elevated pyrazinamide plasma levels were associated with higher ADR risk. Plasma concentrations of first-line antitubercular drugs were not strongly predictive of hepatotoxicity.

Keywords: Adverse drug reactions, fixed-dose combination therapy, plasma drug concentration, therapeutic drug monitoring, tuberculosis

Conflicts of interest

There are no conflicts of interest

Utilization of Deeplex Myc-TB Assay for Rapid Resistance Testing

Rob Warren[1,2,3]; TS Eliot Study Group

[1]Division of Molecular Biology and Human Genetics, SAMRC Centre for Tuberculosis Research, Faculty of Medicine and Health Sciences, Stellenbosch University, [3]National Health Laboratory Service, Green Point Tuberculosis Laboratory, Cape Town, South Africa, [2]Division of Pulmonary and Critical Care Medicine, Zuckerberg San Francisco General Hospital and Trauma Centre, University of California, San Francisco, CA, USA

Background: The implementation of injection-free treatment regimens for rifampicin-resistant tuberculosis (RR-TB) is critical for reducing adverse events as well as improving treatment outcome. It is essential that drug susceptibility testing is implemented together with new treatment regimens to ensure knowledge of the susceptibility to the drugs included in the regimen. Phenotypic drug susceptibility testing remains the gold standard but is time-consuming with a turnaround time of up to 60 days, which means that the initiation of treatment is often done in the absence of knowledge of the drug susceptibility pattern.

Objective: The objective of the study was to test the diagnostic performance of the World Health Organization approved targeted next-generation sequencing kit Deeplex Myc-TB alongside the routine standard of care in a high-throughput diagnostic laboratory in South Africa.

Methods: DNA was extracted from routinely collected *Mycobacterium tuberculosis* isolates using the InstaGene method. Genes known to confer resistance to 15 anti-TB drugs were polymerase chain reaction amplified using the Deeplex Myc-TB kit, and the resulting amplification products were sequenced using a combination of the Illumina DNA Prep Kit and the MiniSeq instrument. Output files were analyzed using the online Deeplex App.

Results: During the period April 2023–January 2024, 701 RR-TB cases were diagnosed by Xpert MTB/RIF Ultra. Of these, 570 (81%) patient sputum specimens were MGIT culture positive, and 401 (70%) were Deeplex Myc-TB successful. Variants in *mmpR5* were identified in 57 (14.2%) isolates. The overall specificity for bedaquiline resistance 96%. Bedaquiline resistance was noted in both rifampicin monoresistant and multidrug-resistant cases. Fifty-eight percent of bedaquiline resistance was noted in new cases, suggesting transmission.

Conclusion: The Deeplex Myc-TB assay provides accurate and rapid genotypic drug susceptibility testing results that are essential to guide treatment, particularly for new and repurposed anti-TB drugs. Implementation of the Deeplex Myc-TB assay adjacent to the standard of care would be a significant step forward for RR-TB care.

Keywords: Bedaquiline, Deeplex Myc-TB, drug resistance, rifampicin-resistant tuberculosis, targeted next-generation sequencing

Conflicts of interest

There are no conflicts of interest

Optimizing Tuberculosis Diagnostics and Surveillance in Italy through Early Whole-genome Sequencing

M. Cirillo Daniela, Saluzzo Francesca

IRCCS San Raffaele Scientific Institute, Milan, Italy

Background: Italy is a low-incidence tuberculosis (TB) country, reporting fewer than 3000 new cases annually. In 2024, 53 rifampicin-resistant or multidrug-resistant (MDR) TB cases were notified. Despite these low numbers, an extensively drug-resistant (XDR) *Mycobacterium tuberculosis* cluster, originating in Eastern Europe in 2020, has emerged and expanded within Italy.

Methods: We investigated the evolution and transmission dynamics of this cluster and evaluated the impact of implementing a whole-genome sequencing (WGS)-based diagnostic algorithm. Under the coordination of the Ministry of Health, phenotypic and genomic data were collected from all TB isolates obtained in five major Italian regions over 2 years. WGS was performed directly from early positive liquid cultures, whereas phenotypic drug susceptibility testing (DST) was restricted to selected cases according to a diagnostic algorithm proposed by the European laboratory network of the ECDC (2025).

Results: The cluster evolved progressively from MDR and pre-XDR to XDR-TB, including resistance to pretomanid. All cases detected since 2021 were reported in Italy, indicating ongoing local transmission. The WGS-based algorithm demonstrated high concordance with phenotypic DST for resistance prediction and clinical management. Implementation of the algorithm allowed phenotypic DST to be avoided in over 90% of cases without loss of diagnostic reliability.

Conclusions: Early WGS from positive liquid cultures provides robust, actionable data for TB case management in low-incidence settings. Adoption of a targeted phenotypic DST strategy substantially reduces laboratory workload while strengthening genomic surveillance. This approach enhances the capacity to detect and respond to emerging XDR-TB clusters and supports timely, coordinated public health interventions.

Keywords: Diagnostic algorithm, extensively drug-resistant tuberculosis, public health surveillance, transmission dynamics, whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Structural Characterization of a Metallothionein from *Mycobacterium leprae*

Gina Marcela López-Ruiz, Dayana Oviedo-Cantillo, Nelson Enrique Arenas

Department of Basic Sciences, Faculty of Medicine, Universidad de Cartagena , Cartagena de Indias, Colombia

Background: *Mycobacterium leprae*, the causative agent of Hansen's disease, can enter Schwann cells and cause neural damage through specific manipulation of host factors. However, this chronic process might require particular nutrients and metals for persistence. Metallothioneins are cysteine-rich proteins that take up metals and have been identified previously in *Mycobacterium tuberculosis*.

Methods: The metallothionein sequence (P9WK09) was retrieved from UniProtKB, and its three-dimensional structure was obtained from the AlphaFold database (AF-P9WK09-F1). SignalP-5.0 was used to identify potential signal peptides. Metal ion-binding sites were predicted via the MIB2 server. Residue conservation was assessed through the ConSurf server, and evolutionary analysis was performed through MEGA version 11 (Molecular Evolutionary Genetics Analysis [MEGA]. Temple University, Philadelphia, Pennsylvania, United States of America).

Results: MYMT (53 aa) has an array of three beta strands, which contain metal ion-binding sites. SignalP analysis revealed a potential signal peptide with a likelihood of 0.576. The metallothionein has seven cysteine residues (13%), which might form thiolate clusters for metal binding (positions 17, 22, 24, 32, 34, 43, and 45) on the basis of the binding site prediction results. Copper affinity was predicted in a binding site delimited with Cys-17, His-19, Cys-22, and Gly-46 (score: 2.14), a second region defined as Cys-17, Cys-22, Cys-43, and Cys-45 for cadmium binding (2.66). Compared with 71 homologs within mycobacteria and related species, cysteine residues were found to be highly conserved (*E* value cutoff = 0.0001).

Conclusions: MymT protects *M. leprae* from copper damage primarily by binding to Cu(I) ions by forming thiolate clusters, thereby sequestering copper or other metals. Moreover, MymT might help this mycobacterium handle oxidative stress and evade the host's innate immune response, particularly phagocytosis, making it a promising target for fighting Hansen's disease.

Keywords: Metal toxicity, metallothionein, mycobacterial survival, *Mycobacterium leprae*, persistence

Conflicts of interest

There are no conflicts of interest

SelektaDx™ Diagnostic Platform: A Rapid Multiplex Molecular Detection of *Mycobacterium tuberculosis* Complex and its Key Drug Resistance

Nikhil Sangith[1,2,3], Manju Purohit[4,5], Vasim Ahmad[6], Narayanan Madaboosi[7], Cecilia Stålsby Lundborg[5], Pavan Asalapuram[1,2,3,5]

[1]Research and Development Unit, Mitti Lifetek AB, [5]Department of Global Public Health, Karolinska Institute, Stockholm, Sweden, [2]Xact Diagnotek Private Limited, TNAU Campus, [3]Tamil Nadu Agricultural University, Coimbatore, [7]Department of Medical Sciences and Technology, Indian Institute of Technology - Madras, Chennai, Tamil Nadu, [4]Department of Pathology, R. D. Gardi Medical College, Ujjain, Madhya Pradesh, [6]Department of Microbiology, Culture and DST Laboratory, Rajan Babu Institute of Pulmonary Medicine, New Delhi, India

Address for correspondence: Dr. Pavan Asalapuram, Research and Development Unit, Mitti Lifetek AB, Stockholm, Sweden. E-mail: pavan@mittilifetek.com

Background: The rise of multidrug-resistant tuberculosis (MDR-TB) continues to pose a major challenge to global TB control. World Health Organization (WHO)-recommended shorter all-oral regimens, such as the 6-month bedaquiline, pretomanid, linezolid, and moxifloxacin regimen (BPaLM), have demonstrated high treatment success rates (>90% in clinical trials) for rifampicin-resistant TB. However, effective implementation of these regimens critically depends on rapid and reliable diagnostics that can simultaneously confirm TB and detect resistance to both first-line drugs (rifampicin and isoniazid) and key second-line drugs (fluoroquinolones and bedaquiline), which directly determine regimen eligibility. While molecular diagnostics such as GeneXpert, Truenat, and line probe assays enable decentralized detection of rifampicin, isoniazid, and fluoroquinolone resistance, substantial gaps remain in decentralized detection of bedaquiline resistance and in comprehensive confirmation of MDR-TB in resource-limited settings. Phenotypic drug susceptibility testing for these agents often requires weeks, specialized biosafety infrastructure, and centralized laboratories, delaying personalized treatment and increasing the risk of resistance amplification and transmission.

Methods: To address these diagnostic gaps, we developed the SelektaDx™ MDR-TB test, a modified intercepting multiplex real-time polymerase chain reaction (PCR) assay incorporating a dual-layer detection architecture. The assay integrates MutaSelect™ probes for mutation-selective amplification of clinically relevant resistance markers with FluoroSelect™ probes for highly sensitive amplicon detection. This design enables concurrent identification of the *Mycobacterium tuberculosis* complex and resistance determinants spanning first-line (rifampicin and isoniazid) and second-line (fluoroquinolones and bedaquiline) drugs directly from sputum samples. The test delivers results within approximately 90 min using standard real-time PCR instruments and basic diagnostic laboratory infrastructure, supporting decentralized deployment in low- and middle-income countries.

Results: In a pilot clinical evaluation of 50 sputum samples, the SelektaDx™ MDR-TB test demonstrated 99% sensitivity and 100% specificity for TB detection and rifampicin/isoniazid resistance when benchmarked against GeneXpert and mycobacteria growth indicator tube culture-based methods. Analytical validation using precharacterized MDR-TB specimens confirmed robust assay performance and reproducibility. A larger prospective clinical evaluation is currently underway in India, enrolling 300 MDR-TB samples, with a specific focus on detecting resistance to rifampicin, isoniazid, fluoroquinolones, and bedaquiline. This evaluation aims to directly inform regimen selection, including eligibility for BPaLM-based therapy, and to strengthen antimicrobial stewardship by enabling earlier, evidence-based treatment decisions.

Conclusions: SelektaDx™ represents a rapid, modular, and cost-effective molecular diagnostic platform that integrates confirmation of TB with clinically actionable resistance detection across both first-line and critical second-line drugs. By enabling early, decentralized identification of MDR-TB and resistance patterns relevant to modern all-oral regimens, the platform supports timely personalized treatment, reduces diagnostic delays, limits resistance amplification, and advances the implementation of WHO-recommended care pathways in alignment with the end TB strategy and the United Nations Sustainable Development Goals.

Keywords: Bedaquiline, pretomanid, linezolid, and moxifloxacin, molecular diagnostics, multidrug-resistant tuberculosis

Conflicts of interest

There are no conflicts of interest

Drug-resistant Tuberculosis: Global Progress, Persistent Gaps in Low- and Middle-income Countries, and India's Pathways to Elimination

Pavan Asalapuram[1,2]

[1]Research and Development Unit, Mitti Lifetek AB, [2]Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden

Background: Tuberculosis (TB) remains one of the leading causes of death from infectious diseases globally, and drug-resistant TB (DR-TB) continues to threaten progress toward the World Health Organization (WHO) End TB Strategy targets. In 2024, an estimated 10.7 million people developed TB worldwide, with multidrug-resistant or rifampicin-resistant TB (MDR/RR-TB) accounting for a growing proportion of cases. Despite major advances in diagnostics and therapeutics, substantial gaps persist between estimated incidence, detection, and treatment initiation – particularly in low- and middle-income countries (LMICs), which bear more than 85% of the global TB burden. These challenges are further exacerbated by persistent funding shortfalls and health system constraints. India sits at the epicenter of the global TB epidemic, contributing approximately 27% of global TB incidence and a significant share of the global MDR/RR-TB treatment gap. Although TB incidence in India declined by about 21% between 2015 and 2024, progress remains insufficient to meet interim elimination targets. Delayed access to high-quality diagnostics at the point of first contact continues to be a critical bottleneck.

Methods: This abstract synthesizes global and India-specific data from WHO reports, national TB program statistics, and published implementation studies to examine recent progress in DR-TB diagnostics, treatment, and prevention. Particular emphasis is placed on the scale-up of rapid molecular diagnostics, decentralized testing strategies in LMIC settings, adoption of all-oral DR-TB regimens, and community-based prevention and care models. Evidence from multi-country pilot evaluations and national programmatic experiences in India and Africa is reviewed to assess operational impact and remaining gaps.

Results: Globally, coverage of rapid molecular diagnostics reached approximately 52% in 2024, leaving nearly half of TB patients – especially those in peripheral and resource-limited settings – dependent on less sensitive diagnostic methods. Swab-based and near-point-of-care platforms such as Truenat MTB Ultima and MiniDock MTB have demonstrated sensitivities ranging from 77.9% to 85.7% with tongue swabs and over 90% with sputum samples, meeting WHO target product profile benchmarks. Multi-country evaluations indicate that decentralized deployment of these tools can reduce diagnostic delays from weeks to hours and increase case detection by 20%–30%. However, reduced sensitivity in paucibacillary disease and among people living with HIV, along with workforce and system-integration challenges, remain important considerations. In parallel, the DR-TB treatment landscape has undergone a major transition toward shorter, all-oral regimens. The WHO-recommended 6-month BPaLM regimen has replaced prolonged injectable-based therapies, improving adherence and patient outcomes. By 2024, more than 100 countries had adopted these regimens, with global MDR/RR-TB treatment enrollment exceeding 160,000 patients and treatment success rates approaching 71%. India has emerged as a leader in scaling up these regimens.

Conclusions: Eliminating TB requires integrated pathways that combine decentralized, high-quality diagnostics, optimized all-oral treatment regimens, strengthened prevention strategies, community participation, and data-driven targeting of interventions. India's evolving TB response – linking rapid diagnostics, modern treatment, preventive therapy, and community engagement – offers a practical and replicable blueprint for LMICs across Asia and Africa seeking to accelerate progress toward TB elimination.

Keywords: Diagnostic gaps in low- and middle-income countries, drug-resistant tuberculosis, India's pathways for tuberculosis elimination

Conflicts of interest

There are no conflicts of interest

Analysis of the c1818 Protein among Drug-susceptible and Drug-resistant *Mycobacterium tuberculosis*

Parissa Farnia, Poopak Farnia, Jalaledin Ghanavi, Ali Akbar Velayti

Mycobacteriology Research Centre, National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Tuberculosis (TB) is one of the most important infectious diseases worldwide, affecting the millions of people annually. Drug resistance represents one of the greatest therapeutic challenges, as multidrug-resistant (MDR), extensively drug-resistant (XDR), and totally drug-resistant (TDR) TB are associated with increased mortality, prolonged treatment duration, and reduced drug efficacy. Members of the PE/PPE, particularly the PE_PGRS family, play important roles in *Mycobacterium tuberculosis* pathogenicity, interaction with the host immune system, and potentially in adaptation to drug pressure. One such gene is Rv1818c, whose corresponding protein, PE_PGRS33, has been reported to contribute to bacterial entry into macrophages, immune evasion, and regulation of inflammatory pathways (Delogu *et al*., 2004; Palucci *et al*., 2016). However, limited information is available regarding the genetic diversity of Rv1818c in isolates resistant to first- and second-line anti-TB drugs and the potential impact of this diversity on protein structure and immune responses. A detailed analysis of mutations in this gene among drug-susceptible and drug-resistant strains may facilitate the identification of new genetic biomarkers and improve understanding of resistance and bacterial adaptation mechanisms.

Objectives: This study aimed to investigate the genetic diversity and mutations of the Rv1818c gene in 150 culture-positive *M. tuberculosis* isolates with different drug-susceptibility profiles, including drug-susceptible, mono-drug-resistant, MDR, XDR, and TDR isolates. We also sought to assess the effects of these mutations on the three-dimensional structure of the PE_PGRS33 protein and their potential influence on host immune interactions and adaptation to drug pressure. Another objective was to compare mutation frequencies between susceptible and resistant isolates and statistically analyze their association with the severity of drug resistance.

Methods: After confirmation of culture positivity in 150 isolates, drug susceptibility testing was performed using standard microbiological assays. Isolates were classified into five groups: drug-susceptible (n = 100), mono-drug-resistant (n = 30), MDR-TB (n = 15), XDR-TB (n = 5), and TDR-TB (n = 5). Genomic DNA was extracted, and the Rv1818c gene was analyzed using the polymerase chain reaction and direct sequencing. Whole-genome sequencing with a coverage depth of ≤ ×100 was performed for all isolates to identify single-nucleotide polymorphisms, insertions/deletions, and structural variations. Sequence data were aligned to the *M. tuberculosis* H37Rv reference genome using bioinformatics tools, and mutations were classified according to type (insertion, deletion, and substitution), position, and predicted effect on the amino acid sequence. The three-dimensional structure of PE_PGRS33 was predicted, and mutations were mapped onto the model. Statistical comparisons of mutation frequencies between susceptible and resistant groups were performed using the χ^2 test with a significance level of 0.05.

Results: Among the 150 isolates analyzed, 100 (66.7%) were drug-susceptible, 30 (20%) showed mono-drug or limited resistance, 15 (10%) were MDR-TB, 5 (3.3%) were XDR-TB, and 5 (3.3%) were TDR-TB. Sequence analysis of Rv1818c revealed that insertions, substitutions, and deletions were markedly more frequent in drug-resistant isolates, particularly MDR, XDR, and TDR strains, compared with susceptible isolates. For example, MDR isolates exhibited insertions at positions 394–396, 401–403, 408–410, and 415–417; XDR isolates showed longer insertions at positions 215–239 and 423–441 and TDR isolates displayed multiple insertion and substitution mutations at positions 33–38, 167–169, 401–403, and 415–417. Statistically, mutation frequencies were significantly higher in resistant groups than in susceptible isolates ($\chi^2 = 28.6$, $P < 0.001$). Three-dimensional structural analysis of PE_PGRS33 indicated that insertions generated additional loop regions, substitutions caused local alterations in protein folding and charge, and deletions resulted in loss of important functional domains. These changes may affect interactions with TLR2, activation of immune signaling pathways, and induction of anti-inflammatory cytokines such as interleukin-10. Notably, some mutations observed in TDR isolates were consistent with experimental latency models, suggesting an enhanced ability of these strains to persist under the adverse conditions.

Conclusion: The findings of this study demonstrate that the Rv1818c gene undergoes multiple mutations, including insertions, substitutions, and deletions, in drug-resistant *M. tuberculosis* isolates – particularly MDR, XDR, and TDR strains – leading to alterations in the three-dimensional structure of the PE_PGRS33 protein and potentially facilitating adaptation to drug pressure and interaction with the host immune system. These results are consistent with previous evidence regarding the role of PE_PGRS33 in pathogenicity and immune evasion and highlight Rv1818c as a potential genetic biomarker for identifying resistant strains and for the design of new therapeutic and vaccine strategies. The study provides a foundation for future investigations into the relationship between PE_PGRS33 genetic diversity, disease severity, treatment response, and the development of rapid molecular diagnostic tools.

Keywords: *Mycobacterium tuberculosis*, Rv1818c, tuberculosis

Conflicts of interest

There are no conflicts of interest

Comparative Analysis of PPE35 Gene Variations in Drug-susceptible and Drug-resistant *Mycobacterium tuberculosis*

Parissa Farnia, Poopak Farnia, Jalaledin Ghanavi, Ali Akbar Velayti

Mycobacteriology Research Centre, National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Tuberculosis (TB) remains one of the most significant global public health challenges, and the emergence and spread of drug-resistant strains – particularly multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) – have seriously hindered disease control efforts. Beyond classical drug-resistance genes, growing evidence indicates that genes involved in host interaction, immune evasion, and intracellular adaptation play important roles in the survival of *Mycobacterium tuberculosis* under drug-induced selective pressure. The PE/PPE gene family, especially Rv1918c (PPE35), has attracted increasing attention due to its high genetic variability, surface localization of the encoded protein, and association with host immune responses.

Objective: This study aimed to investigate and compare genetic alterations in the PPE35 (Rv1918c) gene among drug-susceptible, MDR-TB, and XDR-TB isolates and to interpret the structural and clinical implications of these changes based on published genomic and structural data.

Methods: A total of 120 clinical isolates confirmed as *M. tuberculosis* were analyzed. Isolate identification was based on growth on specific culture media, confirmatory molecular assays, and drug-susceptibility testing. According to susceptibility profiles, 96 isolates (80%) were classified as drug-susceptible, 18 (15%) as MDR-TB, and 6 (5%) as XDR-TB. This distribution enabled comparison of PPE35 genetic variation under different levels of drug selective pressure. Genomic data and reported mutations in PPE35 were extracted from whole-genome sequencing (WGS)–based studies and subjected to comparative and structural analyses.

Results: In drug-susceptible isolates, the PPE35 gene sequence was largely conserved, with only synonymous polymorphisms or minor variations lacking significant functional impact. In contrast, MDR-TB and especially XDR-TB isolates harbored more frequent and deleterious nonsynonymous mutations. The most notable finding was the identification of the dupA.1798c mutation in PPE35, predominantly observed in XDR isolates and in a limited number of MDR isolates. This mutation results from the insertion of an adenine nucleotide within the coding sequence, causing a frameshift near amino acid position ~600. The frameshift leads to substitution of a key amino acid and generation of a premature stop codon, ultimately producing a truncated protein lacking the C-terminal region enriched in β-sheet structures. Structural and host interaction analyses suggest that this alteration disrupts normal protein folding and impairs immune-related functions. Amino acid substitution impact maps indicate that the Thr600→Ala change carries a high functional penalty, markedly reducing protein stability and efficiency. These observations are consistent with previous reports implicating PPE proteins in immune evasion, modulation of cell wall permeability, and enhanced bacterial survival under stress conditions.

Conclusion: Overall, this review and analysis indicate that the PPE35 gene is relatively conserved in drug-susceptible *M. tuberculosis* isolates, whereas MDR-TB and XDR-TB strains exhibit significant structural mutations that may indirectly contribute to bacterial adaptation to therapeutic pressure and the host environment. Although these mutations are not considered classical mechanisms of drug resistance, they likely play roles in drug tolerance, intracellular persistence, and increased complexity of resistance phenotypes. These findings underscore the importance of investigating PPE family genes alongside classical resistance determinants and demonstrate that WGS-based analyses can provide a more comprehensive understanding of hidden survival mechanisms in drug-resistant *M. tuberculosis*. Such insights may facilitate identification of novel biomarkers, improvement of diagnostic strategies, and development of more targeted therapeutic interventions against MDR-TB and XDR-TB.

