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KEY NOTE
SPEAKER SESSION

IMMUNOLOGY OF TUBERCULOSIS

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a significant global health challenge, with over 10 million new cases reported annually. An estimated one-quarter of the global population is latently infected with *M. tuberculosis*, most of whom remain asymptomatic. However, approximately 5–10% of these individuals will develop active disease over their lifetime. The outcome of *M. tuberculosis* infection is largely determined by the host immune response, which is highly complex and involves a coordinated interplay between innate and adaptive immune mechanisms that either contain the infection or allows its progression.

Upon inhalation, *M. tuberculosis* is phagocytosed by alveolar macrophages, where it evades destruction by inhibiting phagosome-lysosome fusion. Innate immune responses are activated through pattern recognition receptors such as Toll-like receptors (TLRs), leading to the production of pro-inflammatory cytokines, including tumor necrosis factor (TNF) and interleukin-12 (IL-12), which are critical for initiating adaptive immunity. Dendritic cells process and present mycobacterial antigens, bridging innate and adaptive responses. Neutrophils, natural killer (NK) cells, and other innate immune cells contribute to early control through cytokine secretion and cytotoxic activity.

The adaptive immune response is dominated by T-helper 1 (Th1) cells, which secrete interferon-gamma (IFN-γ), a key cytokine for activating macrophages and controlling intracellular bacilli. Additional adaptive components, including CD8+ cytotoxic T lymphocytes, other T cell subsets, and B cells, also contribute to pathogen control. Granuloma formation—a hallmark of TB—consisting primarily of macrophages, T cells, and fibroblasts, represents a host strategy to contain infection and limit bacterial dissemination. However, *M. tuberculosis* employs diverse immune evasion strategies that undermine host defenses and promote long-term survival. Consequently, granulomas, while initially protective, can become reservoirs for bacterial persistence and may contribute to chronic inflammation and tissue damage.

Despite intensive research, significant gaps remain in our understanding of the complex interactions between *M. tuberculosis* and host immunity. Clarifying these mechanisms is essential for the development of more effective vaccines and immunotherapeutic strategies against TB. This presentation will provide an overview of the key immunological features of TB in the human host, focusing on both cellular and humoral components of adaptive immunity, critical aspects of innate responses, and the immune dysfunctions associated with major TB risk factors.

TB VACCINES

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The sole licensed vaccine against tuberculosis (TB), the Bacillus Calmette-Guérin (BCG), was administered for the first time in 1921. Despite globally widespread BCG immunization and consistent high coverage in infants, we are far from the world free of TB, as envisioned by the End TB Strategy. BCG's efficacy against pulmonary TB has recently been estimated at 19%, with significant protection only in children younger than three years. Clearly, there is a pressing demand for more effective TB vaccines capable of preventing and controlling *Mycobacterium tuberculosis* infection and its progress to subclinical and clinical TB. The major directions in development of new TB vaccines are prevention of infection (POI), prevention of disease (POD) and prevention of recurrence (POR)/therapeutic (Thp) vaccines. The TB vaccine pipeline currently includes 15 candidates, of which 12 are in active trials. Across vaccine platforms, three live attenuated, three whole cell inactivated, two viral vector, five protein/adjuvant and two mRNA vaccines are in clinical development. BCG revaccination and subunit protein H56:IC31 vaccine were removed from the clinical pipeline due to negative phase IIb results presented in 2024. Six vaccine candidates are currently in phase III trials, including the largest TB vaccine trial mounted in decades that evaluates efficacy of the adjuvanted protein M72/AS01E as POD vaccine among 20,000 adolescents and adults. The M72/AS01E was found to be significantly protective against TB disease (49.7%) in a Phase IIb trial in individuals with evidence of M. tuberculosis infection. Multiple vaccine candidates in the late-stage clinical trials, in particular M72/AS01E, as well as diversification of the pipeline by introduction of mRNA vaccines are important advances in the TB vaccine field. Nonetheless, further advances supported by the sustained research, increased funding and strengthened global collaboration, are needed for development of safe and effective TB vaccines within this decade.

IMPORTANCE OF GENOMICS FOR DRUG-RESISTANT TB DIAGNOSIS AND SURVEILLANCE

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In recent years, the World Health Organization (WHO) has approved the use of next-generation sequencing (NGS) for detecting drug resistance in the diagnosis and surveillance of tuberculosis (TB). NGS applications, including targeted NGS and whole genome sequencing, analyze entire genes to identify specific mutations linked to drug resistance in a single test from clinical samples or isolates. NGS overcomes many challenges associated with conventional phenotypic testing and the limitations of other WHO recommended rapid diagnostics. Additionally, NGS provides valuable epidemiological information, offering insights into the phylogenetics of circulating TB strains and supporting conventional contact tracing. This presentation reviews current evidence and recommendations for the use of NGS, highlighting both its opportunities and challenges. It also outlines key operational considerations for integrating NGS into surveillance systems and diagnostic algorithms at the programmatic level.

NONTUBERCULOUS MYCOBACTERIAL PULMONARY DISEASE - AN OVERVIEW AND RESULTS FROM THE CROATIAN NATIONAL REGISTRY

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Nontuberculous mycobacteria (NTM) are ubiquitous in our environment and NTM pulmonary disease (NTM-PD) is the most common disease form. NTM-PD diagnosis is complex (patients need to have a combination of clinical, radiological and microbiological characteristics), while treatment requires long-term administration of species specific multidrug regimens. The epidemiology of NTM-PD is challenging to determine because reporting is not mandatory and identification of definite disease is difficult. Furthermore, treatment outcomes and long-term survival of NTM-PD in a real-world setting are difficult to assess. In our cohort (all Croatian residents with respiratory NTM isolate from 2006 to 2015, with a follow up to 2020) we showed, based on correlation of NTM-PD rate assessed by microbiological part of the criteria compared with the full criteria, that microbiological criteria are useful in laboratory-based monitoring prevalence of NTM-PD, especially for the NTM species of higher clinical relevance such as *M. avium complex* (MAC). Our cohort was similar to other published cohorts in terms of their characteristics and risk factors. High dose treatment with inhaled corticosteroids was identified as a significant risk factor for the development of NTM-PD (aOR 4.73, 95% CI 1.69-13.23, $p=0.003$). Therapy was started in 71.5% of patients, significantly more often in patients with fibrocavitary disease and/or sputum smear positivity. Unsuccessful treatment outcomes (death or treatment failure) were recorded in 39.8% of patients, while 5-year all-cause mortality amounted to 37.6%. Guideline-based treatment (GBT) was started in 51% of treated patients and followed for the recommended duration in 35.7%. This resulted in a four-time higher chance of being cured. The impact of GBT was clear for MAC disease, but no apparent effect was observed for patients with *M. xenopi* disease.

