# Ecological Study Protocol: Respiratory and Gut Microbiome and Host Gene Expression Regulation in Pulmonary Tuberculosis (TB)

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Date: 2025-08-09  
Protocol format: ICMR-compatible. Submit to Institutional Ethics Committee (IEC) for review.

## 1. Background & Rationale

Pulmonary tuberculosis (TB) remains a leading infectious cause of morbidity and mortality in India. Host immune response and pathogen dynamics are influenced by the respiratory and gut microbiome. Ecological analyses at district and community levels can identify population patterns in microbiome profiles associated with TB incidence and treatment outcomes, informing targeted surveillance and adjunctive interventions.

## 2. Objectives

Primary Objective: To assess area-level associations between respiratory/gut microbiome characteristics and pulmonary TB incidence and treatment outcomes across selected districts in India.

Secondary Objectives:

1. 1. To examine temporal changes in microbiome profiles during anti-TB therapy (ATT) and their association with treatment response.
2. 2. To identify environmental and sociodemographic correlates (air pollution, diabetes prevalence, malnutrition) of microbiome variation linked to TB outcomes.
3. 3. To prioritize districts for targeted microbiome and mechanistic field studies.

## 3. Study Design

Design: Longitudinal ecological panel study + targeted cohort sub-studies.  
Units: District-year observations across a 5–10 year window for panel analyses. For cohort sub-studies, select 3–5 sentinel districts for serial sample collection (baseline, 2 months, end of therapy, 6-month follow-up).  
Sample Size: Panel will include as many districts with high-quality NTEP data as available (aim for >100 districts). Cohort sub-studies: enroll 100–150 pulmonary TB patients per sentinel district for serial sampling.

## 4. Methodology

4.1 Data sources and variables:

* • TB notifications and treatment outcome data from NTEP (district-year).
* • Area-level covariates: PM2.5 (satellite models/CPCB), diabetes prevalence (NFHS/modelled), SES indicators (Census/NFHS), health facility density.
* • For cohort sub-studies: sputum (for respiratory microbiome and MTB testing), stool (gut microbiome), and peripheral blood (transcriptomics and immune phenotyping).

4.2 Sample collection SOP highlights (cohort sub-studies):

* • Sputum: early morning deep-cough sputum into sterile container; triple-packed for transport; processed under BSL-3 or validated inactivation protocol.
* • Stool: collected in stabilizer tubes (OMNIgene or DNA/RNA Shield) for gut microbiome profiling.
* • Blood: PAXgene tubes for host RNA; EDTA plasma for cytokines and metabolomics.

## 5. Laboratory Methods and Biosafety Considerations

Handling of sputum and mycobacterial cultures requires BSL-3 facilities. For nucleic acid extraction for metagenomics, the laboratory must validate an inactivation protocol (chemical/heat) approved by the Institutional Biosafety Committee (IBC) before opening samples in a BSL-2 environment. Coordinate with NTEP and institutional biosafety officer for all procedures.

## 6. Data Analysis Plan

• Panel analyses: fixed-effects and random-effects models with distributed lag structures to examine temporal relationships between exposures (e.g., PM2.5, diabetes prevalence) and TB notification rates.

* • Longitudinal within-subject analyses: changes in microbiome composition and host transcriptomics over therapy; association with sputum conversion and treatment outcomes.
* • Multi-omics integration using time-series network methods and mediation analyses to identify microbe→metabolite→host gene pathways.

## 7. Ethical, Regulatory and Data Governance

Obtain IEC/IRB approval and Institutional Biosafety Committee (IBC) clearance. For use of NTEP surveillance data, secure data-sharing agreements with the state TB program. Ensure participant informed consent for cohort sub-studies including sequencing, data sharing, and future use of samples. De-identify data prior to analysis and deposit sequence data in controlled-access repositories per consent.

## 8. Validation, Mechanistic Follow-up and Translation

• Validate ecological findings through targeted microbiome sampling and individual-level cohort analyses in prioritized districts.

• Mechanistic studies: ex vivo immune assays, in vitro co-cultures, and animal models where appropriate under animal ethics approvals.

• Translate findings into pilot adjunctive therapies (e.g., probiotics, metabolites) to be tested in randomized trials after safety evaluation and regulatory approvals.

## 9. Timeline and Milestones

• Months 0–3: Permissions, data agreements, ethics/IBC approvals, SOP finalization.

• Months 3–9: Data collation, panel construction, sentinel site selection.

• Months 9–18: Cohort enrollment and serial sampling in sentinel sites.

• Months 12–24: Analysis, validation experiments, pilot trial planning.

## 10. Deliverables

• Policy briefs and district-level hot-spot maps for public health action.  
• Peer-reviewed manuscripts.  
• Prioritized list of districts for mechanistic sampling and pilot interventions.  
• SOPs for sample handling and safe processing of TB specimens.