Keywords: Drug resistance, extensively drug-resistant tuberculosis, immune evasion, multidrug-resistant tuberculosis, *Mycobacterium tuberculosis*, PE/PPE gene family, PPE35, protein structure, Rv1918c, tuberculosis, whole genome sequencing

Conflicts of interest

There are no conflicts of interest

Whole-genome Sequencing Analysis of Drug-susceptible Mycobacterium tuberculosis Across Different Spoligotype-based Subtypes

Poopak Farnia, Parissa Farnia, Jalaledin Ghanavi, Ali Akbar Velayati

Mycobacteriology Research Centre, National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Understanding the genetic diversity of *Mycobacterium tuberculosis* (MTB) is essential for elucidating tuberculosis epidemiology and transmission patterns. Spoligotyping is widely used to identify MTB lineages and subtypes; however, due to its limited discriminatory power, it cannot resolve subtle genetic differences. Whole-genome sequencing (WGS) enables a more precise assessment of such variations, particularly among drug-susceptible strains.

Methods: In this study, 200 clinical drug-susceptible *M. tuberculosis* isolates were analyzed. Initial genotyping was performed using spoligotyping, and isolates were classified into different lineages and subtypes based on spoligotype patterns. Representative isolates from both predominant and minor subtypes were subsequently selected for WGS. Genomic analyses included the identification of single-nucleotide polymorphisms (SNPs), comparison of intra- and inter-subtype genetic distances, and assessment of lineage-specific mutational differences.

Results: Spoligotyping revealed that the Central Asian Strain (CAS) lineage was the most prevalent genetic group, comprising approximately 56% of isolates ($n \approx 112$). The East African–Indian (EAI) lineage ranked second with a frequency of 21% ($n \approx 42$). Other lineages included T (9%, $n \approx 18$), LAM (6%, $n \approx 12$), and Haarlem (2%, $n = 4$), while about 10% of isolates were classified as orphan or miscellaneous patterns. No isolates belonging to the Beijing lineage were identified among drug-susceptible strains. WGS analysis demonstrated substantial genetic diversity within each subtype despite identical spoligotype patterns. The mean genetic distance within the CAS lineage was 48 ± 13 SNPs (range: 22–75 SNPs), while the EAI lineage showed a higher mean distance of 68 ± 17 SNPs (range: 35–95 SNPs). The T and LAM lineages exhibited moderate genetic diversity (35 ± 10 SNPs and 40 ± 12 SNPs, respectively). Haarlem isolates, although few in number, displayed genetic differences of approximately 30–45 SNPs, suggesting a lack of recent epidemiological linkage. Lineage-specific mutational patterns included a higher frequency of nonsynonymous mutations in genes related to lipid metabolism and cell wall processes in CAS, and increased polymorphisms in transcriptional regulatory and intracellular adaptation genes in EAI. No known drug-resistance-associated mutations were detected.

Conclusion: The findings indicate that drug-susceptible *M. tuberculosis* strains harbor considerable genetic diversity that cannot be fully captured by spoligotyping alone. The absence of the Beijing lineage among drug-susceptible isolates, together with WGS results, underscores the importance of WGS for the accurate analysis of MTB population structure and transmission dynamics. Integrating spoligotyping with WGS provides a comprehensive framework for molecular epidemiological studies of tuberculosis.

Keywords: Drug-susceptible tuberculosis, genetic diversity, molecular epidemiology, *Mycobacterium tuberculosis*, single-nucleotide polymorphism, spoligotyping, whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Comparative Protein Sequence Analysis of the Rv2752c Gene in Drug-susceptible and Drug-resistant *Mycobacterium Tuberculosis*

Poopak Farnia, Parissa Farnia, Jalaledin Ghanavi, Ali Akbar Velayati

Mycobacteriology Research Centre, National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Tuberculosis (TB) remains one of the most significant infectious diseases threatening global public health, and the emergence and spread of drug-resistant strains – particularly multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) – have created major challenges for disease control and treatment. While classical drug resistance in *Mycobacterium tuberculosis* is commonly attributed to mutations in drug target genes, accumulating evidence indicates that nonclassical mechanisms, such as RNA regulation, cellular stress responses, and drug tolerance, also play crucial roles in bacterial survival under antibiotic pressure. The Rv2752c gene encodes a protein that has recently been identified as a key factor with dual RNase and β-lactamase-like activities. Through its involvement in RNA stability and processing, and potentially in the inactivation of certain β-lactam compounds, this protein may contribute to metabolic adaptation, stress responses, and drug tolerance in *M. tuberculosis*. The importance of such proteins becomes particularly evident in situations where bacteria survive antibiotic exposure without acquiring classical resistance-conferring mutations. Investigating the sequence and mutation profile of the Rv2752c gene in drug-susceptible and drug-resistant isolates can provide new insights into hidden survival strategies, drug tolerance mechanisms, and adaptive evolution of *M. tuberculosis*. In this context, whole-genome sequencing (WGS), with its high discriminatory power, enables precise identification of single-nucleotide polymorphisms (SNPs) and their association with drug-resistance phenotypes. Therefore, the aim of this study was to analyze sequence variations in Rv2752c among susceptible, MDR, and XDR isolates and to evaluate its potential role in drug tolerance and bacterial adaptation.

Methods: In this study, 100 culture-positive *M. tuberculosis* isolates were collected from TB patients, including 80 drug-susceptible isolates, 15 MDR isolates, and 5 XDR isolates. Genomic DNA was extracted using a standard extraction kit, and all isolates were subjected to WGS using Illumina technology with a minimum sequencing depth of × 100. Raw sequencing data were quality-filtered, aligned to the H37Rv reference genome, and analyzed to identify SNPs specifically within the Rv2752c gene.

Results: WGS analysis demonstrated that the Rv2752c gene was completely conserved and remained unaltered in all drug-susceptible isolates. In contrast, a c.27C>T mutation in Rv2752c was identified in all XDR isolates and in three MDR isolates. This mutation resulted in an amino-acid sequence alteration in the corresponding protein and may potentially affect its RNase and β-lactamase-like activities. These findings suggest that preservation of the native Rv2752c function is essential in drug-susceptible isolates, whereas structural alterations in this protein among resistant isolates may contribute to adaptation under antibiotic pressure and enhanced drug tolerance.

Conclusion: This study demonstrates that Rv2752c may function as a key gene involved in nonclassical drug tolerance mechanisms and survival of *M. tuberculosis*. Identification of the specific c.27C>T mutation in MDR and XDR isolates indicates a potential association between this gene and adaptive responses to prolonged drug exposure. Consequently, Rv2752c may serve as a promising molecular marker for assessing the risk of drug resistance and predicting treatment outcomes. Moreover, targeting pathways related to RNA regulation and drug tolerance may support the development of novel and adjunctive therapeutic strategies against drug-resistant TB.

Keywords: Drug tolerance, *Mycobacterium tuberculosis,* Rv2752c, whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Divergent Trends in Tuberculosis Notifications and Treatment Outcomes: An Interrupted Time Series Analysis in Southern Libya (2019–2023)

Shamsi Saad Shamsi[1]*, Aisha Eltaher[2], Abdulhamid Al-Fakhri[3], Huda Al-Griw[4]

[1]Department of Medical Laboratory, Faculty of Medical Technology, Sebha University, [2]Department of Community Medicine, Faculty of Medicine, Sebha University, [3]National Center for Disease Control, Sebha Branch, Sebha, [4]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya

Address for correspondence: Dr. Shamsi Saad Shamsi, Department of Medical Laboratory, Faculty of Medical Technology, Sebha University, Sebha, Libya.
E-mail: sha.saad@sebhau.edu.ly

Background: The COVID-19 pandemic severely disrupted global tuberculosis (TB) control efforts, with the most profound impacts observed in the resource-limited and conflict-affected settings. In Libya, a nation grappling with protracted instability, the pandemic posed a dual threat to its fragile public health infrastructure. This study aimed to quantify the impact of the COVID-19 pandemic on TB program indicators in Sebha, a key urban center in Southern Libya.

Methods: We conducted a retrospective cohort study using TB registry data from the National Center for Disease Control in Sebha from January 2019 to December 2023. An interrupted time series (ITS) analysis using segmented Poisson regression was performed to model trends in monthly TB case notifications, with interruptions at the pandemic onset (March 2020) and the end of the Public Health Emergency of International Concern (June 2023). A multivariable logistic regression was used to identify the predictors of unsuccessful treatment outcomes.

Results: A total of 275 TB cases were analyzed. The ITS analysis revealed a significant 12.7% monthly increase in notifications prepandemic (incidence rate ratio [IRR] = 1.127, 95% confidence interval [CI] [1.033–1.230]), which was immediately reversed by a 57.5% drop in March 2020 (IRR = 0.425, 95% CI [0.183–0.987], $P = 0.047$) [Figure 1]. The odds of an unsuccessful outcome were 3.41 times higher for patients treated during the pandemic compared to the prepandemic period (adjusted odds ratio [aOR] = 3.41, 95% CI [1.48–7.84], $P = 0.004$) [Figure 2]. The pandemic period was characterized by a flattened trend, followed by a strong rebound in 2023. Treatment success deteriorated substantially, with noncompletion rates rising from 27.6% in 2019 to over 50% in 2022–2023 [Figure 3]. Residing outside Sebha city was paradoxically associated with a 47% lower odds of noncompletion (aOR = 0.53, 95% CI [0.31–0.90], $P = 0.018$).

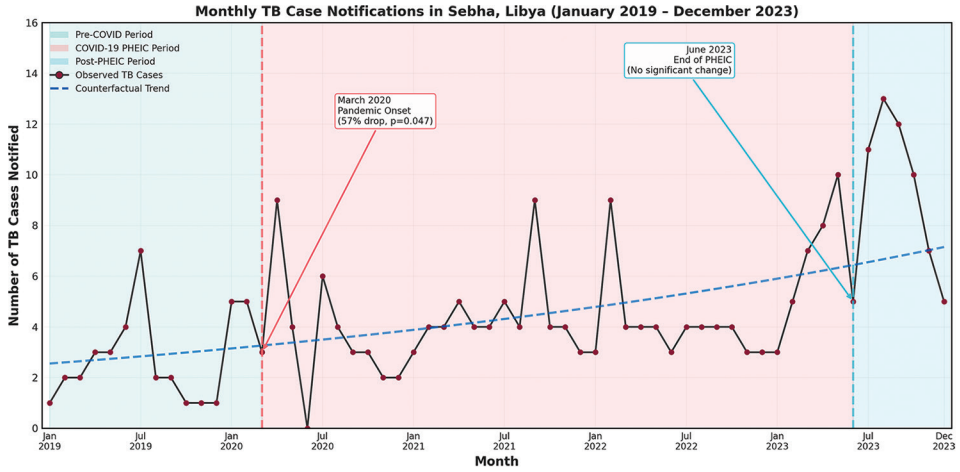

Figure 1: Tuberculosis notifications

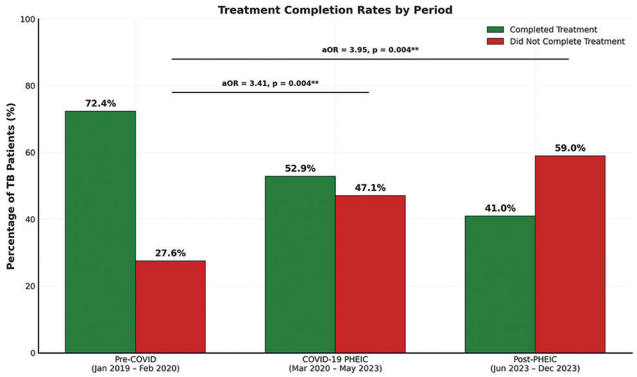

Figure 2: Treatment completion

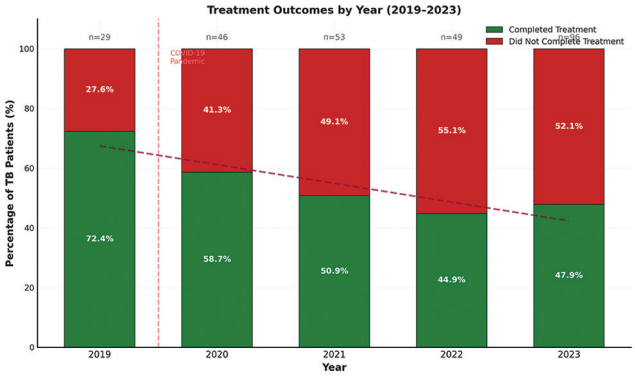

Figure 3: Outcomes by year

Conclusion: The COVID-19 pandemic precipitated an acute, transient decline in TB notifications in Sebha, followed by a surge that suggests a significant accumulation of undiagnosed cases. More critically, the pandemic was associated with a sustained decline in treatment success. These findings underscore the fragility of TB control in conflict-affected regions and highlight the urgent need for resilient, patient-centered strategies to mitigate the long-term consequences of the pandemic.

Keywords: Conflict setting, COVID-19, health systems resilience, interrupted time series, Libya, treatment outcomes, tuberculosis

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There are no conflicts of interest.

Advancing Mycobacterial Treatments: An Integrated Review from the ADVANCE-TB COST Action

Tânia Silva[1,2], Alessandra Aiello[3], Tonino Alonzi[3], M. Alice Carvalho[4], Vladimir Finger[5], Delia Goletti[3], Sven Hoffner[6], Yousra Kherabi[7,8], Jan Korabecny[5], Julia Kulczyńska[9], Agnieszka Kyzioł[9], Alicia Lacoma[10,11,12], Filomena Martins[13], Zorica Nanovic[14,15], Sepideh Hosseiniporgham[16], Martina Hrast Rambaher[17], Leonardo Antonio Sechi[18], Daria Smalchuk[10,11], Grażyna Stochel[9], Lydia Tabernero[19], Nancy L. Tateosian[20,21], Violeta Valcheva[22], Anamarija Zega[17], Mafalda Sarraguça[23]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, [2]ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, [23]LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, Porto, [4]Centre of Chemistry of University of Minho (CQ-UM), Department of Chemistry of School of Science of University of Minho, Campus de Gualtar, Braga, [13]Centro de Química Estrutural, Institute of Molecular Sciences, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Lisboa, Portugal, [3]Translational Research Unit, National Institute for Infectious Diseases L. Spallanzani-IRCCS, Rome, [18]Department of Biomedical Science, University of Sassari, Sassari, Italy, [5]Biomedical Research Center, University Hospital Hradec Kralove, Sokolska, Hradec Kralove, Czech Republic, [6]Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden, [7]Infectious and Tropical Diseases Department, Bichat-Claude Bernard Hospital, Assistance Publique-Hôpitaux de Paris, Université Paris Cité, [8]Université Paris Cité, Inserm, IAME, Paris, France, [9]Faculty of Chemistry, Jagiellonian University, Gronostajowa, Kraków, Poland, [10]Institut d'Investigació Germans Trias i Pujol, Badalona, [11]Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Barcelona, [12]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain, [14]Institute for Lung Diseases and Tuberculosis, Skopje, [15]Faculty of Medical Sciences, University "Goce Delcev", Stip, Republic of North Macedonia, [16]Department of Neuroscience & Experimental Therapeutics, School of Medicine, Texas A&M Health Science Center, Bryan, Texas, USA, [17]University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia, [19]School of Biological Sciences, Faculty of Biology Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK, [20]Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, [21]Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina, [22]Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Acad. Georgi Bonchev str, Sofia, Bulgaria

Background: The global rise in multidrug-resistant tuberculosis and the increasing incidence of nontuberculous mycobacterial (NTM) infections underscore an urgent need for new therapeutic strategies. Current treatments are long, toxic, prone to resistance development, and associated with low patient compliance and low success rates. This work reviews recent preclinical and clinical efforts to develop more effective therapies for mycobacterial infections, spanning direct-acting small molecules, nonclassical approaches, and host-directed therapies (HDTs). This critical review originates from the coordinated expertise of the ADVANCE-TB COST Action 21164: "Toward an improvement in diagnostics and treatment strategies for tuberculosis control," bringing together medicinal chemistry, microbiology, pharmacology, and immunology groups across Europe to address urgent therapeutic gaps in mycobacterial disease.

Methods: A structured analysis of recent literature (2000–2025) was performed, focusing on preclinical and early clinical studies targeting *Mycobacterium tuberculosis* and clinically relevant NTM species. Strategies were grouped into: (i) optimization of existing drug classes and development of new chemical entities targeting essential bacterial enzymes; (ii) formulation-driven approaches to improve pharmacokinetics and delivery; (iii) natural products and phytochemicals; (iv) nonclassical antimicrobials, including antimicrobial peptides (AMPs) and phage therapy; and (v) HDTs aimed at enhancing intracellular mycobacterial clearance. Mechanisms of action, resistance profiles, *in vitro and in vivo* efficacy, and translational potential were assessed.

Results: Significant progress has been made in the development of direct antimycobacterial small molecules. Optimized derivatives of established drug classes, including isoniazid-related compounds, hydrazide-hydrazones, and nitrofurans, show improved activity against drug-resistant strains and enhanced pharmacokinetic properties. Novel chemical entities targeting validated enzymes such as DprE1, InhA, and DNA gyrase demonstrate high potency, with several candidates advancing into clinical trials. Drug repurposing is a complementary strategy. Fusidic acid, approved for staphylococcal infections, shows reproducible *in vitro* activity against *M. tuberculosis*, including drug-resistant strains, with little cross-resistance to TB drugs, and synergistic activity with other antibiotics. Innovative formulation strategies, including cocrystals, co-amorphous systems, and therapeutic deep eutectic systems, markedly improve solubility, stability, and bioavailability of existing drugs. Natural products and phytochemicals contribute structural diversity and often display synergistic effects with standard therapies, though systematic toxicity and mechanism studies remain limited. Nonclassical approaches show particular promise: AMPs combine direct antimicrobial effects with immune modulation and display low propensity for resistance, whereas mycobacteriophage therapy has yielded encouraging results in drug-resistant NTM infections, including compassionate-use clinical cases. HDTs, using repurposed drugs that promote autophagy, phagosome maturation, or immune regulation, enhance intracellular killing and may be especially valuable in patients with comorbidities.

Conclusions: Advancing mycobacterial therapy will require integrated, mechanism-based regimens combining new direct-acting compounds with host-directed and non-classical strategies. While several promising candidates are emerging for TB, translation to NTM disease remains limited and demands NTM-specific screening and validation platforms. Improved delivery systems and rational combination therapies are critical to reduce treatment duration, limit toxicity, and counter resistance. Coordinated efforts across basic research, translational science, and clinical development are essential to deliver shorter, safer, and more effective treatments for both TB and NTM infections.

Keywords: Antimicrobial resistance, antimicrobials, drug development, enzymatic inhibitors, host-directed therapies, natural compounds, nontuberculous mycobacteria, phages, tuberculosis

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Conflicts of interest

There are no conflicts of interest

Antimicrobial Activity of Fusidic Acid against *Mycobacterium Tuberculosis*

Nadiia Tytarenko[1], Adrián Antuori[1,2], Jim Werngren[3], Sven Hoffner[4], José Domínguez[1,5,6], Alicia Lacoma[1,5,6]

[1]Germans Trias i Pujol Research Institute, [2]Department of Microbiology, Metropolitana Nord Clinics Laboratory, Germans Trias i Pujol Universitary Hospital, Badalona, [6]Department of Genetics and Microbiology, Autonomous University of Barcelona, Bellaterra (Cerdanyola del Vallès), Spain, [3]Department of Microbiology, Public Health Agency of Sweden, Solna, [4]Department of Global Public Health, Karolinska Institute, Stockholm, Sweden, [5]Centro de Investigación Biomédica en Red (CIBER), Carlos III Health Research Institute, Madrid, Spain

Background: Drug-resistant tuberculosis (DR-TB) is a major global health challenge in terms of treatment. Repurposed antibiotics represent a promising and alternative strategy to expand the antitubercular drug pipeline. Fusidic acid, an approved antibiotic for the treatment of staphylococcal infections, has shown some activity *in vitro* against *Mycobacterium tuberculosis* (*Mtb*), representing thus a candidate for repurposing. The objectives of this study are (1) to evaluate the antimicrobial activity of fusidic acid against a set of clinical strains of *Mtb* and (2) to test the intracellular and extracellular activity in an infected human macrophage model.

Methods: Fusidic acid (2–32 mg/ml) activity was tested against two multidrug-resistant clinical strains and one drug-susceptible (DS) strain using a standard MGIT system. Phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 macrophages were infected with *Mtb* H37Ra (Multiplicity of infection [MOI] 1:10). After the elimination of extracellular bacteria, the infected cells were treated from day (d) 3 postinfection with fusidic acid (4–120 mg/L), rifampicin (RIF) (4 µg/mL), or no drug. Intracellular and extracellular bacterial loads were quantified by colony-forming units (CFUs) enumeration at d3, d5, and d7 postinfection.

Results: Minimum inhibitory concentration results ranged from 4 to 8 mg/L, regardless of the strain considered. Fusidic acid reduced intracellular CFU in a concentration-dependent manner at d5 and d7 in comparison to the untreated cells ($P < 0.0001$). The highest concentration (120 mg/L) achieved the greatest intracellular inhibition and reduced intracellular CFU by 1.6 $\log_{10}$ at d7 compared with the untreated control, but remained less potent than RIF. Lower concentrations (4–60 mg/L) produced partial but significant reductions. Extracellular CFUs were also reduced by fusidic acid at d7 ($P < 0.05$), although without a clear concentration-response relationship, whereas RIF completely suppressed extracellular growth at all timepoints.

Conclusions: Fusidic acid has antimicrobial activity against DS and DR clinical isolates. Fusidic acid demonstrates measurable intracellular and extracellular antitubercular activity in a macrophage infection model, supporting its further investigation as a repurposed candidate for DR-TB, particularly as part of combination regimens.

Keywords: Antimicrobial resistance, repurposed drug, tuberculosis

Conflicts of interest

There are no conflicts of interest

Development of Minimum Inhibitory Concentration Plate for Extensively drug-resistant *Mycobacterium tuberculosis*

Satoshi Mitarai, Akio Aono, Kinuyo Chikamatsu, Shiomi Yoshida

Department of Mycobacterium Reference and Research, The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Tokyo, Japan

Background: Phenotypic drug susceptibility testing remains essential for the management of multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB). Although molecular resistance prediction is increasingly used, its applicability and operational feasibility remain limited in some settings. The newly developed MTB-NX broth microdilution minimum inhibitory concentration (MIC) panel was designed as a standardized, culture-based tool for comprehensive drug susceptibility testing. A multicenter evaluation was conducted to assess its reproducibility, inter-laboratory variability, and strain-specific performance.

Methods: A multicenter analytical study was performed across 25 laboratories using the MTB-NX MIC panel. The study assessed within-laboratory reproducibility, agreement with reference MIC values, inter-laboratory variability, and strain-specific MIC distributions. Both MIC medians and the spread of MIC distributions (analogous to interquartile range) were analyzed. The panel intentionally included strains with broader MIC distributions to visualize both the strengths and limitations of the measurement system.