SCIENTIFIC PRESENTATIONS BY YRI & PIs

ELUCIDATING THE DIVERGENCE BETWEEN LOCAL AND SYSTEMIC *MYCOBACTERIUM TUBERCULOSIS* IMMUNE RESPONSES

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Mycobacterium tuberculosis (*Mtb*) infection is a term that has been evolving, and currently, it is known that encompasses a spectrum of diverse clinical and immunological states. However, current immune diagnostic tests cannot discriminate between latent, active, and past tuberculosis (TB), nor predict the progression of latency to disease. Moreover, immune mechanisms involved in protection are still poorly understood. It is widely accepted that CD4 T-cells that secrete IFN-γ play a role in the immune responses against mycobacteria, however, they are not sufficient in the control of the infection. Since *Mtb* is contained in local tissues, it is also crucial to study responses at the site of infection for a better understanding of host-pathogen interaction.

Our group has advanced the understanding of TB immunopathology by studying human cell activation markers (CD27, CD38, HLA-DR, and Ki-67) in *Mtb*-specific T-cells for rapid TB diagnosis and treatment monitoring. We have also established an *Mtb*-experimental mice model, and a 34-colour spectral flow cytometry panel to address the goal of local immune response investigation. Analyses reveal that lymphocytes within the lung parenchyma exhibit T-cells with a tissue residency phenotype, that do not recirculate to blood. We are also carrying out studies for future *Mtb* immune response characterization on Collaborative Cross mice, which are a set of recombinant mice models derived from five inbred laboratory strains representing host heterogeneity. Altogether, further research is needed to fully understand the *Mtb* immune mechanisms driving across the different TB stages inside the spectrum. In this sense, networking actions in the core of ADVANCE-TB have generated collaborative work among different Institutions to address this important knowledge gap through multidisciplinary approaches, and integrating expertise from immunology, genomics, and clinical research.

OPTIMIZING GENOMIC ANALYSIS TO REFINE TRACKING OF TUBERCULOSIS TRANSMISSION

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The advent of whole genome sequencing has precipitated a paradigm shift in the field of tuberculosis epidemiology, enabling the characterisation of cases involved in transmission clusters with unparalleled precision. This development signifies the advent of a novel genomic epidemiology era, which is particularly beneficial in intricate socio-epidemiological scenarios. Almería (SouthEast Spain) is a clear example of this complexity, with 78% of total TB cases in 2024 corresponding to migrants and with global incidence rates 4-5 times above the national average. In this population, we have been applying innovative surveillance solutions for more than two decades, with the integrated efforts of microbiologists and epidemiologists. We are now starting a new stage, characterized by a more detailed exploitation of the genomic data and the reduction in response times, to bring as close as possible the precise characterization of clustered cases at the time of their diagnosis, thus facilitating control interventions. A new evolutionary approach for the SNPs analysis within clusters allows us to distinguish between clusters growing mainly due to reactivations of past exposures and others attributable to active recent transmission, where intervention to control is still possible. To optimize the control of these ongoing clusters we increased the speed in the genomic analyses by case-by-case nanopore-based sequencing on primary cultures and even attempting to sequence directly on sputa. The final step in our path to enhance genomic epidemiology strategies is to integrate the sequencing data obtained in independent populations to move on to a new interterritorial, transnational, surveillance scenario. The synergy between microbiologists who ensure a refined and rapid genomic analysis and epidemiologists, supported by community health agents, who are guided by the genomic findings, constitutes an innovative work model that allows for the optimization of the impact of tuberculosis control resources in complex populations.

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INNOVATING PAEDIATRIC TB DIAGNOSIS IN NIGERIA: FROM INSTINT TO BROADER COLLABORATIONS

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Nigeria faces one of the highest burdens of tuberculosis (TB) worldwide, with over 35,000 new paediatric TB cases reported annually. Children, especially those under five, are at greater risk for severe forms of TB, such as miliary TB or TB meningitis. Diagnosing paediatric TB is particularly challenging due to nonspecific clinical presentations and the limited sensitivity of microbiological tests. Malnutrition is highly prevalent among children with TB, particularly in low- and middle-income countries, and significantly worsens outcomes when both conditions coexist. The lack of reliable diagnostic markers for malnutrition further complicates treatment and control strategies.

This proposal seeks to enhance diagnostic methods for both TB and malnutrition in Nigerian children, addressing these intertwined issues to improve outcomes and reduce mortality. A cross-sectional study will be conducted, recruiting children aged 0–14 years from two sites in Abuja, Nigeria. We will explore a urine-based diagnostic test that is suitable for both diagnosing and monitoring TB and malnutrition. The study will also assess social determinants that influence the prevalence and progression of these conditions.

By employing urine metabolomics, the study aims to identify metabolic signatures associated with treatment outcomes for both TB and malnutrition. In parallel, understanding the role of social determinants of health will provide a more holistic approach to tackle these complex challenges. Through collaboration with local healthcare providers and scientists at two healthcare centres in Abuja, the research will facilitate early diagnosis, track treatment effectiveness, and support the broader implementation of improved TB and malnutrition care.

We have already conducted training for local laboratory staff and established protocols, preparing to begin recruitment soon. By integrating biological insights with public health perspectives, this research will generate new knowledge on how TB and malnutrition interact, informing strategies to improve patient monitoring and care.