Results: Overall, MIC distributions for most drugs were centered around the median and fell within ±1 dilution for the central 50% of inter-laboratory results, indicating stable performance. Using a 95% agreement threshold with reference values, most drugs met predefined criteria.

Within-laboratory reproducibility was high, with more than 90% of repeated measurements falling within ±1 dilution. For the reference strain H37Rv, more than 95% of MIC values were within the acceptable range.

Across laboratories, median MICs were largely consistent, and no systematic inter-laboratory bias was observed. However, some facilities showed mild directional bias, likely related to inoculum preparation, incubation timing, or interpretation differences.

Drug-specific interpretation challenges were identified. Visual assessment for pyrazinamide and tailing growth patterns for drugs such as isoniazid and ethionamide contributed to variability. Newer drugs, including bedaquiline and delamanid, also showed reading difficulties due to small colony growth and subtle inhibition patterns.

Conclusions: The MTB-NX MIC panel demonstrated generally good reproducibility and inter-laboratory agreement across multiple centers, supporting its reliability for phenotypic susceptibility testing. The study also identified drug- and strain-specific factors affecting interpretability, providing practical reference data for quality control and future standardization of MIC testing for drug-resistant tuberculosis.

Keywords: Extensively drug-resistant, minimum inhibitory concentration, *Mycobacterium tuberculosis*

Conflicts of interest

There are no conflicts of interest

Determining the Accuracy of Ultrasound in Diagnosing Pleural Effusion in Critically ill Patients Admitted to the Tuberculosis Intensive Care Unit

Seyed Mohammadreza Hashemian[1], Majid Mokhtari[1], Payam Tabarsi[2], Fahimeh Hadavand[3], Batoul Khoundabi[4], Pouya Jalali Fakhr[5], Hamidreza Jamaati[1]

[1]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, [2]Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, [3]Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, [4]Iran Helal Institute of Applied-Science and Technology, Red Crescent Society of Iran, [5]Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Several medical conditions can lead to pleural effusion (PE), which refers to the accumulation of fluid in the pleural space. This study aims to evaluate the diagnostic accuracy of a lung ultrasound protocol in patients admitted to the tuberculosis intensive care unit (ICU).

Methods: Data were collected from 30 patients admitted to the tuberculosis ICU, including clinical variables obtained through measurements and diagnostic tools such as lung ultrasonography and chest radiography. The main study data and variables were extracted from patients' medical records, imaging outputs (including ultrasound findings), and physicians' clinical assessments. These data were recorded in patient-specific checklists and prepared for subsequent analysis.

Results: A total of 30 patients admitted to the tuberculosis ICU were evaluated, of whom 14 patients (47.6%) were diagnosed with PE. Receiver-operating characteristic (ROC) analysis was performed to assess the diagnostic performance of lung ultrasonography for detecting PE based on pleural fluid volume, yielding an area under the ROC curve of 0.857. The sensitivity and specificity of ultrasonography for the diagnosis of PE were estimated at 79% and 81%, respectively. The positive and negative predictive values of ultrasonography were 82% and 79%, respectively. In addition, the Cohen's kappa coefficient, indicating agreement between the two diagnostic methods, was 0.598, reflecting a strong level of agreement.

Conclusion: Despite its inherent advantages, ultrasonography demonstrates acceptable sensitivity and specificity for the diagnosis of PE in patients admitted to the ICU.

Keywords: Chest ultrasound, Intensive care unit, pleural effusion, tuberculosis

Conflicts of interest

There are no conflicts of interest

Screening for Diabetes Mellitus in Human Immunodeficiency Virus Patients in a Referral Center in Iran

Parvaneh Baghaei, Afshin Moniri, Majid Marjani, Zahra Abtahian, Payam Tabarsi

Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: The prevalence of diabetes mellitus (DM) along with tuberculosis (TB) has increased in human immunodeficiency virus (HIV) patients. There is limited information about screening for DM in HIV patients. The aims of this study were to screen HIV patients with and without TB for DM, and the number needed to screen (NNS) to diagnose new cases of DM.

Methods: A prospective cohort descriptive study was conducted in Iranian adults with HIV admitted to Masih Daneshvari Hospital from 2015 to 2016. Sputum smear was utilized for TB diagnosis, and glycated hemoglobin (HbA1c) and fasting blood glucose (FBG) were measured for these patients.

Results: Of the 101 patients included, 61 (60.4%) had TB and 28 (27.7%) had DM. DM was newly diagnosed in 12 (57%) and 9 (43%) patients with and without TB, respectively. The NNS was 4 to identify one new DM case. After adjustment for TB, age ≥40, and gender, only age ≥40 was statistically associated with DM (adjusted odds ratio 2.44, 95% confidence interval 1–6.01). Sensitivities of HbA1c and FBG were 79% and 68%, respectively.

Conclusion: In HIV patients, screening for DM should be performed with an HbA1c test with changing current cutoff point and without considering to exist TB or not.

Keywords: Diabetes mellitus, glycated hemoglobin, human immunodeficiency virus, screening, tuberculosis

Conflicts of interest

There are no conflicts of interest

Acquired Nitroimidazole Resistance during Treatment for Drug-resistant Tuberculosis in People with Sustained Culture Positivity in South Africa

Nabila Ismail[1]*, Erick Auma[1]*, Marianna de Kock[1], Claudia Spies[1], Shatha Omar[1], Sashen Moodley[2], Michelle H. Larsen[3], Max O'Donnell[4,5], Rubeshan Perumal[6,7], Camus Nimmo[8], Yonas Ghebrekristos[1,9], Christoffel Opperman[1,9], Sarishna Singh[1,9], Dawn Davis[1], Megan Folkerts[10], David M Engelthaler[10], MSF Khayelitsha Team, Robin M. Warren[1], Helen Cox[11,12], John Z. Metcalfe[13], Grant Theron[1]

[1]SAMRC Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Department of Biomedical Sciences, Stellenbosch University, [9]National Health Laboratory Service, Greenpoint, Tuberculosis Laboratory, Cape Town, [2]Africa Health Research Institute, Department of Basic and Translational Science, [6]Division of Pulmonology and Critical Care, Department of Medicine, Inkosi Albert Luthuli Central Hospital, University of KwaZulu-Natal, [7]CAPRISA-MRC HIV-TB Pathogenesis and Treatment Research Unit, Durban, South Africa, [11]Division of Medical Microbiology, Department of Pathology, University of Cape Town, South Africa, [3]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, [4]Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Columbia University Irving Medical Center, [5]Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY, [10]Translational Genomics Research Institute, Immunology and Microbial Genomics Division, Flagstaff, USA, [13]Division of Pulmonary and Critical Care Medicine, Zuckerberg San Francisco General Hospital and Trauma Center, University of California San Francisco, USA, [8]Francis Crick Institute, Johnson lab, Systems Chemical Biology of Infection and Resistance Laboratory, London, UK, [12]Prevention of Tuberculosis and Other Respiratory Pathogens, Burnet Institute, Melbourne, Australia

*Contributed equally.

Background: Multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) remains a major global health challenge, with an estimated 390,000 cases in 2024. Shorter, all-oral regimens (containing bedaquiline, linezolid, and levofloxacin or moxifloxacin) improved treatment outcomes, especially when combined with nitroimidazoles (pretomanid and delamanid). Resistance to nitroimidazoles is canonically associated with loss-of-function variants in *ddn*, *fgd1*, and *fbiA–D* (genes belonging to the F420 biosynthesis pathway), but the data describing the frequency of these variants or their sensitivity for predicting phenotypic resistance are limited, despite the increasingly widespread use of these drugs.

Methods: We conducted a retrospective cohort study of individuals ≥12 years with pulmonary MDR/RR-TB who received a nitroimidazole-containing regimen between January 2012 and March 2024 in the Western Cape and KwaZulu-Natal provinces of South Africa. Eligible participants had sustained culture positivity for ≥4 months on treatment. First available isolates for the episode (baseline), interim (±2 months from treatment initiation), and follow-up (≥6 months) TB isolates were analyzed. Minimum inhibitory concentrations for delamanid (0.015–0.25 µg/mL) and pretomanid (0.125–4.0 µg/mL) were determined using the doubling dilutions using the BACTEC MGIT960 platform. Phenotypic drug susceptibility testing for bedaquiline and clofazimine, targeted deep sequencing, and whole-genome sequencing were performed. Notably, whole genome sequencing (WGS) on the growth from drug-containing tubes from the highest drug concentration tube after nitroimidazole minimal inhibitory concentration (MIC) testing was used.

Results: Thirty-four individuals with sustained culture positivity on a nitroimidazole-containing regimen were identified. Overall, the cohort exhibited a high burden of baseline resistance to other drugs, with 16/34 (47%) bedaquiline resistance and 23/34 (68%) fluoroquinolone resistance, and 18/34 (53%) experienced an unfavorable outcome. Nitroimidazole resistance was detected in 15/34 patients (44%): 3/34 (9%) were resistant to both drugs at baseline, 5/34 (15%) acquired resistance to both drugs during treatment, 2/34 (6%) and 3/34 (9%) either acquired resistance or were resistant at baseline to delamanid only and 2/34 (6%) acquired resistance to pretomanid only. WGS from the drug-containing tube showed phenotypic-genotypic concordance for all, except one resistant isolate 14/15 (93%). These variants were predominantly loss-of-function variants in *fbiC*, *fbiD*, *fgd1,* and *ddn*. Nitroimidazole resistance – both baseline and acquired – occurs in treatment-experienced DR-TB patients in South Africa and is strongly associated with loss-of-function mutations in genes within the F420 biosynthesis and activation pathway. These findings highlight an urgent need to optimize molecular diagnostics to detect resistance-associated variants, expand curated resistance catalogues, and refine genotype–phenotype interpretive frameworks. Further research in larger, programmatically representative cohorts is required to define the transmission dynamics, resistance acquisition pathways, and the clinical impact of emerging nitroimidazole resistance.

Conclusions: Nitroimidazole resistance – both baseline and acquired – occurs in treatment-experienced DR-TB patients in South Africa and is strongly associated with loss-of-function mutations in genes within the F420 biosynthesis and activation pathway. These findings highlight an urgent need to optimize the molecular diagnostics to detect resistance-associated variants, expand curated resistance catalogs, and refine genotype–phenotype interpretive frameworks. Further research in larger, programmatically representative cohorts is required to define transmission dynamics, resistance acquisition pathways, and the clinical impact of emerging nitroimidazole resistance.

Keywords: Delamanid, drug-resistant tuberculosis, tuberculosis, nitroimidazole resistance, pretomanid, whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Bacillus Calmette–Guérin-induced Trained Immunity in Pediatric Tuberculosis: A Scoping Review of Mechanisms and Clinical Implications

Maryam Hassanzad[1], Ali Akbar Velayati[2]

[1]Pediatric Respiratory Disease Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, [2]Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Bacillus Calmette–Guérin (BCG) vaccination is the only licensed vaccine against tuberculosis (TB) and is routinely administered during early childhood. While its protective effect against severe forms of pediatric TB is well established, growing evidence indicates that BCG also induces *trained immunity*, a form of innate immune memory driven by epigenetic and metabolic reprogramming of innate immune cells. The extent to which this immunological mechanism influences TB susceptibility, clinical presentation, and disease outcomes in children has not been comprehensively mapped.

Objective: The objective of this study was to map and synthesize available evidence on BCG-induced trained immunity and its mechanistic and clinical relevance to TB in children and adolescents (0–18 years).

Methods: A scoping review was conducted in accordance with PRISMA-ScR guidelines. We systematically searched PubMed, Scopus, Web of Science, and Embase for experimental, clinical, and epidemiological studies published between January 2016 and December 2025 (10 Years). Eligible studies examined BCG vaccination in pediatric populations and reported outcomes related to trained immunity, including epigenetic reprogramming, innate immune activation, or nonspecific immune memory, in relation to *Mycobacterium tuberculosis* infection, latent TB, or active disease. Evidence from all geographic and clinical contexts was considered.

Results: The reviewed literature indicates that BCG vaccination can induce sustained functional reprogramming of innate immune cells, particularly monocytes, macrophages, and natural killer cells, resulting in enhanced cytokine responses upon secondary stimulation. Across pediatric-focused and pediatric-relevant studies, markers of trained immunity were frequently associated with improved control of mycobacterial infection, reduced disease severity, and protection against disseminated forms of TB. However, substantial heterogeneity was observed, influenced by differences in age at vaccination, BCG strain, host immune status, and study design. Direct longitudinal studies linking trained immunity biomarkers to clinical TB outcomes in children remain scarce.

Conclusion: BCG-induced trained immunity offers an important conceptual framework for understanding the variable protection conferred by BCG against pediatric TB. Integrating mechanistic immunological insights with clinical and epidemiological data may help bridge existing evidence gaps and guide the development of improved TB prevention strategies in children.

Keywords: Bacillus Calmette–Guérin vaccine, childhood immunization, epigenetic reprogramming, innate immune memory, pediatric tuberculosis, trained immunity

Conflicts of interest

There are no conflicts of interest

Tuberculosis in the Elderly: An Underrecognized Disease in the Era of Population Aging

Alireza Eskandari, Reza Behfar, Mitra Rezaei, Majid Marjani

Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Mycobacterium tuberculosis remains a major global health problem. While the clinical presentation of tuberculosis (TB) in young adults is well characterized, TB in elderly patients is frequently underrecognized due to atypical manifestations. Age-related immunosenescence, a higher burden of comorbidities, and reduced tolerance to anti-TB medications contribute to diagnostic delays and poorer outcomes. With the ongoing global aging of the population, understanding age-related differences in TB presentation has become increasingly important. The clinical presentation of TB changes with advancing age. Fever is less commonly observed in elderly patients, whereas dyspnea, anorexia, and weight loss are more frequent. Nonspecific symptoms such as weakness and fatigue are common and may mimic other chronic illnesses, leading to delayed diagnosis. Although cough and sputum production occur in both age groups, initial sputum smear positivity is generally lower in elderly patients. Radiologic findings in this population include fewer cavitary lesions and less upper-lobe involvement, with more frequent middle and lower lobe infiltrations, fibrotic changes, and nonclassical patterns. Laboratory findings also differ with age. Elderly patients tend to have higher erythrocyte sedimentation rate levels and a higher prevalence of anemia, while leukocytosis is more frequently observed in younger patients. Comorbid conditions such as diabetes mellitus, cardiovascular disease, chronic obstructive pulmonary disease, renal disorders, and malignancies are significantly more prevalent among elderly TB patients, further complicating diagnosis and treatment. Adverse drug reactions, including gastrointestinal, hepatic, and dermatologic complications, occur more frequently in elderly patients. Treatment adherence and success rates are lower in this group, and mortality rates are higher. With the progressive aging of the global population, TB in the elderly represents a growing yet underrecognized public health challenge. Atypical clinical and radiologic presentations, combined with age-related laboratory changes, contribute to important diagnostic gaps. Increased clinical awareness, age-adapted diagnostic strategies, and careful management of comorbidities and treatment-related adverse effects are essential to improve early detection and outcomes in this vulnerable population.

Keywords: Diagnosis, elderly, treatment, tuberculosis

Conflicts of interest

There are no conflicts of interest

Prevalence and Public Health Significance of Mycobacteria in Cheese: evolving Risks in Traditional and Modern Dairy Systems

Reza Karimi

Department of Food Science and Technology, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

Background: Cheese, a culturally significant and globally consumed dairy product, can serve as a vehicle for pathogenic and opportunistic mycobacteria, presenting a persistent public health challenge. This review synthesizes current knowledge on two primary risks: the classical zoonotic threat from *Mycobacterium bovis* and the emerging concern of environmental nontuberculous mycobacteria (NTM) contamination. The dynamic between these pathogens is particularly relevant in Asian and African contexts, where traditional cheese-making practices, varying levels of bovine tuberculosis control, and diverse environmental reservoirs intersect. A comprehensive evaluation of the literature is necessary to update risk assessments and inform regionally appropriate food safety strategies.

Methods: This narrative review examined scientific literature from 2000 to 2023, sourced from databases including PubMed, Scopus, and Web of Science. Search terms combined "cheese," "mycobacteria," "*Mycobacterium bovis*," "nontuberculous mycobacteria," "prevalence," and "food safety." Eligible studies included original research reporting on the detection, prevalence, survival, or characterization of mycobacteria in any cheese variety. Data on mycobacterial species, detection methods, cheese type, and geographical origin were extracted and analyzed thematically to map trends and evidence gaps.

Results: The reviewed literature reveals a distinct geographical and technological dichotomy in mycobacterial prevalence. *M. bovis* remains a significant, culturally-entrenched hazard in raw milk cheeses from regions where bovine tuberculosis is endemic in cattle, with studies confirming its survival through fermentation and aging. In contrast, in regions with controlled bovine tuberculosis and widespread pasteurization, NTM such as *Mycobacterium avium* complex and rapidly-growing species like *Mycobacterium fortuitum* are the predominant isolates. These ubiquitous environmental contaminants enter the production chain through water, equipment, or postprocessing handling and demonstrate notable resilience in cheese matrices. The review highlights a critical evidence gap: a lack of standardized, culture-independent detection methods that can differentiate between viable and nonviable mycobacteria in cheese, leading to potential overestimation of public health risk from molecular surveys.

Conclusions: The public health significance of mycobacteria in cheese is characterized by a dual and evolving threat profile. While *M. bovis* represents a targeted zoonotic risk linked to specific practices and regions, NTM constitute a broader, systemic challenge of environmental contamination. Future strategies must be bifurcated: in endemic areas, priority must remain on veterinary surveillance and consumer education regarding raw milk cheeses. Globally, food safety frameworks need to integrate specific controls for thermoduric NTM, focusing on hygienic design of processing plants and water quality monitoring. Further research employing viability-based diagnostics is essential to accurately quantify risk and guide effective, evidence-based interventions for the dairy sector.

Keywords: Cheese, food safety, mycobacteria, *Mycobacterium*, prevalence, zoonosis

Conflicts of interest

There are no conflicts of interest

Mycobacteria in the Dairy Industry: Understanding Risks and Mitigation Strategies

Reza Karimi[1], Mojtaba Rezaei[2]

[1]Department of Food Science and Technology, Faculty of Agricultural Sciences, University of Guilan, Rasht, [2]Department of Food Nanotechnology, Research Institute of Food Science and Technology, Mashhad, Iran

Background: Mycobacteria constitute a significant yet frequently underestimated microbiological hazard within the dairy industry, owing to their pronounced environmental persistence, intrinsic resistance to physicochemical stresses, and potential implications for public health. Historically, *Mycobacterium bovis*, the etiological agent of bovine tuberculosis, has represented the primary focus of dairy safety programs. In recent years, however, increasing scientific attention has been directed toward nontuberculous mycobacteria (NTM), including *Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and *Mycobacterium kansasii*. These microorganisms are ubiquitously distributed in soil, water, feed, and farm environments and may be introduced into the dairy production continuum through contaminated raw milk, water utilized in cleaning and processing operations, and biofilms established on milking and processing equipment.

Methods: This review synthesizes current scientific literature to evaluate the sources, persistence, and control of mycobacteria in the dairy chain. A critical analysis is conducted on the pathways of contamination, from farm environments to finished products. The methodological strengths and limitations of techniques for detecting and quantifying mycobacteria in dairy matrices are examined, including culture-based methods, molecular diagnostics (e.g., polymerase chain reaction [PCR], quantitative polymerase chain reaction, viability PCR), immunological assays, and emerging spectroscopic techniques integrated with machine learning. Furthermore, evidence regarding the efficacy of processing interventions (e.g., pasteurization) and sanitation protocols against mycobacterial biofilms is evaluated.

Results: The lipid-rich, hydrophobic cell envelope of mycobacteria confers enhanced tolerance to thermal treatments, resistance to disinfectants, and pronounced adherence to surfaces. Within dairy production systems, MAP is of particular concern due to its role in Johne's disease and its investigated association with Crohn's disease. Although conventional pasteurization is effective against *M. bovis* and reduces MAP populations, evidence suggests that low numbers of MAP and other NTM may survive suboptimal processing or postpasteurization contamination. The persistence of mycobacteria in biofilms within processing equipment impedes hygienic control, as standard cleaning-in-place (CIP) procedures are often inadequate for their eradication, allowing water systems and food-contact surfaces to act as contamination reservoirs.

Conclusions: The effective mitigation of mycobacterial hazards necessitates an integrated farm-to-fork strategy. This encompasses herd health management, stringent raw milk hygiene, validated thermal processing, robust water quality assurance, and optimized CIP protocols designed to disrupt resilient biofilms. Accurate detection remains challenging, driving the adoption of advanced molecular and spectroscopic methods. The systematic incorporation of mycobacteria into dairy-specific hazard analysis and risk assessment frameworks is essential for strengthening food safety governance and ensuring the microbiological integrity of dairy products, particularly for susceptible consumer populations.

Keywords: Biofilm, dairy safety, detection methods, mycobacteria, risk assessment

Conflicts of interest

There are no conflicts of interest

Effects of Pulmonary Rehabilitation on Functional Capacity and Respiratory Symptoms in Patients with Lung Tuberculosis: A Case Series

Atefeh Fakharian, Maryam Sadat Mirenayat, Abouzar Mojibian, Masoumeh Zoghali, Reyhaneh Zahiri, Mohsen Abedi

Pulmonary Rehabilitation Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Respiratory disorders after tuberculosis (TB) may result in extensive pulmonary complications and reduced functional capacity and quality of life of patients. The present case series examines the effect of pulmonary rehabilitation on functional outcomes and disease symptoms of three patients with old TB.

Case Presentation: Three patients (one male, 48–60 years old) with old TB with pulmonary complications, including bronchiectasis and fibrosis, were studied before and after a pulmonary rehabilitation program. Baseline characteristics, functional capacity (distance covered in the six-minute walk test [6MWT]), dyspnea (based on the modified Medical Research Council dyspnea scale (mMRC), mental status (based on the Hospital Anxiety and Depression Questionnaire), handgrip strength, and daily physical function (based on the (activities of daily living) questionnaire and chronic respiratory disease questionnaire [CRDQ]) were recorded. Patient information was extracted from the hospital information system using the patient's case number and unique codes.

Results: The 6MWT distance of two patients increased from 356 to 370 m and from 198 to 270 m, and accordingly, the functional capacity of these two patients improved. Oxygen saturation improved after rehabilitation (from 82% to 87% and from 86% to 93%). Fatigue and shortness of breath caused by activity also decreased in them. Quality of life, St. George's questionnaire scores (total score from 90.06 to 84.42) and other subdomains of this questionnaire improved. Anxiety scores decreased in all cases, while depression improved somewhat in two cases. In the daily activity questionnaire and CRDQ, all subdomains showed improvement in all three patients. However, mMRC scores remained unchanged in two cases. In addition, hand grip strength showed a variable trend and was associated with an increase in one patient.

Conclusion: Based on our observations, pulmonary rehabilitation improved exercise capacity, symptoms, and quality-of-life measures in patients with pulmonary dysfunction associated with old TB. These results support pulmonary rehabilitation as a beneficial adjunctive therapy option for the comprehensive management of old TB patients. Further research in larger populations is recommended to achieve more reliable results.

Keywords: Exercise capacity, pulmonary rehabilitation, quality of life, tuberculosis

Conflicts of interest

There are no conflicts of interest

A Comprehensive Proteomic Analysis of Tuberculosis Glycoprotein Antigens as Emerging Biomarkers for Active Disease Diagnosis

Alireza Hadizadeh Tasbiti[1,2], Shamsi Yari[1,2], Seyed Davar Siadat[1,2], Abolfazl Fateh[1,2], Morteza Maomi[1,2], Maryam Karbasian[1,2], Farid Abdolrahimi[1,2]

[1]Department of Tuberculosis and Pulmonary Research, Pasteur Institute of Iran, [2]Microbiology Research Center, Pasteur Institute of Iran, Tehran, Iran

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*, primarily affecting the lungs. Traditionally, TB diagnosis relies on smear microscopy and Mycobacterial culture. However, microscopy has limited sensitivity, often failing to detect smear-negative TB, and results typically take up to 2 months. In high-income countries, enhanced diagnostic methods such as molecular tests have been developed, but these options are frequently too expensive and complex for routine application in resource-constrained settings where TB is prevalent. Accurate, rapid, cost-effective, and straightforward diagnostic tests are urgently needed for effective TB care and control. In this study, significant efforts have been made to create a rapid TB test centered on Mycobacterium antigen detection. However, our primary goal is to develop a rapid TB antigen detection test utilizing glycoprotein biomarkers. Previous research has focused on identifying host responses related to TB infection. Our approach utilizes the competitive enzyme-linked immunosorbent assay technique to recognize TB antigens. We purified at least 200 glycoproteins through Concanavalin A affinity chromatography and subsequently identified them using Liquid chromatography Mass spectrometry. To analyze posttranslational modifications, we utilized the GlycoPP web server to predict potential N- and O-glycosylation sites within the TB protein sequences. Furthermore, these proteins were assessed using the Immune Epitope Database (IEDB) and B-cell epitope prediction tools to identify linear epitopes from their protein sequences. Sputum samples were collected from patients suspected of having TB. Among these individuals, 54% were confirmed to have TB, while 46% were diagnosed with non-TB conditions. The purified glycoproteins included Rv3873, Rv0934, Rv1078, Rv0954, Rv0173, Rv0583c, Rv1270c, Rv1543, Rv0341, Rv1860, Rv2560, Rv0174, and Rv0838. Notably, Rv0954, Rv1078, Rv0838, Rv0583c, and Rv1860 displayed high compatibility scores (>70%). Receiver operating characteristic curve analysis was performed to determine the optimal cutoff value (≤0.55) for differentiating between TB and non-TB groups. The assay demonstrated a sensitivity of 91.15% and a specificity of 94.24%. These findings highlight the diagnostic potential of the identified glycoprotein biomarkers for TB.

Keywords: Immune Epitope Database and B-cell epitope prediction, mass spectrometry, tuberculosis antigen detection, tuberculosis glycoproteins

Conflicts of interest

There are no conflicts of interest

Experimental Evolution of *Mycobacterium tuberculosis* in the *in vivo* and *in vitro* models

Igor Mokrousov[1], Tatiana Vinogradova[2], Marine Dogonadze[2], Anna Vyazovaya, Violina Angelova[3], Violeta Valcheva[4]

[1]Laboratory of Molecular Epidemiology and Evolutionary Genetics, St. Petersburg Pasteur Institute, [2]Laboratory of Experimental Medicine, St. Petersburg Research Institute of Phthisiopulmonology, Saint Petersburg, Russia, [3]Faculty of Pharmacy, Medical University of Sofia, [4]Department of Infectious Microbiology, Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Different spontaneous mutations emerge in *Mycobacterium tuberculosis* and those beneficial for bacterial survival are selected leading to bacterial drug resistance, tolerance, and persistence. I will present results of our studies of *M. tuberculosis* genetic variation in response to selective pressure of antibiotics and candidate anti-TB compounds in the *in vivo* and *in vitro* models. The C57Bl/6 mice were infected with different clinical strains of the globally spread Beijing and LAM genotypes followed by treatment with moxifloxacin, linezolid, bedaquiline (WHO recommended), and perchlozone (repurposed drug recommended by Russian national guidelines). Bacterial isolates were recovered from the lungs of mice after 2 and 5.5 months of treatment and were subjected to whole-genome sequencing (WGS). For *in vitro* study, H37Rv reference strain was cultured under elevated (×10 MIC) concentrations of new aroylhydrazones; resistant clones were subjected to WGS. The bioinformatics analysis of the WGS data was carried out using Geneious R package (Biomatters, New Zealand) and SAM-TB online tool. This study was supported by Russian Science Foundation (grant 24-44-00004). The *in vitro* study revealed *M. tuberculosis* response to aroylhydrazones involving multiple pathways related to efflux mechanisms and drug tolerance on the whole. Different mutations were found in different clones: frameshift mutations in *ppgK* and *glpK*, and nonsynonymous mutations in *mmpS2* and *Rv3755c*). The *in vivo* study showed that after 5.5 months of treatment of mice, no new resistance mutations emerged. However, an inactivating mutation in dormancy-related lipid metabolism gene *tgs3* was detected in isolates from mice infected with hypervirulent Beijing strain (ancestral sublineage). This mutations have likely promoted the increased growth of the strain in the lings of mice. The *in silico* significant nonsynonymous mutation in the oxidoreductase gene *Rv1856c* was detected in the isolate from one mice infected with low-virulent LAM strain (LAM-RUS clade) after 5.5 months treatment. This enzyme is necessary for mycobacterial persistence and use of the host cholesterol at the later stage of infection. Therefore, these mutations were likely biologically meaningful. To conclude, the short-term primary adaptation of *M. tuberculosis* to selective pressure from the tested compounds *in vitro* is a complex process involving multiple unrelated genes and pathways. These are associated with nonspecific drug tolerance, efflux systems, or mechanisms that counteract oxidative stress. In contrast, during long-term chemotherapy in infected mice – under the influence of the host immune response – the adaptation strategy shifts toward the selection of beneficial mutations in genes not directly linked to resistance, which instead confer enhanced growth.

Keywords: Antibiotic selective pressure, *in vitro* drug tolerance, *in vivo* virulence adaptation, *Mycobacterium tuberculosis genetic variation,* whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Clinical and Functional Effects of Inhaled Pirfenidone in Patients with Posttuberculosis Pulmonary Fibrosis: A Prospective Pilot Study

Atefeh Abedini[1], Baharak Danyaee[1], Arda Kiani[2], Mehdi Khatooni[1], Mohammad Varahram[3], Mehdi Kazempour-Dizaji[3], Zahra Ameri Ahmad[1], Hamed Hamishehkar[4], Atefe Javadi[5], Kimia Taghavi[1,5]

[1]Chronic Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, [2]Tracheal Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, [3]Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [5]Department of Molecular Biology and Genetics, Faculty of Science and Letters, Istanbul Kultur University, Istanbul, Turkey

Background: Pulmonary tuberculosis can lead to persistent lung injury and progressive fibrotic remodeling, resulting in chronic respiratory impairment known as posttuberculosis pulmonary fibrosis (PTPF). Currently, no established antifibrotic therapy exists for this condition, and treatment options are largely supportive. Given the profibrotic molecular pathways shared between PTPF and idiopathic pulmonary fibrosis, inhaled delivery of antifibrotic agents may represent a targeted therapeutic strategy. **Methods:** In this prospective self-controlled pilot study, 31 patients with documented history of pulmonary tuberculosis and established posttuberculosis fibrotic lung disease were enrolled. Participants received inhaled pirfenidone twice daily for 6 months. Clinical, functional, and biochemical assessments were performed at baseline, 3 months, and 6 months, including spirometry (forced vital capacity [FVC] and forced expiratory volume in 1 s [FEV_1]), 6-min walk test (6MWT), symptom scores (Leicester Cough Questionnaire [LCQ], Visual Analog Scale [VAS] dyspnea, and Short Form-12 [SF-12]), high-resolution computed tomography, and serum biomarkers including transforming growth factor-β1 (TGF-β1) and liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]). **Results:** After 6 months, patients demonstrated significant improvements in symptom burden and functional capacity. LCQ and VAS dyspnea scores decreased markedly (both $P < 0.001$), and SF-12 scores increased from 27.0 ± 6.1 to 35.2 ± 4.1 ($P < 0.001$). The mean 6MWT distance improved by approximately 24%, while FEV_1 and FVC increased by 16.4% and 38.1% at 6 months, respectively ($P < 0.001$). Serum TGF-β1 levels decreased significantly from 13.1 ± 2.1 to 9.9 ± 1.5 ($P < 0.001$), indicating suppression of fibrotic activity. Liver enzyme levels (AST and ALT) declined slightly but significantly, confirming hepatic safety. **Conclusion:** Inhaled pirfenidone was feasible, well-tolerated, and associated with meaningful improvements in respiratory symptoms, exercise capacity, and lung function in patients with posttuberculosis pulmonary fibrosis. These findings suggest a potential therapeutic role for inhaled antifibrotic therapy in this underserved population and support further controlled clinical trials.

Keywords: Antifibrotic therapy, fibrotic lung disease, inhaled pirfenidone, pulmonary tuberculosis

Conflicts of interest

There are no conflicts of interest

Closing the Gap: Recent Advances, Shorter Regimens, and Empirical Strategies in Pediatric Tuberculosis

Chishala Chabala

School of Medicine, University of Zambia, Lusaka, Zambia

Pediatric tuberculosis (TB) management has entered a transformative era. Historically reliant on adult data, the field now benefits from pediatric-specific trials that prioritize shorter, all-oral, and child-friendly regimens. However, diagnostic challenges remain a critical barrier, especially in infants and those with human immunodeficiency virus (HIV) co-infection. Here, we review the latest clinical advances in optimizing TB treatment with shorter and empirical regimens. Reviewing the findings of the SHINE, SURE, and EMPIRICAL trials. The SHINE trial evaluated the noninferiority of a 4-month (2HRZ(E)/2HR) regimen for children with nonsevere TB, and the findings are now part of the World Health Organization recommended regimens. The SURE trial evaluated the effectiveness of a 6-month shorter intensive treatment and adjunct aspirin in children with TB meningitis on mortality and neurodisability. The EMPIRICAL trial evaluated whether empirical treatment for TB and congenital cytomegalovirus in high-risk infants living with HIV, hospitalized with severe pneumonia, could improve survival. The landscape of pediatric TB has shifted toward shorter, less toxic, and more evidence-driven care. These trials address the unique challenges of treating TB in children across a spectrum of TB disease.

Keywords: Empirical treatment, human immunodeficiency virus co-infection, pediatric tuberculosis, shorter treatment, tuberculosis meningitis

Conflicts of interest

There are no conflicts of interest

Optimization of Lecithin-enriched Chitosan/Gelatin Nanocarriers for Enhanced Encapsulation Efficiency and Controlled pH-responsive Release of Rifampin

Poopak Farnia, Jalaledin Ghanavi

Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Rifampin remains a cornerstone of first-line antitubercular therapy; however, its clinical performance is constrained by poor aqueous solubility, variable gastrointestinal absorption, and the need for relatively high oral doses. These pharmacokinetic limitations may contribute to subtherapeutic exposure and the emergence of resistant *Mycobacterium tuberculosis* strains. Nanostructured drug delivery systems based on biodegradable polymers offer a rational strategy to enhance solubility, stabilize drug molecules, and provide sustained release. In this study, we developed and optimized a lecithin-enriched chitosan/gelatin nanoparticle platform to improve rifampin loading capacity, physicochemical stability, and pH-responsive release kinetics.

Methods: Rifampin-loaded chitosan/gelatin/lecithin nanoparticles were fabricated using a modified multilamellar vesicle approach followed by ionic crosslinking with tripolyphosphate. Lecithin concentration was systematically varied to determine its influence on particle size distribution, surface charge (zeta potential), encapsulation efficiency, and drug loading. Physicochemical characterization was performed using dynamic light scattering, zeta potential analysis, transmission electron microscopy, and Fourier transform infrared spectroscopy to confirm structural interactions among components. Rifampin quantification was conducted via validated high-performance liquid chromatography. *In vitro* release studies were carried out in dialysis systems under simulated gastric (pH: 1.0), transitional (pH: 3.4), and physiological (pH: 7.4) conditions to evaluate release kinetics over 24 h.

Results: Increasing lecithin concentration markedly improved nanoparticle performance. Particle size was reduced from a heterogeneous range of approximately 250 nm to a more uniform distribution near 150 nm, indicating enhanced electrostatic stabilization during nanoparticle assembly. Zeta potential increased significantly (from +14 mV to +49 mV), exceeding the threshold associated with colloidal stability and suggesting improved dispersion integrity. Drug loading rose from 8% to 20%, and encapsulation efficiency increased from 31% to 67%, demonstrating the critical role of lecithin in promoting hydrophobic drug incorporation within the polymer–lipid matrix. Fourier transform infrared spectra confirmed molecular interactions between rifampin and the chitosan/gelatin backbone without evidence of chemical degradation. Release studies revealed a sustained and pH-dependent kinetic profile. At acidic pH (1.0 and 3.4), rifampin release remained limited during the initial 12 h (approximately 12%–16%), indicating protection against premature gastric liberation. In contrast, at physiological pH 7.4, cumulative release reached approximately 93% within 12 h, significantly exceeding the release rate of free rifampin solution (more than threefold enhancement). Higher lecithin concentrations produced a nearly tenfold increase in drug liberation compared with lower-lecithin formulations, reflecting improved matrix permeability and optimized diffusion pathways. The release pattern followed a controlled, gradual profile rather than burst kinetics, suggesting suitability for sustained systemic exposure.

Conclusions: The optimized lecithin-enriched chitosan/gelatin nanocarrier substantially improves rifampin encapsulation efficiency, colloidal stability, and controlled release behavior. The formulation protects the drug under acidic conditions while enabling efficient release at physiological pH, thereby enhancing bioavailability potential. This nanostructured delivery system represents a promising platform for improving therapeutic outcomes in tuberculosis management and may contribute to reducing dose frequency and minimizing resistance development. Further *in vivo* pharmacokinetic and efficacy studies are warranted to validate translational applicability.

Keywords: Chitosan, controlled release, lecithin, nanoparticles, rifampin

Conflicts of interest

There are no conflicts of interest

Recurrent Complications in a Pediatric Patient with Mendelian Susceptibility to Mycobacterial Disease and IL12RB1 Mutation

Mahsa Rekabi

Pediatric Respiratory Diseases Research Center, National Research, Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction: Mendelian susceptibility to mycobacterial disease (MSMD) is a rare primary immunodeficiency characterized by predisposition to clinical disease caused by weakly virulent mycobacteria, such as BCG vaccines and environmental mycobacteria. It results from genetic defects in the interleukin-12 (IL-12)/interferon-gamma axis. We present a complex case of a 7-year-old boy with MSMD to highlight the multisystem long-term complications of the disease.

Case Presentation: A 7-year-old boy with a known history of MSMD due to an IL12RB1 gene mutation was admitted with melena and lethargy. His history was significant for disseminated BCG infection (BCGosis) at 3 months of age, treated with antituberculosis therapy. His early childhood was marked by recurrent complications, including several hospitalizations for weakness, lethargy, and acute respiratory distress syndrome. Over time, the patient developed multiple sequelae of his condition. Abdominal imaging (sonography and spiral computed tomography) revealed chronic portal vein thrombosis with cavernous transformation, leading to collateral vessel formation, splenomegaly (123 mm), and moderate ascites. An upper endoscopy performed during a previous admission for weakness diagnosed Grade 3 gastritis with active variceal bleeding, explaining the current melena. His pulmonary history was similarly complex, complicated by recurrent infections and a necrotizing pneumonia, which led to loculated pleural effusions, pleural thickening, and the need for chest tube placement. Immunological workup showed an abnormal lymphocyte subset profile (CD3: 50.84%, CD4: 29.38%, and CD8: 18%) and a positive DHR test (164), suggesting a possible concomitant chronic granulomatous disease or a testing aberration, warranting further genetic clarification. The patient's family history is notable for the death of a sibling at 8 months from pulmonary and brain infection.

Conclusion: This case illustrates the severe and progressive multisystem involvement in a patient with MSMD due to an IL12RB1 mutation. Beyond the classic susceptibility to mycobacterial infection, this patient developed life-threatening complications, including portal hypertension with variceal bleeding and destructive pulmonary disease. This highlights the need for comprehensive, long-term multidisciplinary management and close surveillance for vascular, gastrointestinal, and pulmonary sequelae in patients with MSMD.

Keywords: BCGosis, IL12RB1, mendelian susceptibility to mycobacterial disease, pediatric, portal vein thrombosis, primary immunodeficiency, variceal bleeding

Conflicts of interest

There are no conflicts of interest

MiRNAs in Tuberculosis as Diagnostic Biomarkers

Esmaeil Mortaz[1,2]

[1]Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, Netherlands, [2]Chronic Respiratory Disease Research Center, National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background and Aim: Tuberculosis (TB) remains a major global health problem, and improved biomarkers are urgently needed for early diagnosis and understanding of host–pathogen interactions. MicroRNAs (miRNAs), small noncoding RNAs regulating posttranscriptional gene expression, are differentially expressed during Mycobacterium TB (Mtb) infection and may reflect host immune modulation, disease status, and pathogen survival strategies. The objective of current studies was to characterize the expression profiles of miRNAs in TB infection, investigate their mechanistic roles in host responses, and evaluate their potential as noninvasive biomarkers for diagnosis and monitoring of Mtb.

Materials and Methods: A combination of clinical sample analyses, cellular infection models, bioinformatics, and exosomal profiling was employed across studies. In one study, BCG-infected human macrophages were used to induce exosomal miRNA release and identify dysregulated miRNA clusters with downstream network analysis. Serum-derived exosomes from active TB patients and controls were isolated and evaluated by quantitative reverse transcription polymerase chain reaction for selected miRNAs to determine differential expression and diagnostic potential. Additional bioinformatics analyses were performed on dysregulated miRNAs from infected macrophages to explore their target pathways and potential roles in host metabolism and immune regulation.

Results: Our results demonstrated that TB infection alters both cellular and circulating miRNA profiles character. Exosomal miRNAs such as miR-484, miR-425, and miR-96 were significantly upregulated in serum from active pulmonary TB patients compared with controls, and receiver-operating characteristic analysis indicated fair to promising diagnostic accuracy (e.g., area under the curve ≈ 0.66–0.72 for individual miRNAs). Bioinformatics evaluation of miRNA clusters induced by mycobacterial infection revealed enrichment of pathways involved in host energy metabolism and immune response modulation, suggesting these miRNAs may contribute to the reprogramming of macrophage function favorable for intracellular survival of Mtb. Furthermore, BCG infection of macrophages induced specific exosomal miRNA release by cells, supporting the concept that exosomes serve as vehicles for miRNA-mediated possible intercellular communication that may influence local and systemic host responses.

Discussion: Overall, our studies highlight that both intracellular and circulating miRNAs are dynamically regulated in TB and may be considered as functional biomarkers. Exosomal miRNAs in particular represent a promising noninvasive source for TB diagnostic development, potentially augmenting existing diagnostic modalities. The bioinformatics insights into miRNA target networks underscore the role of miRNAs in immune modulation, host metabolism, and pathogen survival strategies, deepening our understanding of TB pathogenesis. Further validation in larger cohort studies and integration into diagnostic algorithms may increase early detection and personalized monitoring of TB disease progression and treatment response.

Conflicts of interest

There are no conflicts of interest

Changes in the Trends of Tuberculosis Incidence in Algeria from 1982 to 2024

Schehrazad Selmane

L'IFORCE, SCORP2, Faculty of Mathematics, University of Sciences and Technology Houari Boumediene, Algiers, Algeria

Background: Tuberculosis (TB) remains prevalent in Algeria in spite of six decades of national control efforts. The aim of the present study was to analyze the trends and changes in incidence rates of smear-positive pulmonary TB (SPPTB) and extrapulmonary TB (EPTB) in the country.

Methods: Data on TB, SPPTB, PTB, and EPTB were retrieved from periodic epidemiological reports published by the Algerian Ministry of Health for 1982–2024. The annual percentage change (APC) and average APC (AAPC) were calculated using the Joinpoint Regression Software (Surveillance Research Program , National Cancer Institute (2025). Joinpoint Regression Software, Version 5.4.0 - April 2025. https://surveillance.cancer.gov/joinpoint) to pinpoint and quantify temporal trends in SPPTB, and EPTB incidence rates.

Results: A total of 744,903 TB cases were reported between 1982 and 2024, including 293,117 SPPTB, 380,953 PTB, and 363,950 EPTB. The annual mean ± standard deviation of TB was 17,323.33 ± 3882.77 (range: 11,039–23,570), of SPPTB was 6816.67 ± 1313.07 (range: 3881–8654), of PTB was 8859.37 ± 1526.87 (range: 5103–10,583), and of EPTB was 8463.95 ± 4179.20 (range: 3035–15,408). Between 1982 and 2024, the incidence rate of TB per 100,000 population ranged from 36.7 and 67.6, showing an overall decline of 41%. The incidence of SPPTB ranged from 8.8 and 33.2, with a decline of 71.1%, whereas the incidence of EPTB ranged from 12.1 and 37.3, showing an increase of 62.5%. The EPTB accounted for 25.6% of TB notifications in 1982 and has exceeded 50% from 2009 to reach 70.6% in 2024. The SPPTB remained the dominant form until 2000, after which EPTB assumed predominance from 2001 onward. Over the full course of the study period, the AAPC was statistically significant: -2.9 (95% confidence interval [CI]: −3.8, −2.2, $P < 0.000001$) for SPPTB, and 1.2 (95% CI: 0.4, 1.9, $P < 0.000001$) for EPTB. Both SPPTB and EPTB showed alternating trends with four joinpoints. The incidence of SPPTB showed a steady decrease from 1982 to 1991, with an APC of -5.0, followed by a slight increase from 1991 to 2005, with an APC of 1.6, then a decrease from 2005 to 2016, with an APC of -3.9, and a further sharp decline from 2016 to 2020, with an APC of -13.6, followed by a slight increase from 2020 to 2024, with an APC of 0.6. The incidence of EPTB showed a decrease from 1982 to 1990, with an APC of -5.2, followed by an increase from 1990 to 2001 with an APC of 7.7, then another slight increase from 2001 to 2017, with an APC of 2.1, then a sharp decline from 2017 to 2020 with an APC of -8.7, followed by a slight increase from 2020 to 2024, with an APC of 0.8. The upward trend from 2020 to 2024 can be ascribed to the COVID-19 and post-COVID period.

Conclusions: The 32.4% decline in TB in Algeria between 2015 and 2024 remains insufficient to meet the future requirements of World Health Organization's End TB strategy, which called for a 50% reduction in TB over 2015–2025. The upward trends in EPTB in some provinces highlight the need for enhanced surveillance and targeted interventions.