INSTIGATION OF NEW TB DRUGS IN PROGRAMMATIC MANAGEMENT: A PRE-XDR CASE STUDY IN BANGLADESH

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Background:

Drug-resistant Tuberculosis is an alarming and rapidly increasing global public health threat, specially in developing countries and a significant portion is extensively drug- resistant TB (XDR TB). Here, we present a complex case of pre-XDR patient in Bangladesh. The objective of this case study is to demonstrate the integration of basic TB control and the identification and treatment of Pre XDR and MDR TB in the ''Programmatic Management of Drug-Resistant TB (PMDT)'' activities under the National Tuberculosis Control Program (NTP).

Methods:

This is a clinical case of a 25 years old non-smoker youngmale presented with cough, hemoptysis, breathlessness, irregular low-grade fever, sweating at night, loss of appetite, weight loss and occasional chest pain for 3 months with previous history of taking Category I and II TB drugs.

Results:

During admission, the patient was conscious, cachexic with no hepatomegaly or splenomegaly. Sputum test for 1st line drug sensitivity revealed resistance to Isoniazid, Rifampicin, Ethambutol and Streptomycin and the 2nd line probe assay showed resistance to Fluroquinolones but sensitive to 2nd line injectable drugs. However, his chest X-ray showedbilateral fibrosis and left pleural thickening with upper lobe collapse. Based on all parameters, he was declared Pre-XDR by PMDT committee and the treatment started with Bidaquiline-Dilamanid-Clofazimine-Linazolid (3 days/week)- Cycloserine (3 days/week)-Vitamin B6. The patient was cured after 24 months of treatment.

Conclusion:

This case illustrates the prevalence of mixed infections from sputum samples, which increase probability of severe form of TB, threat to society for transmission of mutant infection and false interpretation of national TB situation.

EVALUATION OF TUBERCULOSIS PREVENTIVE TREATMENT INITIATION, ADHERENCE AND COMPLETION IN COUNTRY OF GEORGIA

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Tuberculosis (TB) preventive treatment (TPT) is a critical public health strategy aimed at reducing the incidence of active TB, particularly among high-risk populations. However, the utilization and uptake of TPT remain insufficiently explored in low- and middle-income countries. In 2020, Georgia adopted a revised TPT policy aligned with World Health Organization (WHO) guidelines, transitioning from a 6-month isoniazid regimen (6INH) to a 3-month rifapentine-based regimen (3PH) for contacts of both drug-sensitive (DS) and drug-resistant (DR) TB cases. This study aimed to assess the implementation of both regimens within the Georgian National Tuberculosis Program, focusing on treatment initiation, adherence, completion, and the impact on active TB development.

A cohort study was conducted during 2012–2014 and 2021–2022 at the National Center for Tuberculosis and Lung Disease in Tbilisi and in four regions—Kakheti, Kvemo Kartli, Shida Kartli, and Imereti—covering ten TB units. Eligible participants included close contacts of active TB patients and individuals from other high-risk groups who were recommended for TPT.

Among 172 child contacts from 2012–2014, 134 (74%) were exposed to DS-TB. Of those recommended for isoniazid preventive therapy (IPT), 50 (51%) initiated treatment, and 16 (33%) completed it. During 3–5 years of follow-up, 4 children (4%) developed active TB—1 (2%) in the IPT group (treatment interrupted) and 3 (6%) in the non-IPT group (RR=0.33, 95% CI: 0.03–2.97).

In 2021–2022, among 611 contacts, 439 (74.5%) were recommended TPT; 275 (65%) initiated and 131 (47%) completed treatment. Six who did not initiate TPT later developed active TB.

These findings highlight persistent challenges in treatment uptake and completion. Strengthening patient support and monitoring may enhance TPT program effectiveness and reduce progression to active TB in Georgia.

IN VITRO AND *IN VIVO* EVALUATION OF NOVEL COMPOUNDS AS ANTI-TUBERCULOSIS AGENTS

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Objective of the study:

The aim of this study was to evaluate the pharmacological properties of two novel aroylhydrazone derivatives (3a and 3b), along with two nitrofuranyl amides (DO-190 and DO-209), as potential anti-tuberculosis drug candidates.

Methodology:

In vitro antimycobacterial activity was assessed using standard broth microdilution assays. In vivo acute and sub-acute toxicity of all compounds was evaluated in mice following oral and intraperitoneal administration, including behavioral observations, body weight monitoring, and histopathological examination of liver, kidney, and intestinal tissues. Redox-modulating capacity was assessed through in vitro antioxidant assays and in vivo oxidative stress biomarkers. Gastrointestinal permeability was examined under varying pH conditions. Molecular docking studies were conducted to assess the interactions of the selected compounds with key M. tuberculosis protein targets.

Results:

The compounds exhibited low toxicity, with no adverse behavioral or histopathological changes observed. Both aroylhydrazone derivatives demonstrated strong antioxidant activity and high gastrointestinal permeability across a range of pH conditions. The nitrofuranyl amides showed significant in vitro antimycobacterial activity, a favorable safety profile, and strong protein–ligand interactions, with DO-190 displaying the most potent binding affinities among the tested compounds.

Contributions:

Molecular docking and in vivo toxicity studies revealed the potential of both compound classes as promising antitubercular lead candidates.

ADVANCING DRUG DISCOVERY WITH IMPROVED MYCOBACTERIAL INFECTION MODELS

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Mycobacterial infections are difficult to eradicate and require lengthy, multi-drug treatments with poor patient compliance and low success rates. This is primarily due to ineffective drugs, underscoring the need for new and more effective treatments. However, drug discovery efforts targeting mycobacteria have been limited, as traditional drug screening methods are labor-intensive, low-throughput, and do not reliably predict clinical effectiveness.
In this context, we engineered new strains of *Mycobacterium abscessus* that simultaneously express the gene for a fluorescent protein, mScarlet, and the gene for the light-generating enzyme luciferase. The fluorescent signal can serve as a marker of bacterial load, while the bioluminescent signal monitors bacterial metabolism. These strains can be applied to a wide range of in vitro experimental settings, such as biofilm, intracellular, and organoid assays, greatly enhancing the utility of single-reporter bacteria. Moreover, the new double-reporter strains facilitate direct, non-invasive, real- time in vivo bacterial detection and quantification using bioluminescence imaging technology.
The double-reporter strains can be utilized at all stages of the preclinical antimycobacterial drug development pipeline, constituting a highly valuable tool for improving its success. Notably, the constructs used to transform *M. abscessus* can be applied to any other mycobacterial species, creating double-reporters of laboratory strains or clinical isolates, which opens new avenues for host-directed therapies.

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INNOVATIVE INTEGRATED TB AND NON-TB CARE: DEPLOYING DIAGNOSTICS IN REMOTE RURAL COMMUNITIES IN NIGERIA

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Background:
In Nigeria, a country with a high burden of tuberculosis, most cases of TB and other chronic diseases go undetected, especially in remote rural communities, due to limited access to diagnostics and care. This project piloted an integrated, decentralized, and culturally sensitive approach to screening and care that is not only accurate, but is also designed to function at the point of care and community level where it is needed most.

Design/Methods:
Using an AI-enabled mobile digital X-ray system, we conducted 31 outreaches in remote rural communities, supported by community gatekeepers. Participants underwent clinical screening, chest X-ray, and, where necessary, sputum sample collection for GeneXpert testing. Also, treatment initiation and follow-up for all persons diagnosed with TB were decentralized to the community level. In parallel, a community-based TB preventive treatment (TPT) model was rolled out, engaging Patent Medicine Vendors (PMVs) and linkage officers to reach household contacts of TB patients and ensure increased TPT initiation and completion.

Results:
Out of 2,874 people screened, 841 showed abnormal chest X-rays—704 with signs of chronic respiratory disease, and 137 with possible cardiovascular issues. We diagnosed 217 TB cases, and 581 contacts were started on TPT, with a 69.2% completion rate. We found that integrating screening and employing automated diagnostics increased participation, especially among men, and helped reduce delays in detection and care. Cultural beliefs, such as attributing illness to witchcraft, posed challenges to facility use. Community trust and culturally sensitive engagement were key to success

Conclusions:
For diagnostics to make a real impact, they must be accessible, easy to use, and effective where vulnerable populations live, not just in hospitals. Combining technology with community-based delivery can improve TB diagnosis and care.

ADVANCING SOLUTIONSFOR NTM: A COLLABORATIVE HIGH-THROUGHPUT SCREENING APPROACH

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Characterised by a high intrinsic resistance towards antibiotics, treatment of mycobacterial infections relies on multi-drug regimens with high toxicity. This leads to low patient compliance and low cure rates. With the prevalence of nontuberculous mycobacteria significantly increasing, infections caused by *Mycobacterium abscessus* pose the greatest treatment challenge, comparable to multidrug-resistant tuberculosis. Currently, there are no approved drugs specific for its treatment. As such, the scientific community is urged to identify new and effective drugs to treat these infections. However, with most studies focusing on drug development against *M. abscessus* relying on traditional and time-consuming methods (e.g., CFU), the current yield of drug development against *M. abscessus* does not meet its needs.

To overcome this, we set out to develop a new protocol for high-throughput drug screening against mycobacteria, using an in-house developed double-reporter strain of *M. abscessus* capable of emitting both luminescence and fluorescence intrinsically. Combining liquid-handling robotics with automated microscopy and analysis, alongside our double reporter strains, we quickly measure the viability of bacteria either growing planktonically or internalised by macrophages. Since there is no need to add reagents or perform any extra steps, time and variability between assays are reduced – a major advantage for large-scale screenings.

To validate our protocol, we screened a library of 1280 compounds designed for drug repurposing, from Prestwick Chemical libraries®. The available literature confirmed all our findings, thus validating our established assay.

We are now using this protocol to test compounds from ADVANCE-TB members, aiming to find new alternatives to help treat these infections. This presentation will not only highlight the activities of some promising compounds but will also demonstrate how collaboration within this COST action has been beneficial to all parties involved, including short-term scientific missions of ADVANCE-TB students.

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SHORT
TALKS

DERAILING *MYCOBACTERIUM TUBERCULOSIS* ADAPTATION MECHANISMS TO LIPID-RICH ENVIRONMENTS

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Subverting *Mycobacterium tuberculosis* (*Mtb*) nutritional adaptation to the host is a promising strategy to sterilize infections. *Mtb* infection foci are lipid-rich environments containing long-chain free fatty acids (LCFFA), which display antimicrobial activity. Remarkably, *Mtb* not only withstands LCFFA toxicity but also utilizes them as a preferred carbon source during infection. We hypothesized that, beyond metabolizing LCFFA, *Mtb* has evolved specific resistance mechanisms.

Through transposon sequencing, we identified the cyclic AMP (cAMP)-binding universal stress protein TB15.3 of unknown function as required for LCFFA resistance. In this work, we aimed at understanding the role of TB15.3 in *Mtb*'s physiology. We showed that TB15.3 is necessary for a balanced LCFFA uptake and catabolism and that cAMP binding is required for function.

Our results also demonstrated that TB15.3 function on LCFFA resistance is not due to modulation of intracellular cAMP. To test if it can act through protein-protein interaction, we performed a pull-down of TB15.3-Flag, and TB15.3 double mutant (DM)-Flag (unable to bind cAMP) coupled with mass-spectrometry protein identification. Proteomic analysis showed that cAMP alters TB15.3 interactome and identified a possible interacting partner. Molecular docking predicts that this partner forms a stable complex with TB15.3. Although the function of this interacting partner remains unknown, further characterization may offer new insights into how TB15.3 confers LCFFA resistance.

Altogether, our findings show TB15.3 as a central player in *Mtb*'s resistance to LCFFA and highlight its potential as a therapeutic target.