Keywords: Algeria, epidemiology, extrapulmonary tuberculosis, joinpoint regression analysis, pulmonary tuberculosis

Conflicts of interest

There are no conflicts of interest

Prevalence and Treatment Outcome of Drug-resistant Mycobacterium tuberculosis among People Living with Human Immunodeficiency Virus in Two Hospitals of the Southwest Region - Cameroon

Benjamin D. Thumamo Pokam[1,2*], Forbi Catherine Ngum[3], Yangkam Yhiler Nchawa[3], Guemdjom Wabo Prisca[2,4], Cecile Ingrid Djuikoue[2,5]

Departments of [1]Medical Laboratory Sciences and [4]Public Health, Faculty of Health Sciences, University of Buea, [3]Department of Public Health and Administration, School of Health Sciences, Biaka University Institute of Buea, Buea, [2]Central African Network for the Integrated Control of Antimicrobial Resistance, Bafoussam, Cameroon, [5]Department of Veterinary Management of Animal Resources, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

Background: Tuberculosis (TB) is a leading cause of morbidity and mortality among people living with human immunodeficiency virus (PLWHIV), particularly in sub-Saharan Africa. The emergence of multidrug-resistant/rifampicin-resistant TB (RR-TB) poses a serious public health threat by complicating treatment and increasing the risk of poor outcomes. This study aimed to determine the prevalence, associated factors, and treatment outcomes of RR-TB among PLWHIV in the Southwest Region of Cameroon, a high TB/human immunodeficiency virus burden country, from 2020 to 2024.

Methods: A retrospective hospital-based study was conducted at the Regional Hospital Limbe (RHL) and Kumba Regional Hospital Annex (KRHA). Medical records of 322 PLWHIV diagnosed with TB between 2020 and 2024 were reviewed. Data were analyzed using the Statistical Package for the Social Sciences (SPSS, IBM, Chicago - United States). Descriptive statistics summarized demographic and clinical characteristics, and Chi-square or Fisher's exact tests were used to examine associations, with statistical significance set at $P < 0.05$.

Results: Of the 322 patients' files reviewed, the mean age was 39.7 ± 12.5 years; 172 (53.4%) were female, and 242 (75.2%) resided in urban areas. Pulmonary TB accounted for 252 (78.3%) of cases. Nearly all patients (320 [99.4%]) were on antiretroviral therapy during the course of TB treatment. The prevalence of RR-TB was 4.0%, and was slightly higher at KRHA (5.6% [7/124]) compared to RHL (3.0% [6/198]). Prior TB treatment was significantly associated with drug resistance ($P < 0.001$), while no significant association was found with other demographic or clinical factors. Among the total number of RR-TB patients, 11/13 (84.6%) were cured, and 2/13 (15.4%) died. Treatment success was highest in 2020, with all 6 patients (100%) cured, while the only patient treated in 2024 died.

Conclusion: RR-TB among PLWHIV in the Southwest Region of Cameroon is relatively low, with favorable treatment outcomes. Previous TB treatment was the only factor significantly associated with resistance, highlighting the need for improved surveillance and tailored interventions for such patients.

Keywords: Cameroon, human immunodeficiency virus co-infection, rifampicin resistance, southwest region, treatment outcome, tuberculosis

Conflicts of interest

There are no conflicts of interest

Repurposing TrueNat™ Residual DNA for Low-cost Detection of Zoonotic Tuberculosis

Hindol Maity[1,2*], Supriya Meshram[1], Keshao Hiwale[1], Pratibha Narang[2], Vijayshri Khairkar[2]

[1]Department of Pathology and Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Higher Education and Research, Sawangi, [2]Department of Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, Maharashtra, India

Background: *The Mycobacterium tuberculosis* complex (MTBC) comprises multiple subspecies, including *M. tuberculosis, Mycobacterium bovis, Mycobacterium caprae,* and *Mycobacterium orygis,* which differ in host range, epidemiology, and drug susceptibility. Routine diagnostic tests in India, including the widely deployed TrueNat™ platform, do not differentiate MTBC species, leading to underrecognition of zoonotic tuberculosis (zTB). Since *M. bovis* is intrinsically resistant to pyrazinamide, species-level identification has direct therapeutic implications. TrueNat™ produces ~90 µL of DNA eluate per test but uses only ~12 µL for analysis; the remaining ~78 µL is discarded. Leveraging this unused DNA could facilitate species-specific MTBC identification without additional patient sampling or cost. This study evaluated the operational feasibility of repurposing residual TrueNat™ DNA eluates for subspecies differentiation using a simple real-time polymerase chain reaction (PCR) workflow.

Methods: A cross-sectional laboratory feasibility study was conducted from January 2021 to October 2025 in a rural molecular diagnostics laboratory in Central India. Residual DNA eluates (~78 µL) were collected from 1115 clinical samples (sputum, pus aspirates, cerebrospinal fluid, and tissue biopsies) that were MTBC-positive by TrueNat™ with high bacillary load (≥10,000 or 10^4 CFU/ml). A two-step real-time PCR protocol, adapted from validated assays, was used. Step 1 confirmed MTBC DNA via IS1081 gene and primer–probe sets for human-adapted (MTCHum) and animal-adapted (MTCAni) strains of MTBC. Step 2 employed species-specific targets for *M. bovis*, *M. caprae*, and *M. orygis*, to be used only if MTCAni turned positive.[1] PCR was performed on an Applied Biosystems QuantStudio 5 system. Each run included species-specific positive controls and multiple negative controls. A random 10% subset of samples was retested to evaluate reproducibility. Any *M. bovis*-positive result underwent confirmatory region-of-difference (RD) PCR, culture on Löwenstein–Jensen and Mycobacterium Growth Indicator Tube (BD) media, and MPT64 antigen testing to differentiate wild-type *M. bovis* from Bacillus Calmette–Guérin (BCG) strains.

Results: All 1115 eluates yielded valid amplification results. Of these, 1114 (99.91%) were identified as *M. tuberculosis* sensu stricto, while one sample (0.09%) tested positive for *M. bovis*. This isolate originated from a 2-year-old child with cervical lymphadenitis. RD-PCR and culture confirmed the organism as *M. bovis* BCG (MPT64 negative). No samples were positive for *M. caprae* or *M. orygis*. All negative controls remained negative, and positive controls performed as expected. Repeat testing of 10% of samples showed 100% concordance. The workflow required no change to TrueNat™ testing procedures and integrated seamlessly into routine laboratory operations.

Conclusions: Repurposing residual DNA eluates from TrueNat™ is a feasible, low-cost strategy for MTBC subspecies differentiation. This approach can be implemented without additional sample collection, infrastructure, or disruption to routine workflows, making it suitable for high-burden, resource-limited settings. Detection of a pediatric *M. bovis* BCG case underscores the clinical and surveillance importance of species-level identification, particularly for zTB. Wider adoption of this workflow could enable national programs across Asia and Africa to incorporate real-time zTB surveillance into existing diagnostic platforms, aligning with One Health goals.

Keywords: *Mycobacterium bovis*, *Mycobacterium tuberculosis* complex, One Health, real-time polymerase chain reaction, residual deoxyribonucleic acid, TrueNat™, zoonotic tuberculosis

Reference

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Conflicts of interest

There are no conflicts of interest

Express High-performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry Analysis for the Determination of Linezolid and Bedaquiline Concentration in the Blood Plasma of Tuberculosis Patients for Therapeutic Drug Monitoring

Pavel Vadimovich Sobolev, Alena Igorevna Merenkova, Anastasia Alexandrovna Kazyulina

National Medical Research Center of Phthisiopulmonology and Infectious Diseases, Ministry of Health, Moscow, Russia

Background: Tuberculosis is a widespread infectious disease of humans and animals, caused by various species of mycobacteria from the Mycobacterium tuberculosis complex. This infection requires antituberculosis therapy, which often needs adjustment. Therapeutic drug monitoring based on high-performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) allows for establishing the correspondence of drug concentrations in a patient's blood to the therapeutic range and for developing recommendations for dosage regimen correction. We have developed an express method for the simultaneous determination of linezolid and bedaquiline concentrations in human blood plasma using HPLC-MS/MS.

Methods: Plasma obtained from the blood of patients at the NMRC Phthisiopulmonology and Infectious Diseases was treated with acetonitrile followed by centrifugation for 15 min at 13,500 rpm. Analysis was performed on an HPLC-MS/MS HELICON 5210 system. Calibration graphs for active pharmaceutical substances of linezolid and bedaquiline were plotted to determine concentrations. The method was tested on 36 plasma samples (from 2 patients). For the plotting of the calibration graphs, solutions of pure pharmacopoeial substances with known concentrations of linezolid (CAS# 165800-03-3, Nanjing Pars Biochem CO., China) and bedaquiline (CAS# 845533-86-0, Nanjing Pars Biochem CO., China) were used.

Results: The obtained calibration curves had high linearity R2 values (0.9967 for linezolid and 0.9943 for bedaquiline). The concentrations of the drugs in 36 plasma samples in triplicate from 2 patients with infiltrative tuberculosis were calculated using the calibration graphs. One hour after plasmapheresis, the average concentration of linezolid in patients was 0.96 μg/mL, and the average concentration of bedaquiline was 0.067 μg/mL. After 2 h: 0.69 μg/mL (linezolid) and 0.066 μg/mL (bedaquiline). After 3 h: 0.43 μg/mL for linezolid, 0.063 μg/mL for bedaquiline. After 4 h: 0.19 μg/mL for linezolid, 0.064 μg/mL for bedaquiline. After 5 h: 0.39 μg/mL (linezolid) and 0.104 μg/mL (bedaquiline), which correlates with the pharmacokinetic data of the drugs. Thus, no adjustment of the bedaquiline and linezolid dosage regimen was required for these patients. The chromatographic analysis time for linezolid was 4.2 min, for bedaquiline, 5 min. The total time spent on the analysis, including sample preparation, was 45 min.

Conclusions: The study demonstrates the successful application of the developed express analysis protocol based on HPLC-MS/MS for the simultaneous determination of bedaquiline and linezolid in the blood plasma of tuberculosis patients. The use of this highly accurate and selective analytical method can help to correctly adjust drug dosages, which could potentially increase the number of successful treatment outcomes for patients with tuberculosis.

Keywords: Bedaquiline, high-performance liquid chromatography-mass spectrometry/mass spectrometry, infiltrative tuberculosis, linezolid, therapeutic drug monitoring

Conflicts of interest

There are no conflicts of interest

The Exploration of Phytomedical Compounds as a Novel Approach for Therapeutics in Tuberculosis

Nerges Mistry, Ambreen Shaikh, Tania Shroff, Deepti Pai

Department of Tuberculosis, The Foundation for Medical Research, Dr. Kantilal J. Sheth Memorial Building, Mumbai, Maharashtra, India

Background: Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (*Mtb*) remains a major global health challenge. With the rising emergence of drug-resistant strains in addition to the long duration and frequent side effects associated with existing treatment regimens, the need for innovative strategies to combat the disease has never been greater. Besides gaining recognition for extensive medicinal properties, plant-derived bioactive compounds have long been studied for their antimycobacterial activity. Literature associates phytocompounds with the advantages of flexibility and a multi-target approach, leaving little likelihood of resistance development. Notable medicinal-plant-based studies have been conducted at The Foundation for Medical Research (FMR), with an aim of identifying the most effective natural anti-TB agents. Six plants, namely *Acorus calamus* (rhizome), *Alpinia galanga* (tubers), *Andrographis paniculata* (leaves), *Ocimum sanctum* (leaves), *Piper nigrum* (seeds), and *Pueraria tuberosa* (tubers), were tested for their anti-TB activity using axenic cultures of *Mtb* reference strain (*Mtb* H37Rv), and clinical strains (drug-sensitive and drug-resistant), followed by intracellular assay systems. Among the six plants, *A. galanga, A. paniculata,* and *P. nigrum* were found to be most promising as they exhibited anti-TB activity against all strains including drug sensitive and resistant clinical strains, through multiple modes of action. The *Mtb* genome houses several noncanonical deoxyribonucleic acid (DNA) structures that are crucial in regulating replication and transcription processes. Recent studies have implicated the formation of these structures in regulating the expression of key bacterial genes responsible for survival, virulence, and drug-resistance. Researchers have been successful in targeting these structures using synthetic and natural ligands. Plant-derived flavonoids, alkaloids, and polyphenols are reported to show binding interactions with noncanonical DNA that can be exploited for developing therapeutic strategies. The binding and stabilization of noncanonical DNA structures formed in key *Mtb* genes, by quercetin and kaempferol has been successfully validated in the preliminary studies conducted at FMR.

Methods: In the future, assessing the role of phytomedical compounds in binding and targeting noncanonical DNA of *Mtb* through *in silico*, biophysical, and *in vitro* studies could provide insights into their potential in inhibiting *Mtb* growth, reducing cytotoxicity to the host, and modulating drug resistance. This can generate comprehensive evidence to support them as prospective drug candidates for therapeutic interventions.

Results: The expected outcomes would include the identification of phytocompounds as novel drug candidates or adjuncts to conventional therapy, capable of combating drug-resistant TB and contributing to the development of effective, phytomedicine-based therapies in the future.

Conclusion: Phytomedicine may hold promise for exerting a synergistic pharmacological effect through multiple modes of action including targeting of noncanonical DNA. This avenue warrants deep investigation, both with crude extracts and single/multi- phytocompounds.

Keywords: Noncanonical deoxyribonucleic acid, phytomedical compounds, tuberculosis

Conflicts of interest

There are no conflicts of interest

Clonal Clusters and Adaptive Genomic Features of Extrapulmonary *Mycobacterium monacense*

Christoffel Opperman[1,2,3], Aysha Ahmed[1], Marianna De Kock[2], Claudia Spies[2], Michael Biggel[4], Tristen Lourens[5], Sarishna Singh[1,2], Yonas Ghebrekristos[1,2], Robin Warren[2], Wynand Goosen[2,5], Giovanni Ghielmetti[2,6]

[1]National Health Laboratory Service, Green Point TB-Laboratory, [2]Division of Molecular Biology and Human Genetics, SAMRC Centre for Tuberculosis Research, Faculty of Health Sciences, Stellenbosch University, [3]Department of Pathology, Division of Medical Microbiology, Faculty of Health Sciences, University of Cape Town, Cape Town, [5]Department of Microbiology and Biochemistry, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa, [4]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, [6]Section of Veterinary Bacteriology, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Background: *Mycobacterium monacense* is a rapidly growing nontuberculous mycobacterium (NTM) increasingly detected in clinical specimens; however, its genomic diversity, epidemiology, and antimicrobial susceptibility remain poorly characterized. This study aimed to characterize extrapulmonary *M. monacense* isolates from the Western Cape province of South Africa, evaluating their genetic relatedness, virulence potential, and antimicrobial resistance (AMR) profiles.

Methods: Whole-genome sequencing (WGS) was performed on eight extrapulmonary *M. monacense* isolates using the Oxford Nanopore Technologies PromethION and MinION platforms (Oxford, UK). Base-calling was done with Dorado, quality-checked using FastQC and pycoQC, and taxonomically screened with Kraken2. High-fidelity *de novo* assemblies were generated with Flye, polished using Medaka, and compared against type strain genomes using the Type Strain Genome Server, incorporating MASH and Genome BLAST Distance Phylogeny analyses. Core genome and whole-genome multilocus sequence typing (MLST) profiles were constructed with pyMLST, and variants were identified through alignment using Minimap2 and variant calling with BCFtools. Plasmid content was assessed using MOB-Suite, and genome annotation was performed with Bakta. AMR determinants were screened with AMRFinderPlus, ResFinder, and the Comprehensive Antibiotic Resistance Database. Virulence-associated loci were identified via the Virulence Factor Database. Patient residential locations were mapped in ArcGIS Pro (ESRI, USA) to assess potential geographic clustering. Phenotypic antimicrobial susceptibility testing was conducted using the Sensititre RAPMYCO microdilution plate (Thermo Fisher Scientific, USA) and proportional method with critical concentrations. Molecular detection of macrolide- and aminoglycoside-associated resistance markers was performed using the GenoType NTM-DR line-probe assay (Hain Lifescience, Germany).

Results: Eight viable extrapulmonary isolates collected between 2019 and 2023 were confirmed and analyzed. Two distinct clonal clusters were identified, separated by more than 8000 single-nucleotide polymorphisms and geographically associated with the Central Karoo district (Cluster 1) and Cape Metro region (Cluster 2), approximately 300 km apart. Genomic analysis revealed cluster-specific features: cluster 1 lacked plasmids but uniquely harbored the chromosomal siderophore-associated *mbtH* gene, whereas Cluster 2 contained plasmids encoding ferredoxin and cytochrome P450 genes involved in redox processes. The microdilution plates demonstrated susceptibility to aminoglycosides, macrolides, and fluoroquinolones with consistently elevated minimum inhibitory concentrations for β-lactam antibiotics. No inducible macrolide resistance was observed, and molecular assays detected no resistance-associated mutations in *rrs*, *rrl*, or *erm* genes. The proportional phenotypic method suggested reduced susceptibility to isoniazid, whereas rifampicin, bedaquiline, and linezolid remained active. All patients were alive at the 1-year follow-up, with more than half being HIV-immunocompromised.

Conclusions: This study provides the first integrated genomic and clinical characterization of extrapulmonary *M. monacense* infections in South Africa. The presence of two clonal clusters with distinct genomic adaptations, plasmid-mediated redox functions in Cluster 2 and chromosomal iron-acquisition pathways in Cluster 1, suggests divergent ecological strategies. Variable concordance between genomic resistance markers and phenotypic susceptibility highlights the need for expanded NTM specific databases and continued integration of WGS with conventional laboratory testing. Susceptibility to several key antimicrobials supports potential therapeutic relevance, including in the context of coinfection with *Mycobacterium tuberculosis* complex. Expanded environmental sampling and ongoing genomic surveillance are warranted to elucidate transmission pathways and guide clinical management.

Keywords: Antimicrobial susceptibility, clonal, *Mycobacterium monacense*, nontuberculous mycobacteria, phylogenomics, plasmidome, South Africa, whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Management of Tubercular Empyema in a Tertiary Care Tuberculosis Facility: A 25-year Experience

Ravindra Kumar Dewan

National Institute of TB and Respiratory Diseases, New Delhi, India

Background: Tubercular empyema continues to remain a major clinical challenge in high-burden countries. Unlike parapneumonic empyema, tuberculosis (TB) empyema is characterised by chronicity, thick pleural peel, multiloculation, lung entrapment, and frequent association with bronchopleural fistulae. Surgical management plays an indispensable role in halting chronicity, preventing morbidity, and restoring lung function. This study presents a 25-year surgical experience in managing tubercular empyema at a tertiary care TB center with one of the world's largest dedicated empyema units.

Methods: A retrospective analysis was conducted of all patients with tubercular empyema managed between March 2000 and December 2025. Surgical interventions were performed according to a standardized institutional protocol aimed at (1) controlling primary TB infection, (2) evacuating purulent material, (3) eradicating the empyema cavity, and (4) achieving maximal lung re-expansion. The procedures included percutaneous needle aspiration, image-guided tube thoracostomy (±rib resection), decortication (video-assisted thoracoscopic surgery [VATS]/open), open window thoracostomy (pleurocutaneous window), and space-reducing thoracoplasty.

Results: A total of 8216 procedures were performed over 25 years. These included:

- Tube thoracostomy: 2605 cases – Performed as first-line intervention in early and minimally septated empyema. Routine drain flushing and computed tomography-based reassessment ensured adequacy of drainage
- Open window thoracostomy (Pleurocutaneous window): 3915 cases – The most common procedure in chronic TB empyema, providing effective, ambulatory drainage with excellent symptom relief and promoting gradual lung expansion
- Decortication: 1240 cases – Utilized for chronic organized empyema with significant visceral peel. VATS was increasingly adopted for Stage II and selected Stage III empyema, reducing postoperative morbidity. Conversion to thoracotomy was reserved for uncontrollable bleeding, poor visualization, or inability to achieve therapeutic goals
- Space-reducing thoracoplasty: 456 cases – Performed for persistent pleural spaces, multiloculated disease, or lung nonexpansion despite adequate drainage. Cosmetic concerns were minimal, and functional outcomes were gratifying.

Radiological follow-up at 3, 6, and 12 months demonstrated:

- Complete lung expansion: Majority of early-stage and well-drained chronic cases
- Partial expansion: Observed in long-standing TB empyema but associated with clinical improvement
- No expansion: In patients with destroyed lung, multiple bronchopleural fistulae, or advanced parenchymal disease, wherein thoracoplasty or permanent window proved beneficial.

Discussion: Tubercular empyema requires a flexible, individualized, and staged surgical strategy. Aggressive early drainage avoids chronicity, whereas decortication and VATS significantly improve outcomes in organized disease. Open window thoracostomy remains a highly effective procedure in chronic and debilitated patients – a finding that contrasts with many global practices but is validated by this large-volume TB-specific dataset. Thoracoplasty continues to have an essential role in selected cases with persistent space or nonexpandable lung. The "Empyema Diamond" framework – considering etiology, patient status, and disease stage – guided decision-making effectively.

Conclusion: This 25-year experience demonstrates that optimized, stage-appropriate surgical intervention forms the cornerstone of tubercular empyema management. No single procedure guarantees universal success; instead, outcomes depend on individualized planning, institutional expertise, and multidisciplinary coordination. Our data strongly support a flexible protocol-based surgical approach tailored to disease chronicity and patient physiology, ensuring consistent, reproducible, and function-restoring results in tubercular empyema.

Keywords: Decortication, empyema, surgery, thoracoplasty, tube thoracostomy, tubercular, window thoracostomy

Conflicts of interest

There are no conflicts of interest

Development and Approval of a Real Time Polymerase Chain Reaction Test for Detecting Fast and Slow Metabolizers of Isoniazid by rs1495741 of the NAT2 *Gene*

Oleg B. Ogarokov[1], I. V. Malov[2], E. Yu. Zorkaltseva[1], S. N. Zhdanova[1], N. P. Pertolchina[2], I. G. Kondratov[1], S. I. Malov[2], S. V. Kulinich[1], V. V. Sinkov[1]

[1]Institute of Epidemiology and Microbiology, "Scientific Centre for Family Health and Human Reproduction Problems", [2]Federal State Budgetary Educational Institution of Higher Education "Irkutsk State Medical University" of the Ministry of Health of the Russian Federation, Irkutsk, Russia

Introduction: Standard tuberculosis treatment includes the use of isoniazid; however, its application may be complicated by the drug's hepatotoxicity. The polymorphism of the *NAT2* gene affects isoniazid metabolism, and genotyping of this gene helps predict acetylation speed and the risk of toxic effects.

Materials and Methods: A laboratory sample of a reverse transcription polymerase chain reaction (RT-PCR) test based on the tag SNP rs1495741 has been developed, allowing for a high-accuracy prediction of the NAT2 phenotype and risks associated with isoniazid treatment. As positive controls, fragments of the *NAT2* gene "rs1495741G" and "rs1495741A" were cloned into the pJET1.2/blunt plasmid. Testing was performed on DNA from buccal swabs of 150 children receiving isoniazid during treatment or prophylaxis.

Results: In carriers of the rs1495741A allele, regardless of the presence or absence of the second rs1495741G allele, adverse reactions occurred significantly more often ($\chi^2 = 2.75$, $P < 0.05$), including toxic hepatitis, accompanied by high ALT and AST levels.

Conclusion: The testing of the laboratory sample of the RT-PCR test on the rs1495741 NAT2 gene for identifying slow or fast isoniazid metabolizers indicates the need for expanded clinical trials of the test for more comprehensive coverage of clinically significant target patient groups.