GENETICALLY DIVERSE *MYCOBACTERIUM TUBERCULOSIS* ISOLATES MANIPULATE INFLAMMASOME ACTIVATION AND IL-1b SECRETION OF MACROPHAGES INDEPENDENTLY OF MACROPHAGE METABOLIC REWIRING

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The diversity of *Mycobacterium tuberculosis* impacts the outcome of tuberculosis. We previously showed that *M. tuberculosis* isolates obtained from patients with severe disease induced low inflammasome activation and interleukin (IL)-1b production by infected macrophages. Here, we questioned whether this differential modulation of macrophages by *M. tuberculosis* isolates depended on distinct metabolic reprogramming. We found that the macrophage metabolic landscape was similar regardless of the infecting *M. tuberculosis* isolate. Paralleling single Toll-like receptor (TLR) activated macrophages, glycolysis inhibition during infection impaired IL-1b secretion. However, departing from TLR-based models, in infected macrophages, IL-1b secretion was independent of mitochondrial metabolic changes and hypoxia-inducible factor (HIF)-1a. Additionally, we found an unappreciated impact of a host metabolic inhibitor on the pathogen, and showed that inflammasome activation and IL-1b production by macrophages require metabolically active bacteria. Our study highlights the potential confounding effect of host metabolic inhibitors on the pathogen and uncoupling of *M. tuberculosis*-inflammasome modulation from the host metabolic reprogramming.

THE POTENTIAL OF *MYCOBACTERIUM TUBERCULOSIS* PHASE-SPECIFIC ANTIGENS FOR TARGETED TB DIAGNOSIS

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Tuberculosis (TB) remains a major global health threat, ranking as the second leading cause of death from infectious diseases after COVID-19, and surpassing HIV/AIDS in mortality. Current TB diagnostics, including interferon-gamma release assays (IGRAs), fail to differentiate between latent infection and active TB, and offer limited predictive value for disease progression. These limitations highlight the urgent need to develop next-generation IGRA tests that incorporate novel antigens with improved diagnostic and prognostic capabilities.

The proposed study targets a critical gap in TB diagnostics by identifying phase-specific antigens of *Mycobacterium tuberculosis* that capture the entire range of infection stages. These novel biomarkers could transform TB diagnosis by enabling the development of more accurate, stratified, and predictive tests. Utilizing advanced molecular biology, bioinformatics, and in silico epitope prediction, we will systematically screen and characterize antigens associated with distinct infection phases. These include latency-associated proteins expressed during dormancy and actively secreted antigens during bacterial replication, known to elicit robust Th1 immune responses involving both CD4$^+$ and CD8$^+$ T cells.

By moving beyond the traditional binary classification of latent versus active TB, the identification of phase-specific antigens could pave the way for next-generation IGRAs that stratify infection stages and predict disease progression. These diagnostic advancements will be crucial for timely treatment decisions, precision-targeted interventions, and more effective TB control efforts.

DEVELOPMENT OF RAPID ON-CHIPANTIBIOTIC SUSCEPTIBILITY ASSAYSFOR TB TREATMENT

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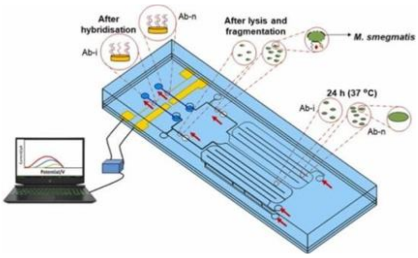
The main objective of the study was to develop a rapid, simple, cost effective and comprehensive antibiotic susceptibility/ resistance test based on rapid nucleic acid profiling. To do so, we first developed an electrochemical detection methodology to determine antibiotic susceptibility of slow-growing TB by measuring the changes in antibiotic incubation-dependent nucleic acid levels. To do so, we have applied a low- cost commercially available electrode platform, screen-printed gold electrode (SPGE). We then integrated a microelectrode sensor within a microfluidic chip that combined bacterial incubation, lysis,and electrochemical detection chambers in a single simple set-up.

As a case study, *Mycobacterium smegmatis* was investigated as a surrogate organism for *Mycobacterium tuberculosis*. The novelty of the work lies in developed capability of performing incubation, lysis, fragmentation, and detection process in a comprehensive yet simple lab-on-a- chip device.



The chip consists of a bioreactor for bacterial incubation, lysis and a microelectrode functionalised with nucleic acid sequences specific – 16sRNA in this case. The detection time was reduced to less than a day while the automation for on-field application are ongoing. The detection scheme can be easily applied to other bacterial strains and further functionalised for multiplexed detection such as drug and multi-drug resistant TB detection in low resource settings.

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ADVANCES IN DEEP LEARNING FOR PEDIATRIC TB DETECTION FROM CHEST X-RAYS

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Introduction and objectives:

The diagnosis of pediatric tuberculosis (TB) remains challenging due to non-specific findings and the need for radiologist interpretation. Since 2022, the World Health Organization (WHO) has recommended the use of chest X-rays (CXR) and computer-assisted tools to improve TB diagnosis; however, tools specifically designed for pediatric populations are limited. This study develops an artificial intelligence (AI) model to identify CXRs consistent with pediatric TB in different settings.

Methods:

A deep learning model was developed using two pediatric cohorts (N=782): pTBred (Spain, N=564) and CISM (Mozambique, N=218). CXRs were classified as positive if TB was confirmed or unconfirmed, and negative if TB was unlikely, latent, or from controls. The model was pre-trained on adult CXRs from public sources (N=367,924) and then trained using 652 frontal (AP/PA) and 264 lateral (LAT) CXRs (pTBred, N=502; CISM, N=150). It was validated on an independent set (N=130; pTBred, N=62; CISM, N=68) with 130 AP/PA and 130 LAT CXRs, using area under the curve (AUC), F-score, sensitivity, and specificity. The impact of including LAT CXRs on model performance was also assessed.

Results:

During validation, the model without pre-training or LAT CXRs achieved an AUC of 0.724 and F-score of 0.685. With pre-training, it reached an AUC of 0.763 and an F-score of 0.735. Including LAT CXRs further improved the AUC to 0.797 and the F-score to 0.753 (+3.43% AUC; +1.76% F-score), with sensitivity and specificity of 74.4% and 60.4%, respectively. Activation maps showed consistency with TB-related radiological findings.