Keywords: *NAT2* gene, pharmacogenetics, phthisiopediatrics, reverse transcription polymerase chain reaction test, rs1495741, tuberculosis

Conflicts of interest

There are no conflicts of interest

Molecular Identity of Mycobacteria Isolated from Zoos in Alborz Province, Iran

Nader Mosavari[1], Zohre Ahmadi[2], Alireza Pardis[1]

[1]Bovine Reference Laboratory, Razi Vaccine and Serum Research Institute, AREEO, [2]Department of Biology, Faculty of Basic Sciences, Karaj Branch, Islamic Azad University, Karaj, Iran

Mycobacteria represent a diverse genus that includes both environmental and pathogenic species affecting humans, animals, and birds. This study aimed to isolate and molecularly identify mycobacteria from wildlife in zoos located in Alborz province, Iran.

Fecal samples were collected from 40 animals, including camelids, bears, primates, birds, big cats, zebras, deer, horses, and other taxa. The samples were decontaminated with NaOH and cultured on Lowenstein–Jensen medium. The mycobacterial genus was identified using 16S rRNA polymerase chain reaction (PCR). To distinguish members of the *Mycobacterium tuberculosis* complex from nontuberculous mycobacteria (NTM), IS6110 PCR was utilized. Further species-level confirmation was attempted through PCR-RFLP of the oxy-R pseudogene.

Direct smears after decontamination did not reveal any acid-fast bacilli; however, 29 out of 40 cultures produced colonies, of which 11 contained acid-fast bacilli. All 11 isolates were confirmed to be of the *Mycobacterium* genus via 16S rRNA testing. Of these, two isolates were positive for IS6110, indicating that they belonged to the *M. tuberculosis* complex, specifically identified as *Mycobacterium bovis*. The remaining nine isolates were classified as NTMs. Oxy-R confirmation was conducted only on the camel and sheep isolates, while other samples tested negative.

Among the 40 samples studied, 11 yielded *Mycobacterium* that tested positive for 16S rRNA, with 2 isolates identified as *M. bovis* within the *M. tuberculosis* complex and 9 as NTMs. Oxy-R confirmation was limited to the camel and sheep isolates. These findings underscore the presence of environmental NTMs in zoo settings and indicate a limited occurrence of *M. bovis*, highlighting the importance of ongoing surveillance for animal health and environmental exposure in zoos.

Keywords: 16S rRNA, IS6110, isolation, molecular identification, mycobacteria, oxy-R, polymerase chain reaction, RFLP

Conflicts of interest

There are no conflicts of interest

Diagnostic Challenge: Bilateral Testicular Abscesses with Renal Calculi Revealing Genitourinary Tuberculosis

Farha Siddiqui[1], Anindita Datta[2], Varshith Veeramachaneni[1], Shashank Purwar[1], Kumar Madhavan[3], Anand Kumar Maurya[1]

Departments of [1]Microbiology and [3]Urology, All India Institute of Medical Science, Bhopal, Madhya Pradesh, [2]Department of Microbiology, All India Institute of Medical Science, Guwahati, Assam, India

Background: Genitourinary tuberculosis (GUTB) is the second most prevalent kind of extrapulmonary tuberculosis (TB). The infection can spread to the ureters, bladder, prostate, epididymis, and, less commonly, the testes; however, the kidneys are the primary organs affected. It constitutes about 15%–20% of cases in endemic areas, especially in South Asia and sub-Saharan Africa. Testicular TB is uncommon and frequently poses a diagnostic dilemma because it might resemble pyogenic abscesses or carcinoma, particularly when bilateral involvement is seen. This case report highlights the importance of distinguishing TB in GUTB accounts from other individuals with conditions that exhibit unusual clinical, scrotal, radiological, and renal microbiological abnormalities. Antitubercular treatment (ATT) must be initiated as soon as possible to avoid complications such as organ damage, chronic infection, and infertility.

Case History: We documented a case of a 42-year-old man with bilateral testicular abscesses, when combined with renal calculi and GUTB, who presented with intermittent fever (38.5°C), edema, and persistent bilateral testicular pain. His symptoms persisted after receiving empirical antibiotic treatment for suspected epididymitis, which led to a referral for additional testing. Laboratory tests showed increased C-reactive protein (48 mg/L) and leukocytosis (white blood cell count: 16,000/mm^3). A urinary tract infection was suggested by the severe pyuria found in the urinalysis. Microbiological confirmation was obtained via Fluorescent Microscopy and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT-Cepheid GeneXpert®MTB/RIF) on semen samples, which detected *Mycobacterium tuberculosis* without Rifampicin resistance. Fine-needle aspiration cytology, which provided histological support, further confirmed the diagnosis of bacterial infection. On radiology, the Ultrasonography scrotal showed both testes to be heterogeneous and hypoechoic. Magnetic resonance imaging and contrast-enhanced computed tomography of the abdomen and pelvis showed bilateral multiloculated testicular abscesses with extension into the epididymides. Intravenous antibiotics and empirical ATT were started. Following 6 months of ATT treatment, he experienced ATT-induced hepatotoxicity and lichenoid eruptions.

Results: The patient was later readmitted with fever, burning micturition, and right flank pain. Imaging showed mild hydronephrosis, reduced right proper renal function, and a persistent right mid-ureteric calculus. After performing a right ureteroscopic lithotripsy once more and obtaining total stone clearance, the DJ stent was positioned. The diagnosis of tuberculous epididymo-orchitis complicated by secondary bacterial superinfection was made based on the patient's clinical presentation, imaging data, and histological findings. After showing signs of improvement, the patient was released in stable condition, and the DJ stent was finally removed. The patient had resolved abscesses, retained left renal function, and was still asymptomatic at the 1-year follow-up.

Conclusion: This case highlights the importance of maintaining a high index of suspicion for GUTB in patients with ongoing testicular symptoms, particularly in endemic regions. For optimal patient outcomes, a multidisciplinary approach that combines medical treatment, surgery, and close observation is necessary.

Keywords: Bilateral testicular abscesses, genitourinary tuberculosis, renal calculi

Conflicts of interest

There are no conflicts of interest

Genetic-code Features Underlying Variability and High Adaptive Potential of Mycobacterium tuberculosis

Veranika Slizen

Department of Tuberculosis Diagnosis and Treatment, Republican Scientific and Practical Center for Pulmonology and Phthisiatry, Minsk, Belarus

Background: To sustain high adaptive fitness, *Mycobacterium tuberculosis* (Mtb) may maintain genomic mechanisms with promutagenic potential that enable mutational variation at loci crucial for survival under heterogeneous selective pressures. Despite the absence of horizontal gene transfer, Mtb demonstrates remarkable genetic variability, particularly within the PE_PGRS gene family. This variability may be driven by specific features of the Mtb genetic code that influence the analogue layer of deoxyribonucleic acid (DNA) information and predetermine physicochemical interactions within DNA. This study aims to identify the features of the Mtb genetic code that may underlie the high variability of PE_PGRS genes and contribute to the high adaptive potential of Mtb.

Methods: We quantified tetranucleotide abundances (CGGC, GCGC, CTGC, GGG, and GGGG) and out-of-frame stop codons (TGA, TAG, and TAA) across Mtb coding genes and compared them with genomes from diverse bacterial taxa (88 genomes, 74 species). Analyses were performed using a Java-based tool (https://github.com/ashakirin/microbilogy-2). Secondary DNA structures were predicted in silico using the Quikfold web server (m-fold).

Results: PE_PGRS genes exhibited a significantly higher proportion of CGGC tetramers (1.70%–7.14%) compared with the Mtb genomic average (1.62%; $P < 1 \times 10{-}27$). In contrast, several Mtb genes were completely devoid of CGGC motifs. PE_PGRS genes were also markedly depleted in out-of-frame stop codons, which are associated with robustness against 1-nt and 2-nt frameshifts. Mtb harbors a distinct pool of genes with a high density of sterically active CGGC motifs (>2.5 per 100 bp; $n = 223$). The size of this pool varied substantially among species, including *Mycobacterium leprae* (10), *Mycobacterium avium* (1021), *Mycobacterium kansasii* (601), *Mycobacterium fortuitum* (257), *Pseudomonas aeruginosa* (659), *Pseudomonas fluorescens* (31), *Yersinia pestis* (659), *Yersinia enterocolitica* (7), and *Bordetella pertussis* (744). Ranking mycobacterial species by CGGC abundance corresponded to their epidemiological success (*M. avium*, *Mycobacterium intracellulare* > Mtb > *M. leprae*). In silico analyses showed that mutation-prone regions, including loci associated with antituberculosis drug resistance, frequently form short DNA hairpins (7–13 nt) with stems with CGGC or related tetramers. Such structures may alter local electrostatic and hydrophobic interactions, promoting nucleotide misincorporation and replication-associated mutagenesis. Elevated CGGC content in genes, including PE_PGRS, may promote mutational events through the formation of distinct DNA conformations and the redistribution of physicochemical interaction forces during replication.

Conclusions: Mtb exhibits genetic-code features that are also characteristic of other highly successful human pathogens, such as *Y. pestis*, *P. aeruginosa*, and *B. pertussis*, namely the presence of gene subsets enriched in CGGC motifs and depleted in out-of-frame stop codons. CGGC-driven secondary DNA structures may locally modulate physicochemical interactions within DNA, increasing mutational susceptibility. These findings indicate that the Mtb genetic code exhibits promutagenic properties, providing them exceptional adaptive potential.

Keywords: Constrained spontaneity of mutations, *Mycobacterium tuberculosis* evolution, promutagenic architecture of PE_PGRS genes

Conflicts of interest

There are no conflicts of interest

Emerging Burden of Nontuberculous Mycobacteria: Clinical Presentation and Species Distribution

Bhavya Sharda[1], Anand Kumar Maurya[1], Falguni Agrawal[1], Alkesh Khurana[2], Sagar Khadanga[3], Debasis Biswas[1], Shashank Purwar[1]

Departments of [1]Microbiology, [2]Pulmonary Medicine and [3]General Medicine, All India Institute of Medical Sciences, Bhopal, Madhya Pradesh, India

Background: Nontuberculous mycobacteria (NTM) refer to mycobacterial strains distinct from *M. tuberculosis* and *M. leprae*. NTM are called by several names, including environmental mycobacteria, atypical mycobacteria, or anonymous mycobacteria, mycobacteria other than *Mycobacterium tuberculosis* (Mtb) (MOTT). NTM disease is a growing concern in public health. Highlighting species-level identification using advanced molecular techniques, such as LPA, is crucial for enhancing diagnostic accuracy and improving patient outcomes, underscoring its clinical relevance. Through this study, we aimed to evaluate the clinical spectrum and species distribution of NTM infections in a tertiary care setting and to assess the diagnostic utility of Line Probe Assay (LPA) for rapid and accurate differentiation of NTM from the Mtb complex.

Methods: We conducted a hospital-based observational study from October 2024 to September 2025 in a tertiary care centre to evaluate the epidemiology, clinical features, and diagnostic utility of NTM using the LPA. The data collected included demographics, clinical presentation, infection site, comorbidities, and microbiological results from microscopy, culture, the MPT64 test, and LPA. Molecular identification via LPA was used to rapidly differentiate NTM species from the Mtb complex, highlighting its importance in improving diagnostic accuracy and treatment decisions.

Results: The Line Probe Assay identified a total of 18 distinct Mycobacterium species, including mixed infections and pure isolates, demonstrating substantial species diversity within the study population. Pulmonary infection was the most common clinical presentation, characterized by chronic cough, sputum production, and radiological abnormalities. Extrapulmonary cases included soft-tissue infections, immunosuppression, and postsurgical site infections. A significant proportion of patients had pre-existing lung conditions or a history of tuberculosis. Compared to traditional biochemical techniques, the use of LPA significantly reduces diagnostic turnaround time and improves accuracy by enabling fast and accurate species identification directly from culture isolates. LPA made it easier to distinguish between NTM infections and tuberculosis early on, thereby avoiding unnecessary treatment.

Conclusion: The results underscore the importance of routine molecular diagnostics, such as LPA, for rapid and accurate identification of NTM species. Incorporating such techniques into diagnostic algorithms can empower clinicians to improve patient outcomes and strengthen infection surveillance efforts.

Keywords: Clinical manifestations, epidemiology, line probe assay, nontuberculous mycobacteria

Conflicts of interest

There are no conflicts of interest

Diagnostic Utility of GeneXpert *Mycobacterium tuberculosis*/RIF in Genital Tuberculosis: A Systematic Review of Male and Female Genital Tract Disease

Farha Siddiqui[1], Anindita Datta[2], Anuja Singh[1], Varshith Veeramachaneni[1], Anand Kumar Maurya[1]

[1]Department of Microbiology, All India Institute of Medical Science, Bhopal, Madhya Pradesh, [2]Department of Microbiology, All India Institute of Medical Science, Guwahati, Assam, India

Background: In high-burden settings, genital tuberculosis (GTB), which affects both the male and female genital tracts, is a significant but not widely recognized cause of infertility and chronic pelvic or scrotal illness. Because GTB is usually paucibacillary and mimics other inflammatory or neoplastic diseases, diagnosis is challenging, and conventional methods such as smear microscopy and culture are time-consuming and insensitive. Extrapulmonary and GTB are now frequently diagnosed using cartridge-based nucleic acid amplification tests (CBNAAT-Cepheid GeneXpert®MTB/RIF), which offers quick and automated detection of *Mycobacterium tuberculosis* (MTB) and rifampicin resistance.

Materials and Methods: A systematic review approach was conceptualized, including electronic searches of PubMed, Scopus, and Google Scholar for studies assessing CBNAAT (CBNAAT-Cepheid GeneXpert®MTB/RIF) in genital or urogenital tuberculosis (TB) among males and females. Eligible studies used samples from the genital tract, semen, prostate, epididymis, endometrium, pelvis, or urine. They compared GeneXpert with composite reference standards that incorporated histopathology, culture, imaging, laparoscopy, and clinical criteria. Extracted outcomes included sensitivity, specificity, and the detection of rifampicin resistance. The results were synthesized narratively, with attention to sex-specific patterns and to GeneXpert's position in diagnostic algorithms.

Results: Studies on premenstrual endometrial biopsies and other pelvic specimens in females demonstrate that GeneXpert's sensitivity for genital TB is usually between 7% and 50%, and its specificity is consistently near 100% against composite standards, indicating an insignificant bacillary load. Although GeneXpert provides quick confirmation and information on rifampicin resistance in a subset of instances, it often performs less accurately than histology or more comprehensive TB-polymerase chain reaction. Male genital involvement typically affects the epididymis, prostate, and seminal tract; imaging, culture, histology, and nucleic acid testing on semen, prostatic secretions, abscess material, or urine are used to make the diagnosis. Although there is not much data specifically about GeneXpert in isolated male GTB, studies involving urogenital TB and urine-based Xpert indicate moderate sensitivity with excellent specificity, suggesting utility as a quick supplement but not as a stand-alone exclusion test. When bacillary load is adequate, GeneXpert on semen or lesion-derived samples can confirm male GTB and identify medication resistance, as demonstrated by case reports and short series.

Conclusion: In line with other extrapulmonary sites, GeneXpert MTB/Rifampicin (RIF) exhibits high specificity but inconsistent and frequently low sensitivity for both male and female genital TB. It is primarily used as a quick rule-in test to supplement, rather than replace, a thorough diagnostic investigation that includes imaging, laparoscopy, surgical exploration, histopathology, and culture. Therefore, GTB in both sexes can be described as a disease where multimodal assessment remains crucial for case discovery and management; however, GeneXpert provides significant value for the rapid confirmation and detection of rifampicin resistance.

Keywords: Cartridge-based nucleic acid amplification test, extrapulmonary tuberculosis, GeneXpert *Mycobacterium tuberculosis*/RIF, genital tuberculosis

Conflicts of interest

There are no conflicts of interest

The Frequency of the rs4588AA Genotype of the *GC* gene (Vitamin D-binding Protein) is Elevated among Tuberculosis Patients in the Irkutsk Region (Russia)

Elizaveta Orlova, Sophia Kulinich, Oleg Ogarkov

Institute of Epidemiology and Microbiology, Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia

Background: Susceptibility to tuberculosis (TB) depends on multiple factors, including host genetic predisposition. Numerous single-nucleotide polymorphisms (SNPs) have been implicated in TB susceptibility or resistance, among them variants of the *GC* gene encoding Vitamin D-binding protein (VDBP), the primary carrier of Vitamin D metabolites. Given the established role of Vitamin D in antimycobacterial immunity, variation in VDBP may influence immune pathways involved in TB pathogenesis. Associations between SNPs and TB vary across ethnic and geographic groups. The rs4588C allele of the *GC* gene is the most common worldwide, with frequencies from 71% in South Asians to 92.9% in African populations. The rs4588A allele is minor, and the frequency of rs4588AA homozygotes among Europeans and Asians generally does not exceed 9.2%, underscoring the need for population-specific studies to identify genetic markers of TB susceptibility. This study aimed to assess the distribution of rs4588 polymorphisms in TB patients and healthy individuals from the Irkutsk region (Russia).

Methods: The study was approved by the Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems. Real-time polymerase chain reaction (PCR) genotyping of the rs4588 SNP was performed in 430 residents of the Irkutsk region, predominantly of Slavic ethnicity. The cohort included 303 healthy donors and 127 TB patients; 32.3% and 57.5% of them were men, respectively, with median ages of 50 (33; 63) and 40 (34; 50) years. Samples were collected between 2020 and 2025 during routine public health screening for socially significant infections. PCR was carried out on a CFX96™ Real-Time System C1000 Touch Thermal Cycler (Bio-Rad) system using custom-designed primers and TaqMan probes specific to the rs4588 C/A alleles.

Results: In healthy individuals, the genotype frequencies were CC – 51.2%, CA – 43.6%, and AA – 5.3%; allele frequencies were C – 72.9% and A – 27.1%. Among TB patients, genotype frequencies were CC – 50.4%, CA – 33.1%, and AA – 16.5%; allele frequencies were C – 66.9% and A – 33.1%. The rs4588AA genotype was significantly more frequent in TB patients ($\chi^2 = 15.6$; $P = 0.0004$). Genotype distribution in healthy individuals conformed to Hardy–Weinberg equilibrium ($\chi^2 = 3.2$; $P = 0.1$), whereas TB patients showed deviation ($\chi^2 = 8.1$; $P = 0.004$), possibly reflecting disease-associated selection or sampling structure.

In the recessive inheritance model (AA vs. CC + CA), the rs4588AA genotype was strongly associated with increased TB risk (odds ratio [OR] = 3.6, 95% confidence interval [CI] [1.8, 7.1]; $z = 3.6$; $P = 0.0003$). No association was observed in the dominant model (CA + AA vs. CC; OR = 1.03, 95% CI [0.7, 1.6]; $z = 0.14$; $P = 0.9$).

Conclusions: The rs4588AA genotype of the *GC* gene is associated with a significantly increased risk of TB in the Irkutsk region, contrasting with findings reported for South Indian populations. Further studies with larger cohorts and broader geographic coverage are warranted to validate these observations and clarify the role of VDBP genetic variation in TB immunopathogenesis.

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Keywords: rs4588, single nucleotide polymorphism, tuberculosis, Vitamin D-binding protein

Conflicts of interest

There are no conflicts of interest

Species Identification of Actinobacteria Is a Key Etiological Factor in the Differential Diagnosis of Respiratory Diseases

Platon Eliseev, Natalya Gorshkova, Ekaterina Gosteva, Evgeny Kuznetsov, Anastasia Samoilova

Microbiological Laboratory, The National Medical Research Center of Phthisiopulmonology and Infectious Diseases under the Ministry of Health of the Russian Federation, Moscow, Russia

Background: Actinobacteria encompass the key unifying order *Mycobacteriales*, which includes families such as *Mycobacteriaceae, Nocardiaceae, Gordoniaceae*, and *Tsukamurellaceae*. Accurate identification of the family, genus, and species of the pathogen is essential for determining further clinical management. *Nocardia* spp., *Gordonia* spp., and *Tsukamurella* spp. are Gram-positive, aerobic, partially acid-fast microorganisms that form branching filaments. Partial acid-fastness is a diagnostic feature for the initial identification of clinically significant Actinobacteria, particularly in immunocompromised patients. The aim of the study was to evaluate the frequency of *Nocardia* spp., *Gordonia* spp., and *Tsukamurella* spp. among patients with detected acid-fast bacilli (AFB).

Methods: From August 1, 2024, to August 1, 2025, a study was conducted at the Clinical Microbiology Laboratory of the National Medical Research Center for Phthisiopulmonology and Infectious Diseases of the Ministry of Health of Russia. Clinical samples (sputum, bronchoalveolar lavage, and tissue biopsies) were analyzed for AFB identification. The samples were cultured on solid and liquid media, followed by microscopy and, if AFB were detected, identification of the isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Results: Microbiological analysis of 1136 samples positive for acid-fast microorganisms revealed the following distribution: *Mycobacterium tuberculosis* accounted for the majority (52.9%; n = 601). Nontuberculous mycobacteria were detected in 46.2% (n = 525) of cases. In 0.9% (n = 10) of samples, *Nocardia, Gordonia,* and *Tsukamurella* were identified. Further analysis showed a predominance of *Nocardia* spp. (n = 7), with *Nocardia asteroides* (n = 4) being the most common, followed by *Nocardia farcinica* (n = 1), associated with antimicrobial polyresistance, and *Nocardia cyriacigeorgica* (n = 1), a cause of pulmonary infections. One isolate required further species-level verification (*Nocardia* spp.). Among the rare Actinobacteria (n = 3), *Tsukamurella paurometabola* (n = 2) and *Gordonia aichiensis* (n = 1) were identified, opportunistic pathogens capable of causing bacteremia in immunocompromised individuals.

Conclusions: Although the prevalence of *Nocardia* spp., *Gordonia* spp., and *Tsukamurella* spp. was relatively low (0.9%) among patients with detected acid-fast microorganisms, a diagnostic approach aimed at precise species identification enabled the determination of appropriate clinical strategies for etiotropic therapy.

Keywords: Acid-fast bacilli, Actinobacteria, mycobacteria, *Nocardia*

Conflicts of interest

There are no conflicts of interest

Mycobacterium bovis was absent among Tuberculosis Patients in Russia

Platon Eliseev, Egor Shitikov, Anastasia Kazyulina, Tatyana Tyulkova, Anastasia Samoilova, Irina Vasilyeva

Microbiological Laboratory, The National Medical Research Center of Phthisiopulmonology and Infectious Diseases under the Ministry of Health of the Russian Federation, Moscow, Russia

Background: Tuberculosis (TB) is caused by members of the Mycobacterium tuberculosis complex (MTBC), which includes Mycobacterium bovis. This pathogen primarily affects cattle and can be transmitted to humans through consumption of unpasteurized dairy products or raw meat, as well as via aerosols from infected animals. Human-to-human transmission of *M. bovis* is considered rare. While zoonotic TB may contribute significantly to overall TB incidence in endemic regions, the proportion of *M. bovis* cases remains poorly characterized due to the inability of standard TB diagnostic tests to differentiate MTBC species. According to WHO estimates, over 100,000 people globally are infected annually with *M. bovis*, which also poses substantial risks to livestock industries. The aim of this study was to assess the prevalence of *M. bovis* among TB patients in Russia.

Materials and Methods: From 2022 to 2024, MTBC cultures from confirmed TB cases were referred to the Laboratory of the National Medical Research Center for Phthisiopulmonology and Infectious Diseases (Moscow, Russia) for phenotypic and genotypic species identification and drug susceptibility testing to guide diagnosis and treatment. All isolates were subjected to whole-genome sequencing for definitive species assignment within the MTBC, with specific emphasis on detecting *M. bovis*.