Conclusions:

The developed AI model demonstrated effective detection of CXRs suggestive of pediatric TB. Both pre-training on adult CXRs and the inclusion of LAT views enhanced performance. The proposed solution is a promising option for resource-limited settings with scarce access to radiologists and highlights the value of diverse, high-quality pediatric datasets for AI-assisted TB diagnosis.

REDEFINIG THE ROLE OF THE SIGNALLING BY NUCLEAR RECEPTORS PATHWAY IN TUBERCULOSIS

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Tuberculosis (TB) continues to pose a major global health challenge, with host imune responses playing a crucial role in shaping disease outcomes. In this study, we revisit the role of the “Signalling by Nuclear Receptors” (SNR) pathway in TB by integrating whole blood transcriptomics from different cohorts of TB patients with experimental validation in preclinical models. We identified the SNR pathway as a transcriptional signature associated with TB severity across independent patient datasets. In mouse models that mirror the immune landscape of human TB, we observed a progressive, time-dependent upregulation of the SNR component liver-X-receptor (LXR) genes in both resistant (C57BL/6) and susceptible (C3HeB/FeJ) strains upon *Mycobacterium tuberculosis* infection. Notably, pharmacological activation of the LXR pathway during later stages of infection significantly reduced pulmonary bacterial burdens and prolonged survival of susceptible mice. This effect was accompanied by enhanced cholesterol efflux and improved macrophage-mediated control of *M. tuberculosis*, suggesting that LXR- driven modulation of host lipid metabolism can restrict bacterial access to a critical nutrient during chronic infection. Importantly, this antibacterial effect occurred independently of major inflammatory changes or tissue damage. Our findings position the SNR/LXR axis as a biomarker of TB severity and also as a promising target for host- directed therapy. By bridging clinical data with mechanistic insight, this study establishes the temporal activation of nuclear receptor signalling as a tractable and potentially transformative strategy to improve TB treatment.

DEVELOMPENT OF LACTOFERICCIN17-30 FUNCTIONALIZED PLANT OIL-BASED NANOGELS AS ANTIMICROBIAL AGENTS

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Intracellular infections caused by *Mycobacterium avium* represent a major therapeutic challenge due to the bacterium's impermeable cell wall and its ability to persist within host macrophages, thereby limiting the efficacy of conventional antibiotic regimens.1 The cationic antimicrobial peptide lactoferricin17-30 (LFcin17-30) has demonstrated the ability to disrupt bacterial membranes and enhance host immune responses; however, its therapeutic application is restricted by rapid degradation and limited stability in biological environments.2 To address these challenges, LFcin17-30 was covalently conjugated via nucleophilic reaction to an epoxy-functionalized monomer derived from castor oil, acrylated methyl ricinoleate (EAMR).The resulting conjugates were then incorporated into PEG-based nanogels through UV-induced photopolymerization.3 EAMR provides reactive epoxy groups that facilitate stable covalent binding of the peptide. Physicochemical characterization, including FTIR spectroscopy, dynamic light scattering, and zeta potential measurements, confirmed successful conjugation and the formation of nanoscale, biocompatible hydrogels.3 Additional characterization studies—such as the evaluation of conjugation efficiency, peptide loading, and cytotoxicity—are currently in preparation. Further future studies, including in vitro antimicrobial assays against *M. avium*, are planned to assess the therapeutic potential of the system. It is anticipated that this covalent conjugation strategy will enhance peptide stability, enable localized and sustained delivery within infected macrophages, and ultimately lead to improved bacterial clearance compared to free peptide formulations.2 Overall, this system may represent a promising injectable nanoplatform for the treatment of persistent intracellular mycobacterial infections.

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DISRUPTING THE ELECTRON RESPIRATORY CHAIN BY INHIBITING NADH-TYPE II DEHYDROGENASE OF *MYCOBACTERIUM TUBERCULOSIS*

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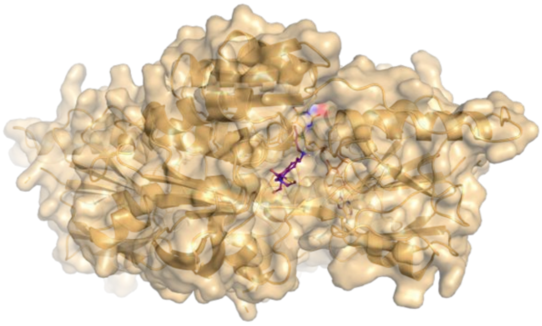
Tuberculosis (TB) is amongst the major worldwide health threats. Yet in 2023, after COVID-19 pandemics, TB returned to being the world's leading cause of death from a single infectious agent and caused almost twice as many deaths as HIV/AIDS [1]. Despite progresses witnessed in the last two decades [2-3], the ability to fight multidrug-resistant *Mtb* strains is still challenging, underscoring the urgency of developing new TB drugs with novel mechanisms of action.

NADH-type II dehydrogenase of *Mycobacterium tuberculosis* (*Mtb*), specifically Ndh is an optimal candidate for drug discovery since Ndh is i) essential for the bacillus,[4] ii) a key component in the electron transport chain, iii) absent from the mammalian genome, which minimizes undesired off-target effects, and iv) druggable, as noted by the discovery of compounds acting as Ndh inhibitors with submicromolar potency [5].

It is worth noting that the structure for Ndh of *Mtb* has not been crystallized. However, very recently the structure of Ndh from *M. smegmatis* has been crystallized, which has been a key point to refine our preliminary 3D model of M. tuberculosis. Based on this model, we have identified three putative binding sites: i) the NADH binding site, ii) the quinone (Q)-binding site, and iii) an allosteric pocket. Furthermore, preliminary docking studies have led to a potential mechanism of action of inhibitors towards the different binding sites.

Our objective is to find novel small organic compounds able to bind to specific cavities of NADH cofactor and/or ubiquinone, inhibiting the first steps of the electron transfer mechanism, and disrupting the survival of the bacillus. Here, we present the first molecules identified at different pockets and our computational protocol to improve its inhibitory potency.