Results: A total of 1372 MTBC cultures from all federal districts of Russia were analyzed. None of the isolates were identified as *M. bovis*. All specimens belonged to various lineages of *M. tuberculosis*.

Conclusions: No cases of *M. bovis* infection were detected in this nationwide sample, suggesting that this pathogen does not currently contribute significantly to the human TB epidemic in Russia. However, sporadic cases may still occur in regions with zoonotic TB endemicity. Further studies integrating veterinary surveillance data are needed to comprehensively evaluate the zoonotic TB transmission dynamics.

Keywords: *Mycobacterium bovis*, tuberculosis, zoonotic tuberculosis

Conflicts of interest

There are no conflicts of interest

Prospects for Using Artificial Intelligence-driven Models in Tuberculosis Diagnostics for Cases with Focal Lung Lesions and Bacteriologically Unconfirmed Status

Valeria Sergeeva, Tatiana Tyulkova, Valentina Tinkova, Daria Kutuzova

Department of Scientific Research Coordination, Federal State Budgetary Institution "National Medical Research Center for Phthisiopulmonology and Infectious Diseases" of the Ministry of Health of the Russian Federation, Moscow, Russia

Background: Limited pulmonary involvement is characterized by the presence of a few foci localized within a restricted area of one or both lungs and involving 1–2 segments. The etiology of such lesions can be diverse and to include tuberculosis. It can be extremely difficult to detect the pathogen due to the lack of sputum, a small amount of the pathogen, etc. Additional diagnostic procedures, including invasive diagnostic methods, are required to verify the etiological factor. In some cases, this makes it difficult to diagnose tuberculosis and delays the start of therapy, which contributes to the spread of infection and the preservation of its reservoir in society. The study aims to evaluate the prospects for using artificial intelligence (AI)-driven model (artificial intelligence model) for tuberculosis diagnosis based on a set of clinical and laboratory parameters.

Methods: The study used a method for diagnosing tuberculosis in the presence of focal lung opacity (the patent RU 2814414 C1 [https://www1.fips.ru/registers-doc-view/fips_servlet]) and bacteriologically unconfirmed status. This method has been developed based on the formula of decision rule: $R = 0.055 \times X1 + 2.456 \times X2 + 0.67 \times X3 - 1.294 \times X4 + 2.589 \times X5 - 1 \times X6 - 0.416 \times X7 - 0.025 \times X8 - 2.645$ (if the value of $R \leq 0$ the patient has tuberculosis [TB], if the value of $R > 0$ the patient does not have TB [non-TB]), where X1 – Age, X2 – The fact of belonging to TB follow-up groups, X3 – The fact of alcohol consumption, X4 – The fact of detection upon application, X5 – The fact of having cancer now or in the anamnesis, X6 - Rg – Focal formation, X7 – Band neutrophils, and X8 – Lymphocytes. The result of processing the AI-driven model data according to the described formula is a protocol specifying TB/non-TB. The prospects of using an AI-driven model for the diagnosis of tuberculosis with limited lung damage and unconfirmed bacterial excretion were assessed by calculating sensitivity and specificity (Sp).

Results: The prospects of using the AI-driven model were assessed on a sample (n = 123) of patients with limited lung damage. Among them were patients with tuberculosis (n = 72) and diseases of nontuberculosis etiology (n = 52), confirmed by clinical epidemiological and histomorphological data. The use of the AI-driven model demonstrated 89.9% sensitivity (Se) and 78.9% Sp. The study showed that the AI-driven model in people with limited lung damage with unconfirmed bacterial excretion increases the accuracy of tuberculosis diagnosis by 24.5%.

Conclusions: The use of the AI-driven model for the diagnosis of tuberculosis in clinical practice is promising in cases where the patient has a limited lesion (1–2 segments on one or both lungs) with unconfirmed bacterial excretion. The AI-driven model allows timely detection of tuberculosis patients and chemotherapy, reducing the risk of infection and maintaining public health.

Keywords: Artificial intelligence, diagnostics, tuberculosis, unconfirmed bacterial tuberculosis

Conflicts of interest

There are no conflicts of interest

Identification of mutations associated with resistance of *Mycobacterium avium* to linezolid, clarithromycin, and rifampicin

Platon Eliseev, Alexandra Bayrakova, Anastasia Kazyulina, Evgeny Kuznetsov[1], Anastasia Samoilova, Irina Vasilyeva

Laboratory of Microbiology, The National Medical Research Center of Phthisiopulmonology and Infectious Diseases Under the Ministry of Health of the Russian Federation, Moscow, Russia

Background: The effective treatment of diseases caused by various nontuberculous mycobacteria (NTM) is complicated by the frequent involvement of multiple organs and the diversity of pathogens. *Mycobacterium avium* is a leading cause of these infections, but gaps exist in our knowledge of its resistance profile, particularly to essential drugs such as linezolid, clarithromycin, and rifampicin. To address this, our study focused on characterizing the molecular genetic basis of resistance in *M. avium* isolates resistant to this triad of commonly used antimicrobials.

Methods: NTM were detected using cultural methods. Inoculation of diagnostic material was performed on solid Löwenstein–Jensen medium and in liquid Middlebrook 7H9 broth, followed by incubation in BACTEC MGIT 960 (Becton Dickinson, USA). Differentiation of NTM from *Mycobacterium tuberculosis* complex was carried out by immunochromatographic assay using the MGIT TBc Identification Test (Becton Dickinson, USA). NTM species were identified by MALDI-TOF mass spectrometry. Phenotypic drug susceptibility testing was performed by broth microdilution (MIC) according to CLSI M24 guidelines; results were interpreted using CLSI M24S-2023. A total of 61 *M. avium* isolates resistant to linezolid, clarithromycin, and rifampicin, based on MIC values, were selected for further analysis. Whole-genome sequencing was performed using the MGISEQ-200RS platform (BGI, China). DNA extraction was carried out with the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germany). Samples were fragmented ultrasonically using a Covaris ML230 sonicator (Covaris, USA), and libraries were prepared with the MGI Universal Library Prep Set (BGI, China). Intermediate quantification of dsDNA and ssDNA was performed using a Qubit fluorometer (Thermo Fisher, USA); fragment length distribution was assessed on an Agilent 2100 Bioanalyzer (Agilent, USA).

Results: Analysis of genomic sequences from 61 *M. avium* isolates revealed 48 genes with mutations associated with phenotypic drug resistance (elevated MIC) to linezolid, rifampicin, and clarithromycin. Nearly half (51%) of the mutations were located in intergenic regions, 20% were frameshift mutations, 18% were nonsynonymous point mutations, 5% were insertions, 5% were stop-codon mutations, and 1% were deletions. The highest number of mutations (59 polymorphisms) was associated with linezolid resistance. Among clarithromycin-resistant isolates, three insertions were identified: one causing a frameshift in the TetR/AcrR family protein (a repressor regulating expression of tetracycline resistance genes capable of activating efflux pump expression) and two located in intergenic regions between PPE family/ATP-binding protein and GDP-mannose 4,6-dehydratase/IS3 family transposase, involved in substance transport and cell wall synthesis.

Conclusion: Further investigation of drug resistance in NTM to widely used antibacterial agents requires integrated analysis combining phenotypic and molecular genetic markers of resistance. Understanding cross-resistance mechanisms is essential for rational therapeutic strategy design.

Keywords: Drug resistance, *Mycobacterium avium*, nontuberculous mycobacteria

Conflicts of interest

There are no conflicts of interest

Molecular Epidemiology of *Mycobacterium tuberculosis* Lineage 2 in Kazakhstan

Sabina Atavliyeva[1,2], Dana Auganova[1], Nour Gharbi[3,4], Elena Zholdybayeva[1], Yuriy Skiba[1,5], Akmaral Akisheva[6], Anna Tsepke[6], Thierry Wirth[3,4], Christophe Sola[7,8], Pavel Tarlykov[1,2]

[1]National Center for Biotechnology, [2]Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, [6]City Center for Phthisiopulmonology of the Akimat of Astana, Astana, [5]Almaty Branch of the National Center for Biotechnology, Almaty, Kazakhstan, [3]Department of Origins and Evolution, EPHE, PSL University, [4]Institute of Systematics, Evolution and Biodiversity (ISYEB), National Museum of Natural History, CNRS, Sorbonne University, EPHE, University of the French West Indies, [7]IAME, INSERM, Université Paris Cité, Université Sorbonne Paris Nord, [8]Université Paris-Saclay, Paris, France

Kazakhstan ranks among the highest-burden countries for multidrug-resistant tuberculosis (MDR-TB) in the World Health Organization European Region, with *Mycobacterium tuberculosis* lineage 2 (Beijing) representing a major driver of transmission. Despite the epidemiological importance of this lineage, comprehensive data on the genomic diversity, population structure, and evolutionary dynamics of circulating *M. tuberculosis* strains in Kazakhstan remain limited. In particular, the contribution of dominant sublineages and compensatory mutations to the persistence and spread of MDR-TB has not been fully characterized at the national level. The aim of this study was to characterize the genomic diversity, population structure, and transmission dynamics of *M. tuberculosis* lineage 2 isolates circulating in Kazakhstan, with a particular focus on drug resistance profiles, compensatory mutations, and historical population dynamics. A total of 177 *M. tuberculosis* lineage 2 (Beijing) clinical isolates were analyzed, collected from newly diagnosed and previously treated TB patients across 15 regions of Kazakhstan between 2010 and 2022. Phenotypic drug susceptibility testing was performed using the BACTEC MGIT 960 system, and lineage assignment was confirmed by real-time polymerase chain reaction-based genotyping. Whole-genome sequencing was conducted on the Illumina MiSeq platform, followed by read mapping to the H37Rv reference genome and variant calling using MTBseq, TB-Profiler, and TB-Annotator pipelines. Phylogenetic reconstruction was based on high-quality single-nucleotide polymorphism (SNP) alignments using a maximum likelihood approach, while Bayesian coalescent analyses implemented in BEAST were applied to infer historical population dynamics and effective population size changes over time. Whole-genome sequencing of 177 *M. tuberculosis* lineage 2 isolates revealed a high burden of drug resistance, with 46.9% classified as MDR and 14.7% as pre-extensively drug-resistant. Phylogenetic analysis demonstrated the dominance of the Central Asian Outbreak (CAO, L2.2.M4.9.1) sublineage, accounting for 67.2% of isolates and showing widespread geographic distribution across Kazakhstan. Rifampicin resistance was detected in 72.3% of isolates, predominantly driven by the rpoB S450 L mutation, and 89.6% of rifampicin-resistant CAO isolates harbored putative compensatory mutations in rpoA, rpoB, or rpoC genes, displaying strong sublineage specificity. High-resolution phylogenetic reconstruction identified multiple closely related clusters consistent with recent transmission, including six previously unrecognized historical clusters defined by distinct SNP signatures. Bayesian skyline analysis revealed a marked expansion in the effective population size of lineage 2 strains during the late 1990s and early 2000s, coinciding with periods of major socioeconomic instability in Kazakhstan. Genomic analysis demonstrates that the CAO sublineage of *M. tuberculosis* lineage 2 has played a central role in sustaining the MDR-TB epidemic in Kazakhstan through widespread transmission and clonal expansion. These findings highlight the need for strengthened genomic surveillance and timely drug susceptibility testing to limit further dissemination and prevent the emergence of extensively drug-resistant TB. Data presented in this abstract are derived from our previously published, peer-reviewed study.[1]

Keywords: Drug resistance, Kazakhstan, lineage 2, *Mycobacterium tuberculosis,* whole-genome sequencing

Conflicts of interest

There are no conflicts of interest

Reference

1. Auganova D, *et al*. Genomic characterization and epidemiology of Mycobacterium tuberculosis lineage 2 isolates from Kazakhstan. Scientific Reports. 2025.

Diagnostic Value of Different Clinical Specimens in the Laboratory Diagnosis of Spinal Tuberculosis

Leonid Lavrenchuk, Tatiana Minogina, Sergey Skornyakov, Diana Vakhrusheva

National Medical Research Center of Phthisiopulmonology and Infectious Diseases of the Ministry of Health of the Russian Federation, Yekaterinburg, Russia

Background: Against the background of a steady decline in tuberculosis (TB) incidence and mortality in the Russia, the relative proportion of extrapulmonary TB, particularly osteoarticular forms, has been increasing. In most patients, the tuberculous etiology of spondylitis can only be confirmed after surgical acquisition of bone tissue; consequently, prior to surgery, clinicians often lack both microbiological confirmation and, critically, information on drug resistance patterns. Timely diagnosis and adequate chemotherapy of spinal TB are hampered by difficulties in obtaining diagnostic material from the lesion, which is usually accessible only during invasive surgical procedures, as well as by the limited diagnostic yield of available specimens. These limitations highlight the need to evaluate alternative diagnostic materials that are directly related to the spinal lesion but can be obtained using noninvasive or minimally invasive procedures. In addition, the clinical relevance of nonlesion specimens, such as respiratory samples and urine, for providing meaningful information about the causative pathogen remains an open question.

Methods: Clinical specimens obtained from 333 patients with confirmed spinal TB were classified into two major categories:

- Lesion-specific specimens (540 samples): wound or fistula discharge (57; 10.65%), lymph node aspirates (17; 3.18%), abscess aspirates (43; 8.04%), and surgical bone tissue (418; 78.13%)
- Nonlesion specimens (2570 samples): respiratory specimens (1370; 53.31%), urine (1170; 45.52%), and cerebrospinal fluid (30; 1.17%).

Diagnostic methods included Auramine-based fluorescence microscopy, culture on Löwenstein–Jensen and Finn media, and real-time polymerase chain reaction (PCR). Mutations associated with drug resistance were detected using real-time PCR-based assays and the TB-Test system, based on microarray.

Results: Molecular genetic methods demonstrated the highest diagnostic sensitivity, ranging from 82% to 95%, with no statistically significant differences among different types of lesion-specific specimens [Figure 1]. Nonsurgical lesion-specific specimens proved to be particularly valuable: despite their minimal invasiveness, their diagnostic yield was comparable to that of surgical bone tissue, enabling confirmation of TB and early characterization of the infecting strain at the preoperative stage. The diagnostic yield of respiratory specimens and urine was relatively low. Nevertheless, molecular methods detected *Mycobacterium tuberculosis* DNA in 28.3% of respiratory samples and 13.15% of urine samples [Figure 2]. A key issue was whether drug resistance patterns identified in nonlesion specimens reliably reflected resistance in spinal lesions. Phenotypic drug susceptibility testing was available for 14 paired cultures (11 respiratory and 3 urine), demonstrating concordance in 91.5% of cases (95% confidence interval [CI]: 86.01–94.97). Similarly, 21 paired genotypic comparisons showed concordant resistance-associated mutations in 96.77% of cases (95% CI: 88.98–99.11).

Conclusions: Minimally invasive lesion-specific specimens, such as wound or fistula discharge and aspirates from abscesses or lymph nodes, should be prioritized over surgical specimens for molecular genetic testing, as their diagnostic value is equivalent to that of surgical bone tissue and allows clinically meaningful preoperative assessment. Furthermore, drug resistance profiles of *M. tuberculosis* obtained from respiratory specimens or urine largely correspond to those identified in lesion-specific samples, indicating that nonlesion specimens may be used to guide chemotherapy regimens both prior to surgery and in cases where surgical material is unavailable or noninformative.

Keywords: Extrapulmonary tuberculosis, spondylitis, tuberculosis

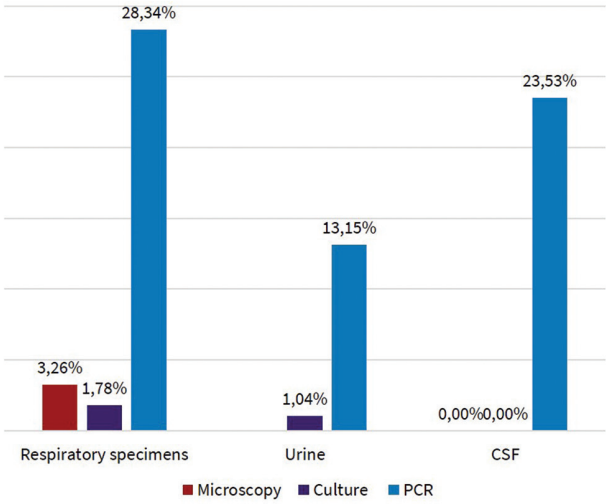

Figure 1: Detection rate of *Mycobacterium tuberculosis* in different types of lesion-specific diagnostic specimens (proportion of positive samples). PCR: Polymerase chain reaction, CSF: Cerebrospinal fluid

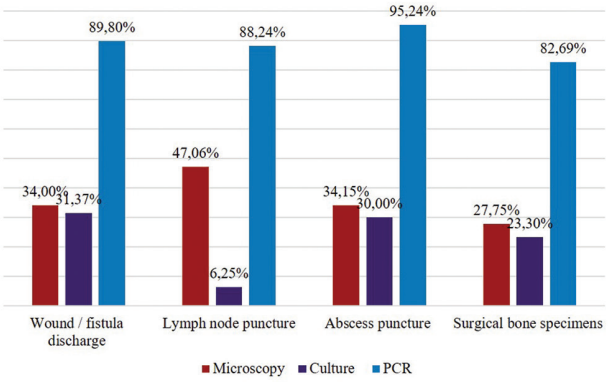

Figure 2: Detection rate of *Mycobacterium tuberculosis* in nonbone diagnostic specimens (proportion of positive samples). PCR: Polymerase chain reaction

Conflicts of interest

There are no conflicts of interest

Features of Nutritional Status and Systemic Inflammation in Various Forms of Pulmonary Mycobacteriosis

Ruslan Khachetlov, Roman Ladyu, Maria Romanova, Anna Abramchenko, Anastasia Gaida, Valentina Tinkova, Anastasia Samoilova, Irina Vasilyeva

National Medical Research Center of Phthisiopulmonology and Infectious Diseases, Moscow, Russia

Background: Pulmonary mycobacteriosis (PM) is characterized by variability in its clinical course. Nutritional status indicators, such as body mass index (BMI) and total protein levels, as well as C-reactive protein (CRP) levels as a marker of systemic inflammation, reflect the general condition of the organism and can be used to assess the severity of the process and plan complex treatment. The objective of this study was to assess the features of nutritional status and the severity of systemic inflammation in patients with various forms of PM.

Methods: A retrospective analysis was conducted on data from 82 patients with PM observed at a specialized medical center from 2023 to 2025. The diagnosis of PM was established using molecular-genetic or culture methods from sputum (twice), bronchoalveolar lavage, or surgical material. Patients were divided into two groups based on chest computed tomography findings: Group 1 included patients with the nodular bronchiectatic form ($n = 38$) and Group 2 included those with the cavitary form ($n = 44$). BMI, total protein levels, and CRP were determined for all patients before the initiation of therapy. Statistical analysis was performed using the Shapiro–Wilk and Mann–Whitney tests, and Spearman's correlation analysis. Differences between groups were considered statistically significant at $P < 0.05$.

Results: The median BMI (normal range 18.5 − 24.9 kg/m^2) in patients with the cavitary form was 19.62 kg/m^2 (Q1 − Q3: 18.05 − 23.32), whereas in the nodular bronchiectatic form it was significantly higher at 22.26 kg/m^2 (Q1 − Q3: 20.81 − 25.40) ($P = 0.001$). The CRP level in the group with the cavitary form exceeded that of the nodular bronchiectatic group: median 9.09 mg/L (Q1 − Q3: 2.10 − 22.73) versus 4.42 mg/L (Q1–Q3: 1.76–8.37) ($P = 0.0428$). The total protein level in patients with the cavitary form was 72.5 g/L (Q1 − Q3: 67.0 − 76.3), and in the nodular bronchiectatic form, it was 70.1 g/L (Q1 − Q3: 67.2 − 73.0); the differences were statistically insignificant ($P = 0.62$).

Conclusions: Patients with the cavitary form of PM more frequently exhibit signs of nutritional deficiency (lower BMI) and a higher level of systemic inflammation (CRP) compared to the nodular bronchiectatic form. Indicators of BMI, total protein, and CRP reflect the general condition of patients and may be useful for assessing disease severity and planning complex therapy; however, their diagnostic significance regarding the form of mycobacteriosis requires further study.

Keywords: Body mass index, cavitary form, C-reactive protein, nodular bronchiectatic form, nutritional status, pulmonary mycobacteriosis, total protein

Conflicts of interest

There are no conflicts of interest

Trends in Prevalence of Multidrug-resistant Tuberculosis in the Kingdom of Bahrain (2013–2023)

Alhasan Al-Mojtaba Al-Hammaqi, Ali Salman Bin Thani

Department of Biology, College of Science, University of Bahrain, Sakhir, Public Health Directorate Laboratories, Manama, Bahrain

Background: Drug-resistant tuberculosis (DR-TB), particularly multidrug-resistant TB (MDR-TB), represents a major challenge to TB control programs worldwide. Continuous monitoring of resistance trends is essential to guide effective treatment and public health interventions.

Methods: A retrospective analysis was conducted on TB cases reported in the Kingdom of Bahrain between 2013 and 2023. A total of 1158 TB-positive samples were reviewed, of which 310 were identified as DR. Drug susceptibility testing was performed using the BACTEC MGIT 960 system for first-line anti-TB drugs, including streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide.

Results: The prevalence of MDR-TB peaked at 5.48% in 2018. Pyrazinamide resistance showed a notable increase, reaching 32.65% in 2023. DR-TB was more frequent among males (69.7%) and individuals aged 21–40 years (60.2%). Nonnational cases, particularly among South Asian expatriates, constituted the majority of resistant cases. A marked disruption in TB diagnostic services and drug susceptibility testing was observed in 2021, coinciding with the COVID-19 pandemic.

Conclusions: Although MDR-TB prevalence in Bahrain remains comparable to other Gulf Cooperation Council countries and lower than global high-burden regions, increasing pyrazinamide resistance and demographic disparities warrant concern. Strengthening drug susceptibility testing capacity, improving surveillance systems, and implementing targeted public health interventions are essential to control the spread of DR-TB.

Keywords: Bahrain, drug susceptibility testing, epidemiology, multidrug-resistant tuberculosis, tuberculosis

Conflicts of interest

There are no conflicts of interest

Hepatoprotective and Cardioprotective Effects of Coenzyme Q10 under Conditions of Experimental Toxicity Induced by a Complex of Antituberculosis Drugs

Galina Mozhokina[1,2], Anastasia Gaida[1], Eleonora Pereverzeva[2], Sophia Yazerian[2], Anastasia Samoilova[1], Irina Vasilyeva[1]

[1]Federal State Budgetary Institution "National Medical Research Center for Phthisiopulmonology and Infectious Diseases", Scientific Department, [2]Federal State Budgetary Scientific Institution "G.F. Gause Research Institute for Development of New Antibiotics" Laboratory of Pharmacology and Chemotherapy, Moscow, Russia

Background: Drug intolerance remains a major challenge in tuberculosis chemotherapy, with hepatotoxicity being the most prevalent adverse effect. The introduction of newer agents such as bedaquiline, delamanid, clofazimine, and fluoroquinolones into treatment regimens further elevates the risk of cardiotoxicity. A novel strategy to enhance treatment tolerability is the adoption of an integrated approach aimed at mitigating the range of toxic effects associated with antituberculosis drugs (ATDs).