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EFFECTIVENESS OF MYCOBACTERIOPHAGE D29 IN TARGETING INTRACELLULAR *MYCOBACTERIUM SMEGMATIS*

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Considering the growing problem of antibiotic resistance in tuberculosis worldwide and the need to find new treatment approaches, this study focuses on the potential of phage therapy. The aim of this study is to initially evaluate the efficacy of mycobacteriophage D29 in reducing intracellular and extracellular populations of *Mycobacterium smegmatis* in human macrophages.

Materials and Methods:

Human monocytic cell line was differentiated into macrophages using Phorbol 12-myristate 13-acetate and subsequently infected with *M. smegmatis* at a multiplicity of infection of 10 (MOI 10). Infected macrophage cells were treated with mycobacteriophage D29 (MOI 10) and samples were taken during 72 hours infection course to assess phage and bacterial load both extracellularly and intracellularly. The phage plaque assay was used to quantify phage titres, while intracellular bacterial counts were determined to assess the efficacy of phage therapy.

Results:

Bacterial presence in the supernatant was detected throughout the duration of the experiment, with a decline observed after 60 hours, coinciding with an increase in mycobacteriophage activity. The detection of bacteria in the supernatant can be due to the lysis of macrophages and the subsequent release of intracellular bacteria into the extracellular environment, where mycobacteriophage replication is more efficient. During the first 24 hours post-infection, the intracellular bacterial load remained stable and was comparable to that observed in the control (untreated macrophages). Following the point at which phage concentration exceeded bacterial concentration, a reduction in intracellular bacterial load was observed, while phage levels plateaued after 60 hours. In contrast, bacterial concentrations in the control continued to increase throughout the experiment.

Conclusions:

These findings suggest that mycobacteriophage D29 can reduce *M. smegmatis* populations both extracellularly and intracellularly, though with a delayed intracellular efficacy. But, considering the duration of our experimental protocol, the mycobacteriophage alone could not fully eradicate the bacterial load. In future, combining phage therapy with antibiotics could provide a solution, potentially shortening treatment duration and reducing the required antibiotic dosage.

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POSTER PRESENTATIONS

EPIDEMIOLOGICAL PROFILE OF TUBERCULOSIS IN NEPAL: A PUBLIC HEALTH CALL TO ACTION

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Tuberculosis (TB) remains one of the leading causes of death in Nepal and poses a significant public health burden. Based on projections from the National Population and Housing Census 2011, Nepal's population in 2020 was approximately 29.9 million. According to the National TB Prevalence Survey 2018/19, the incidence of TB is estimated at 245 per 100,000 population, translating to approximately 69,000 new drug-sensitive TB (DSTB) cases annually—of which 65% are male and 35% female. TB mortality is estimated at 16,000 deaths per year, reflecting a substantial increase (3.1 times higher) over previous estimates. Additionally, TB is now recognized as one of the major leading causes of death in the country.

An estimated 47% of Nepal's population is infected with latent TB, posing a risk of future reactivation and transmission. In 2020, a total of 40,776 DSTB cases and 756 drug-resistant TB (DRTB) cases were notified. However, the estimated annual burden of DRTB is around 3,000 new cases, underscoring a significant detection gap. Nepal has consequently been categorized by the World Health Organization as one of the high-burden countries for drug-resistant TB.

These findings call for an urgent scale-up of TB control efforts, with a focus on improving case detection, addressing gender disparities, expanding drug-resistance surveillance, and tackling latent TB infection. The revised data provides a critical foundation for policy reform and targeted interventions needed to reduce the TB burden and move toward elimination.

CURRENT ADVANCES IN TUBERCULOSIS AND PULMONARY MYCOBACTERIOSIS: IMMUNOLOGICAL INSIGHTS AND DIAGNOSTIC STRATEGIES

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Tuberculosis (TB), primarily caused by *Mycobacterium tuberculosis* (*Mtb*), and non-tuberculous pulmonary mycobacterioses (NTM) remain significant global health challenges. Despite widespread Bacillus Calmette-Guérin (BCG) vaccination and anti-mycobacterial therapy, TB remains a leading cause of infectious mortality, while the incidence of NTM infections, particularly in immunocompromised and elderly populations, is rising. Recent advances in immunology have elucidated the complex interplay between host immune responses and mycobacterial pathogens. Novel insights into innate immunity, including the roles of macrophage polarization, autophagy, and pattern recognition receptors such as Toll-like receptors (TLRs) and C-type lectins, have uncovered critical mechanisms of early host defense. Adaptive immunity, particularly the function of Th1 and Th17 cell responses and regulatory T cells, has also been shown to shape disease progression and outcome. Simultaneously, diagnostic innovations are improving early detection and differentiation of Mtb from NTM species. Interferon-gamma release assays (IGRAs) and nucleic acid amplification tests (NAATs) have enhanced sensitivity and specificity, though challenges remain in distinguishing active from latent infection. Whole-genome sequencing and transcriptomic biomarkers are emerging as powerful tools for precision diagnostics and monitoring therapeutic responses. Furthermore, advances in point-of-care diagnostics and imaging modalities offer promise for implementation in resource-limited settings. Together, these developments highlight a paradigm shift toward integrated immunological and molecular approaches in the diagnosis and management of mycobacterial lung diseases. Continued investment in translational research is essential for the development of targeted immunotherapies and next- generation diagnostic platforms.

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TUBERCULOSIS IN MIGRANT WORKERS:SOCIAL AND HEALTHCARE CHALLENGES

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The increasing employment of foreign workers from high tuberculosis (TB) incidence countries has introduced significant public health challenges in Croatia. While initial hospitalization for TB is legally guaranteed regardless of insurance status, continuation of treatment post-discharge requires access to primary care, which is frequently hindered by a lack of health insurance, unregistered residency, or the absence of a designated general practitioner. Additional barriers include language difficulties, undocumented employment, and the absence of prior medical records. These factors contribute to delayed diagnosis, advanced disease presentation, heightened infectivity, and increased transmission risk in densely populated living and working conditions. Irregular employment and frequent changes of employers further complicate epidemiological contact tracing. The most recent amendments to the Foreigners Act have not adequately addressed healthcare access for this population, nor have sufficient preventive health measures been implemented. In the post-pandemic period, a rising TB incidence in Croatia has been observed, with a notable proportion attributable to foreign workers. Comprehensive legislative reform, improved access to healthcare services, mandatory health screenings, and targeted preventive interventions are urgently needed to mitigate TB transmission and ensure effective disease management.