Research Objective: To evaluate the potential of coenzyme Q10 to reduce hepatotoxic and cardiotoxic reactions induced by a combination of ATDs in a toxicological experiment.

Materials and Methods: Inbred female rats were divided into three groups of ten animals each. In the experimental groups (EG1 and EG2), rats were administered five ATDs (moxifloxacin, linezolid, cycloserine, bedaquiline, and pyrazinamide) in a 1% starch gel. Rats in EG2 additionally received coenzyme Q10 in 0.9% sodium chloride solution 30 min prior to ATD administration. The doses of ATDs and coenzyme Q10 used were equivalent to human therapeutic doses. Control group rats received the 1% starch gel. All substances were administered orally once daily for 21 days. Hepatotoxicity was assessed by changes in biochemical liver profile markers and morphological signs, whereas cardiotoxicity was evaluated by electrocardiography (ECG).

Results: Administration of coenzyme Q10 in rats led to the normalization of liver enzyme levels and an increase in albumin and total protein to control levels, which had been reduced by the ATD combination. Coenzyme Q10 reduced the severity of structural liver damage by 40.6%. Administration of the ATD combination led to altered cardiac rhythm in rats: a statistically significant decrease in heart rate (HR) and prolongation of QT and RR intervals on ECG. In the group receiving coenzyme Q10, HR, QT, and RR intervals did not differ from control values. Coenzyme Q10 administration reduced the intensity of toxic cardiomyopathy by 43.3% at the histological level.

Conclusion: The experiment proved the efficacy of coenzyme Q10 in simultaneously reducing hepatotoxicity and cardiotoxicity induced by a combination of ATDs. This finding opens prospects for its use as a modifier of toxic reactions during tuberculosis chemotherapy.

Keywords: Antituberculosis drugs, coenzyme Q10, experiment, toxic reactions, toxicity modifier

Conflicts of interest

There are no conflicts of interest

The Role of Telemedicine in Tuberculosis Management

Lida Fadaizadeh[1], Farnia Velayati[1], Hassan Shojaee-Mend[2]

[1]Telemedicine Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, [2]Department of General Courses, Infectious Diseases Research Center, Faculty of Medicine, Gonabad University Of Medical Sciences, Gonabad, Iran

Background: Tuberculosis (TB) continues to represent a significant global public health concern, exerting considerable pressure on low- and middle-income countries. The advent of telemedicine, encompassing remote consultations, video-observed therapy (VOT), and digital monitoring tools, has surfaced as a pivotal strategy to address disparities in access to specialized health care, improve treatment adherence, and enhance resource management in TB care. This review consolidates findings from recent studies on the role of telemedicine in TB.

Methods: This review was conducted in 2025 with a comprehensive search in PubMed, Web of Science, Scopus, and ProQuest databases. Following screening and duplicate removal, relevant data were extracted and analyzed.

Results: Teleconsultations within TB management provide access to expert knowledge, facilitating early diagnosis, the development of tailored treatment plans, and ongoing remote monitoring, ultimately leading to improved cure rates, particularly in resource-constrained settings. Furthermore, both synchronous and asynchronous VOT reduce the time required from healthcare workers and decrease the costs by reducing travel, when also enhancing patient satisfaction through increased flexibility, especially vital in remote or underserved populations. This approach is congruent with the World Health Organization's End TB Strategy, as it promotes patient-centered care, underpins policies for digital health integration, and encourages innovative practices.

Conclusion: Telemedicine presents a viable and scalable solution that aligns with international initiatives aimed at TB elimination, in accordance with the World Health Organization's End TB Strategy. Future initiatives should emphasize equitable access and implementation in regions with high TB burdens to optimize management outcomes.

Keywords: Teleconsultations, telemedicine, tuberculosis, tuberculosis management, video-observed therapy

Conflicts of interest

There are no conflicts of interest

The Effect of the Linezolid Dose on the Dynamics of Peripheral Neuropathy Markers and the Development of Hematotoxicity

Galina Mozhokina[1,2], Anastasia Gaida[1], Eleonora Pereverzeva[2], Sophia Yazerian[2], Anastasia Samoilova[1], Irina Vasilyeva[1,3]

[1]Federal State Budgetary Institution "National Medical Research Center for Phthisiopulmonology and Infectious Diseases" of the Ministry of Health of the Russian Federation, Scientific Department of Diagnostics and Differential Diagnosis of Tuberculosis and Combined Infections, [2]Gause Institute of New Antibiotics, [3]Department of Phthisiology, Pirogov Russian National Research Medical University, Moscow, Russia

Background: Hematotoxicity and peripheral neuropathy are the most serious adverse events when using linezolid, which can lead to changes in the dose and treatment regimen of patients with multidrug-resistant tuberculosis. More often, toxic reactions occur with prolonged use, especially at a dose of 1200 mg. The aim of the study was to study in an experiment on rats the dependence of changes in markers of peripheral neuropathy and signs of hematotoxicity on the amount of the applied dose of linezolid.

Materials and Methods: Inbred female rats were divided into two experimental and 1 control group of 10 individuals each. Linezolid was administered orally to rats daily for 14 days in a single dose of 54 (1 experimental group) and 108 (2 experimental group) mg/kg, which is equivalent to doses of 600 mg and 1200 mg for humans, respectively. The rats in the control group received an oral solvent (0.9% sodium chloride solution). The follow-up was continued for 30 days after the course of injections. After the 8th and 14th administration and on the 15th and 30th days after the end of the administration period, blood was taken from rats for clinical analysis and biochemical examination. Creatine kinase (KK), cholinesterase (HE), and lactate dehydrogenase (LDH) were determined as markers of peripheral neuropathy.

Results: When linezolid was administered at a dose of 54 mg/kg, a significant increase in the level of CE was observed in the blood serum of rats. There was also a tendency to increase creatinine clearance, but without statistically significant differences with the control. Administration of linezolid in a single dose of 108 mg/kg resulted in a statistically significant increase in all markers (CC, CE, and LDH). At the end of the experiment (30th day of follow-up), the creatinine clearance level in both experimental groups was higher than in the control group rats. The HE index in Group 1 rats decreased to the control values, and in Group 2 rats, it was lower than in the control. LDH values did not exceed the control limits. Clinical blood analysis showed that linezolid at a dose of 54 mg/kg did not cause significant changes in the peripheral blood of rats. In Group 2 rats, after a course of linezolid injections at a dose of 108 mg/kg, there was a significant decrease in erythrocytes, hemoglobin, and hematocrit compared with the control, followed by normalization of these indicators by the 15th day of follow-up.

Conclusion: Linezolid at a dose of 108 mg/kg, even with a short course of use, leads to the development of anemia in rats. At the same time, 15 days after the course, spontaneous recovery of blood parameters occurs. Significant changes in peripheral neuropathy markers were noted in rats of both groups, but were more pronounced when the drug was administered at a dose of 108 mg/kg and persisted for 30 days after its withdrawal.

Keywords: Hematotoxicity, linezolid, neurotoxicity, rats relevance

Conflicts of interest

There are no conflicts of interest

Role of Health Information Systems in Early Detection and Management of Pediatric Tuberculosis

Ali Valinejadi[1], Maryam Hassanzad[2], Leila Mohammadpour-Belvirdy[2]

[1]Department of Health Information Technology, Khomein University of Medical Sciences, Khomein, [2]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Early detection and effective management of pediatric tuberculosis (TB) are critical for reducing morbidity and transmission. Health information systems (HIS) play a central role in supporting timely diagnosis, clinical decision-making, and continuity of care.

Objective: This scoping review aimed to examine the role of health information systems in the early detection and management of pediatric TB and to identify key challenges and opportunities in their implementation.

Methods: A scoping review of the literature was conducted using major databases, including PubMed, Scopus, and Web of Science. Studies addressing the use of HISs, electronic health records, surveillance systems, and reporting platforms in pediatric TB care were included in the study.

Results: HISs were found to facilitate early case identification, improve documentation accuracy, enhance patient follow-up, and support multidisciplinary care. Electronic registries and surveillance systems improved the reporting and monitoring of pediatric TB cases. However, challenges such as data fragmentation, limited interoperability, and data quality issues were frequently reported.

Conclusion: HISs are essential components of effective pediatric TB control strategies. Strengthening system integration, data quality, and user training can significantly improve early detection and management outcomes in pediatric TB care.

Keywords: Early detection, electronic health records, health information systems, pediatric tuberculosis, tuberculosis surveillance

Conflicts of interest

There are no conflicts of interest

The Structure of Drug Resistance in *Mycobacterium tuberculosis* Isolates from Children of Different Age Groups

Anna Narkevich, Liubov Parolina, Olga Otpushchennikova, Irina Lizinfeld

Federal State Budgetary Institution "National Medical Research Center of Phthisiopulmonology and Infectious Diseases" Ministry of Health of the Russian Federation (NMRC PhPI), Moscow, Russian Federation

Background: According to the World Health Organization, 10.7 million people developed tuberculosis in 2024, including nearly 700,000 children aged 0–14 years. Currently, a positive trend is observed in the tuberculosis epidemic situation among children in Russia: The incidence of tuberculosis in the 0–14 years age group decreased from 16.7/100,000 children in 2005 to 6.1/100,000 children in 2024. At the same time, the proportion of children aged 0–14 years with multidrug-resistant tuberculosis increased. The aim of this study was to analyze the structure of drug resistance in *Mycobacterium tuberculosis* isolates obtained from pediatric tuberculosis patients aged 0–18 years.

Methods: A retrospective, cross-sectional, and descriptive study was conducted using the data from the Federal Register of Tuberculosis Patients in 2025. The study included 2057 patients who met the inclusion criteria. The inclusion criteria were as follows: children aged 0–18 years with newly diagnosed tuberculosis and available results of culture on solid media. Patients were divided into five age groups: Group I – 16 patients aged 0–1 year; Group II – 276 patients aged 1–3 years; Group III – 300 patients aged 4–6 years; Group IV – 488 patients aged 7–12 years; and Group V – 977 patients aged 13–18 years. The structure of *M. tuberculosis* drug resistance included monoresistance, polyresistance, multidrug resistance (MDR), preextensively drug-resistant (pre-XDR), and XDR tuberculosis. Mycobacterial isolation and determination of drug susceptibility/resistance were performed by the culture on the liquid and solid media. The categorical variables are presented as frequencies and percentages, whereas quantitative variables are presented as means and standard deviations. Comparative analysis was performed using the χ^2 test; for small expected values, the χ^2 test with Yates' correction was applied. Differences were considered statistically significant at $P < 0.05$.

Results: Among the examined patients, 46.1% were male and 53.9% were female. The mean age was 10.9 years. Bacterial excretion was significantly more frequent in Group I than in Groups II, III, and IV (31.3% vs. 5.1%, 1.0%, and 4.9%, respectively; $P < 0.001$). Bacterial excretion was also more common in Group V compared with Groups II, III, and IV (21.8% vs. 5.1%, 1.0%, and 4.9%, respectively; $P < 0.001$). Analysis of *M. tuberculosis* drug resistance showed that in Group II, compared with Group V, monoresistance predominated (28.6% vs. 4.7%, $P = 0.003$). In Group III, XDR tuberculosis was more prevalent compared with Group V (33.3% vs. 1.4%, $P < 0.001$). No statistically significant differences were observed for other types of drug resistance across the age groups.

Conclusions: The study demonstrated a high frequency of bacterial excretion among children aged 0–1 year and adolescents, while bacterial excretion was considerably less common in other age groups. Pre-XDR and XDR *M. tuberculosis* predominated in patients aged 1–6 years. Monoresistance, polyresistance, and MDR were evenly distributed across all age groups.

Keywords: Drug resistance, *Mycobacterium tuberculosis*, Pediatric Tuberculosis (TB)

Conflicts of interest

There are no conflicts of interest

Tuberculosis or Nontuberculous Mycobacterial Disease in Chronic Obstructive Pulmonary Disease? Key Clinical Features for Differential Diagnosis

Irina Lizinfeld, Liubov Parolina, Ilona Gudieva, Olga Otpushchennikova, Anna Narkevich

Federal State Budgetary Institution "National Medical Research Center of Phthisiopulmonology and Infectious Diseases" Ministry of Health of the Russian Federation (NMRC PhPI), Moscow, Russian Federation

Background: Chronic obstructive pulmonary disease (COPD) is often complicated by tuberculosis (TB) or mycobacterial infections, which worsen the disease course and pose a significant clinical and diagnostic challenge, with their impact on prognosis still insufficiently studied. To compare the clinical, laboratory, and radiological characteristics of patients with COPD and TB versus those with COPD and nontuberculous mycobacterial (NTM) infections, and to identify key features facilitating differential diagnosis.

Methods: A retrospective cohort study including 113 patients was conducted at a National Research Center between March 1, 2024, and March 15, 2025. Adult patients aged 18–79 years with established COPD and microbiologically confirmed TB (COPD + TB, $n = 60$) or NTM disease (COPD + NTM, $n = 53$) were enrolled. Patients with human immunodeficiency virus infection, malignancy, or critical illness were excluded from the study. Clinical symptoms, smoking history, laboratory parameters, microbiological findings, and radiological data were extracted from medical records. Microorganism identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry after obtaining pure cultures. Statistical analysis included descriptive statistics, the Mann–Whitney *U*-test, and Pearson's χ^2 test; $P < 0.05$ was considered statistically significant.

Results: The mean age of patients was 57.4 ± 2.0 years. Male patients predominated in both groups (75.4% in COPD + TB vs. 70.0% in COPD + NTM). Active smoking was more frequent in the COPD + TB group (69.3%), with a higher mean smoking index (33.5 pack-years) compared with the COPD + NTM group (26.4% and 12.3 pack-years, respectively; $P < 0.04$). Patients with COPD + NTM more commonly reported weakness (77.3%) and productive cough (77.3%) compared with COPD + TB patients (59.3% and 65.4%, respectively; $P < 0.02$), whereas hemoptysis was more frequent in the COPD + TB group (13.3% vs. 2.1%; $P < 0.04$). A positive Diaskintest result was observed in 5.7% of patients in the COPD + NTM group and in 92% of patients in the COPD + TB group ($P < 0.012$). Lung destruction was observed more frequently in COPD + TB patients (69.0%) than in those with COPD + NTM (31.0%; $P < 0.042$), predominantly involving the upper lobes (55%) and affecting one or two lobes in 71% of cases. In the COPD + NTM group, a significant decrease in diastolic blood pressure ($P = 0.023$), an increased Tiffeneau index ($P = 0.040$), and elevated serum creatinine levels ($P = 0.003$) were observed. Among nontuberculous mycobacteria, unidentified species were most common (40.0%), followed by *Mycobacterium chimera* (14.3%) and *Mycobacterium smegmatis* (8.6%). Nonspecific bacterial flora was detected in 81 patients in both groups, without statistically significant differences in pathogen distribution.

Conclusions: Differential diagnosis between TB and NTM in patients with COPD is challenging due to substantial overlap in clinical and radiological features. TB is more often associated with a history of heavy smoking, hemoptysis, a positive skin test with a TB allergen (Diaskintest), and destructive lesions of the upper lung lobes. NTM, in contrast, is characterized by more pronounced general weakness, productive cough, a lower frequency of destructive pulmonary changes, and systemic alterations, including elevated creatinine levels and reduced Tiffeneau index. Despite these differences, microbiological confirmation remains essential for accurate diagnosis and optimal treatment selection, especially in regions with a high TB burden.

Keywords: Chronic obstructive pulmonary disease, differential diagnosis, mycobacterial lung disease, nontuberculous mycobacteria, tuberculosis

Conflicts of interest

There are no conflicts of interest

Molecular Identification and Characterization of Nontuberculous Mycobacteria in the Irkutsk Region, Russia

Sofiia Kulinich, Ilya Kondratov, Elizaveta Orlova, Oleg Ogarkov

Scientific Centre for Family Health and Human Reproduction Problems, Irkutsk, Russia

Background: Infections caused by nontuberculous mycobacteria (NTM) present a diagnostic challenge due to nonspecific symptoms that overlap with other chronic lung diseases. Unlike tuberculosis, which mainly affects people with weakened immune systems, NTM infections usually develop more gradually and are more often associated with chronic lung disease, such as bronchiectasis. The epidemiological situation in the Irkutsk region remains insufficiently studied. These data gap hinders effective monitoring. Traditional verification of the pathogen often does not allow for accurate species identification. As a result, there is an urgent need to introduce molecular genetic methods for high-precision differentiation. This study aimed to develop and evaluate an approach for species identification of NTM to characterize the structure of circulating NTM species in the Irkutsk region, Russia, with a high tuberculosis burden.

Methods: The study objects were 54 clinical isolates of mycobacteria from patients at the Irkutsk Regional Clinical TB Hospital (Irkutsk, Russia). Standard identification was inconclusive. The first stage involved polymerase chain reaction (PCR) screening of the *groEL2* (Rv0440) gene, which was performed to differentiate the *Mycobacterium tuberculosis* complex from NTM. For subsequent accurate species identification of NTM, we analyzed a genomic region encompassing the 23S rRNA gene and the internal transcribed spacer (ITS) region (23S rRNA/ITS locus), as it providesgreater discriminatory power than the conserved 16S rRNA gene. After optimizing the method, amplification of a highly variable fragment of the 23S rRNA/ITS locus (approximately 1,000 bp) was performed, followed by Sanger sequencing. Species affiliation was established by analyzing the obtained nucleotide sequences and comparing them with reference strains of the genus *Mycobacterium* in international databases.

Results: PCR analysis of the *groEL2* gene allowed for initial differentiation: 45 isolates (83.3%) were identified as NTM, 4 (7.4%) belonged to the *M. tuberculosis* complex, and 5 (9.3%) represented other microorganisms. However, this method provided information only at the genus level; its resolution was insufficient for species identification. Reliable species identification of clinical NTM isolates was achieved by sequencing the 23S rRNA/ITS locus, which was supported by a high degree of homology (>98%) with reference sequences. Of the 45 NTM isolates, 35 (77.8%) were slow-growing mycobacteria and 10 (22.2%) were fast-growing mycobacteria. The species *Mycobacterium avium* (57.8% of all NTM) and *Mycobacterium intracellulare* (17.8%) were predominant. Within the slow-growing NTM subgroup, *M. avium* accounted for 74.3% (26/35). Among fast-growing NTM, the species *Mycobacterium abscessus* predominated (40%, 4/10). *Mycobacterium paragordonae* was also identified (8.9% of all NTM).

Conclusions: A molecular method for precise identification of clinical NTM isolates was developed, based on sequencing the 23S rRNA/ITS region. This first regional study in Irkutsk revealed a predominance of *M. avium* and *M. intracellulare*. The results highlight the need to integrate molecular techniques into routine practice for better diagnosis, surveillance, and treatment of NTM diseases.

Keywords: 23S rRNA and ITS region, nontuberculous mycobacteria, species molecular diagnostics identification

Conflicts of interest

There are no conflicts of interest

Unmasking Nontuberculous Mycobacteria among Presumptive Tuberculosis Cases: Implications for Diagnostic Strengthening

Anand Kumar Maurya[1], Falguni Agrawal[1], Shaina Gaikwad[1], Antisha Tiwari[1], Bhavya Sharda[1], Jitendra Singh[2], Alkesh Khurana[3], Sagar Khadanga[4], Shashank Purwar[1], Debasis Biswas[1]

Departments of [1]Microbiology, [2]Translational Medicine, [3]Pulmonary Medicine, [4]General Medicine, All India Institute of Medical Sciences, Bhopal, Madhya Pradesh, India

Background: Nontuberculous mycobacteria (NTM) have emerged as clinically significant pathogens worldwide. Recognizing their importance in regions like Central India can help microbiologists and public health officials better address these infections, underscoring the need for their expertise to improve diagnostics and surveillance.

Objective: The objective of this study was to characterize NTM among presumptive tuberculosis (TB) cases, highlighting opportunities to improve diagnosis in a Tertiary Care Hospital in Central India.

Methods: Clinical samples were routinely tested for TB/NTM culture at a tertiary care center in Central India from January 2025 to September 2025. Mycobacterial culture-positive isolates suspected of NTM, although MPT64 Ag testing negative, were subjected to molecular identification using the GenoType® Mycobacterium CM assay, followed by further characterisation with the GenoType® Mycobacterium AS panel to detect additional NTM species.

Results: Laboratory results showed the detection of 28 NTM among presumptive TB cases, with a notable proportion of mycobacterial isolates identified as NTM. NTM further characterize, pulmonary samples constitute the majority of isolates. Both rapid-growing NTM (*Mycobacterium fortuitum 4 (14.3%) and M. abscessus 4 (14.3%)) were the most common*, and slow-growing NTM species *M. intracellulare 3 (10.7%), M. Simiae 3 (10.7%), and M. chimaera 2 (7.1%)* were prominent, and the rest were mixed infections reported.

Conclusion: NTM infections are an emerging and clinically relevant entity in Central India. Enhancing laboratory diagnostics and establishing regional surveillance can empower public health officials to control better and understand these infections, ultimately improving patient outcomes.

Keywords: Emerging infections, mycobacterial diagnostics, nontuberculous mycobacteria, tuberculosis

Conflicts of interest

There are no conflicts of interest

Lymphadenitis as a Complication of BCG Vaccination in Kuwait: A Case Series

Noura M. Al-Mutairi[1], Hanaa S. Eldeen[2], John Talat[2], Eiman Mokaddas[1], Suhail Ahmad[1]

[1]Department of Microbiology, College of Medicine, Kuwait University, Jabriya, [2]Kuwait National TB Unit, Shuwaikh, Kuwait

Introduction: Tuberculosis is a major infectious disease caused primarily by *Mycobacterium tuberculosis* (Mtb). *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) is used as a vaccine against tuberculosis. Since BCG is a live attenuated vaccine with a potential pathogenic action, vaccination can cause several complications, both locally near the inoculation site and remotely through blood dissemination. Here we report four separate cases of BCG-related disease (lymphadenitis) which occurred during 2016 to 2024 in Kuwait.

Methods: Clinical (swab) samples obtained from vaccine site wound of four patients (age 9-24 months) were tested for Mtb complex DNA by GeneXpert MTB/RIF qPCR assay and were cultured by using BACTEC MGIT 960 System. For specific BCG identification and its differentiation from Mtb and *M. bovis*, DNA was extracted from MGIT cultures and 4 separate uniplex PCR assays were performed by using four Mtb complex-specific primer pairs targeting *esxA*, *mce3A, mce1A* and *rrs* genes with appropriate positive and negative controls.

Results: All four infants had wound with pus at the site of inoculation. Only 1 patient was immunocompromised (with severe combined immunodeficiency disease) while the other 3 were immunocompetent including 1 patient for whom extensive immunological screening was performed. GeneXpert MTB/RIF qPCR assay identified Mtb complex DNA in all 4 clinical samples. DNA from all 4 MGIT cultures was positive for BCG as PCR amplicons were obtained for *mce1A* and *rrs* genes but not for *esxA* and *mce3A*, as expected. All 4 patients responded to appropriate anti-TB therapy.

Conclusion: Although BCG vaccine has a good safety record for most newborns, adverse reactions can still occur in some infants and should be promptly investigated. Our data also reinforce previous observations that BCG vaccination should be delayed in suspected immunocompromised infants.

Key words: *M. bovis* BCG, vaccination, lymphadenitis