ACCELERATING *MYCOBACTERIUM ABSCESSUS* DRUG DISCOVERY WITH NOVEL DOUBLE-REPORTER STRAINS

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Mycobacterium abscessus (*Mab*) is one of the most challenging pathogens for human health, often called an "incurable nightmare". Management of *Mab* infections is comparable to multi-drug-resistant tuberculosis due to intrinsic antibiotic resistance and complex pathogenesis involving biofilms and granulomas. There are no approved drugs specific for this bacterium. Despite multi-drug regimens taken for months to years, severe secondary effects and low patient compliance lead to clinical success below 50%. Thus, finding new, more efficient drugs to combat this pathogen is imperative.

To develop predictive assays and improve drug screening setups, we have engineered novel *Mab* double-reporter strains capable of emitting strong luminescent and fluorescent signals [1]. Taking advantage of these strains and a robotic and automated drug screening platform, we developed a protocol for high-throughput drug screening against *Mab* growing in broth cultures, biofilms and inside macrophages [2]. These strains allow the real-time, non-destructive quantification of bacteria based on cell mass (fluorescence) and metabolic activity (luminescence). We have validated these strains for drug screening, showing the transformation is stable without the selection antibiotic (genomic integration), with no phenotypical alterations in the original strain, either in growth or antibiotic susceptibility. Importantly, the luminescent and fluorescent signals correlate with colony-forming unit (CFU) quantification, reinforcing their usefulness in drug screening setups. The use of these strains simplifies the screening process and enhances reliability, representing a significant advancement from CFU-based methods.

Using these strains and the developed high-throughput protocol, we screened a commercial library from ChemBridge containing 10,000 new small molecules. We found two highly active compounds against intracellular Mab that were not toxic to macrophages. We are now working on testing these hits against in-vivo infection models.

We have provided the scientific community with a tool to assess the in vitro anti-*Mab* activity of any compound. Most importantly, they can be used to determine the drug's effect in-vivo without sacrificing the animals and obtaining the results immediately.

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ESCAPE-TB - EUTECTIC SOLUTIONS FOR CONTROLLED AND ADVANCED PULMONARY ENHANCEMENT IN TUBERCULOSIS

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The ESCAPE-TB project targets the urgent need for improved tuberculosis (TB) treatments by exploring deep eutectic solvents (DESs). Facing increasing drug resistance, the initiative aims to enhance current TB drugs and create novel formulations. Key objectives include boosting drug efficacy, optimizing formulations using DESs, investigating new treatment options, and tackling TB eradication challenges.

TB's global impact is worsened by multidrug-resistant strains. DESs, eco-friendly solvents, offer low toxicity and high biodegradability. Therapeutic DESs (THEDESs) can improve drug solubility and permeability, enhancing bioavailability and patient compliance. Natural Deep Eutectic Solvents (NADESs) show antimicrobial potential and can improve drug palatability.

ESCAPE-TB will develop DES-based drugs (DESs-TB), including THEDESs of existing TB drugs and new NADESs. Encapsulation in lipidic nanoparticles (LNPs) will improve drug stability and bioavailability, enable targeted delivery to macrophages, control release, and reduce side effects. Project deliverables include DES-based formulations, innovative nanomedicines for drug-resistant TB, and progression to in vivo studies within 36 months.

The anticipated outcome is at least one DESs-based formulation with enhanced efficacy, palatability, solubility, and permeability, potentially shortening treatment and improving adherence. Despite the novelty of DESs in clinical applications, the project employs risk mitigation and sustainable green synthesis. Collaboration within the ADVANCE-TB COST Action will promote knowledge sharing and scalability, contributing to the global goal of ending TB by 2035.

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CLINICAL INTERPRETATION OF XPERT MTB/RIF ULTRA 'TRACE DETECTED' RESULTS IN TUBERCULOSIS DIAGNOSIS: A RETROSPECTIVE STUDY FROM NORTH MACEDONIA

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In the context of tuberculosis (TB), North Macedonia is a low incidence country. In 2023, there were 157 new cases of TB, with notification rate of 8.7 per 100,000. Our country has a tuberculosis laboratory network consisting of 3 first level laboratories (microscopy only), 2 second level (microscopy and culture) and one National Reference Laboratory (NRL). NRL is the only TB laboratory that performs molecular testing, both for diagnosis and susceptibility testing. Testing palette consisting of Xpert MTB/RIF Ultra, Hain GenoType MTBDRplus, GenoType MTBDRsl, AnyplexTM MTB/NTMe and Allplex™ MTB/MDR/XDRe, GenoType Mycobacterium CM and AS, Genotype NTM-DR.

Objective:
To evaluate the clinical significance of Xpert MTB/RIF Ultra "MTB trace detected" results in tuberculosis diagnosis by comparing them with culture outcomes and treatment initiation patterns.

Materials and methods:
Retrospective analysis of all "MTB trace detected, rifampicin resistance not detected" results from Genexpert Ultra assays performed between 2020-2024 (n=54 trace results from 3,224 tests). Culture confirmation (LJ/MGIT) and microscopy results were reviewed for 51 trace results (49 patients), excluding pediatric cases (n=3).

Results:
51 trace results originated from 49 patients, and only 18 (36.7%) were confirmed by culture. Treatment was initiated for all culture positive patients. 7 (14.3%) patients started with treatment without being culture confirmed: one was positive in a sputum sample, 4 were positive from a bronchoalveolar lavage, and 2 were positive from pleural punctate. 6 patients were previously or at the time treated for TB.

Conclusion:

Trace results demonstrate Ultra's improved sensitivity at the same time increasing false positives. Therefore, clinical interpretation must account for treatment history and sample type. Culture remains critical but insufficient alone for treatment decisions in trace-positive cases, especially those with prior TB treatment. The 6 treated patients explain part of the culture-negative results, as WHO notes trace calls may detect non-viable bacilli in patients treated within 5 years

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