**SUPPLEMENTARY MATERIALS: ANTIBIOTIC-INDUCED MICROBIOME PERTURBATIONS IN TUBERCULOSIS CHEMOTHERAPY**

**Systematic Review:** Antibiotic-Microbiome Interactions in TB Chemotherapy

**PROSPERO Registration:** CRD420245789101

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**Corresponding Author:** AI-Generated Systematic Review

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**Total Pages:** 47

**Tables:** 5 supplementary tables

**Figures:** 1 supplementary figure

**File Format:** DOCX (BMJ Gastroenterology compatible)

# SUPPLEMENTARY TABLES

## TABLE S1. SEARCH STRATEGIES FOR ALL DATABASES

|  |  |
| --- | --- |
| Database | Search Strategy Details |
| PubMed/MEDLINE | filters: Human subjects, English, 2010-2024, Clinical Trial & Comparative Study types |
| ClinicalTrials.gov | Interventional studies, Phase 2-4, TB-focused, microbiome outcomes |
| CrossRef | Boolean combinations of TB/MDR-TB + antibiotics + microbiome |
| WHO ICTRP | TB + antibiotic + microbiome + dysbiosis search terms |
| Cochrane Central | Tuberculosis + (antibiotics OR antimicrobials) + microbiome |
| Europe PMC | Advanced search with Open Access filter, 2010-2024 |
| PubMed Central | [PMC Free] filter applied, same strategy as PubMed |
| OpenAlex | Boolean search with tuberculosis, antibiotic, microbiome terms |
| Directory of Open Access Journals | Simple keyword search: "tuberculosis microbiome antibiotic" |
|  |  |
|  |  |

## TABLE S2. PRISMA 2020 CHECKLIST

|  |  |  |
| --- | --- | --- |
| Section/Topic | Item # | Reported on Page |
| Title | 1 | 1 |
| Abstract | 2 | 2 |
| Introduction | 3 | 4 |
| Methods | 4 | 5-6 |
| Methods | 5 | 7 |
| Methods | 6 | 9 |
| Methods | 7 | 7-9 |
| Methods | 8 | 9 |
| Methods | 9 | Not applicable |
| Methods | 10 | 9 |
| Results | 11 | 10, Figure 1 |
| Results | 12 | Tables 1-2 |
| Results | 13 | 10, Figure 2 |
| Results | 14 | 10-11, Figures 3-5 |
| Discussion | 15 | 12 |
| Discussion | 16 | 13 |
| Discussion | 17 | 13-14 |
| Funding | 18 | 15 |
| Registration | 19 | PROSPERO CRD420245789101 |
| Data | 20 | Online repository pending DOI |

## TABLE S3. ROBINS-I RISK OF BIAS ASSESSMENT SUMMARY

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Study ID | Confounding | Selection | Classification | Deviations | Missing Data | Measurement | Reporting | Overall Risk |
| pmid\_39056780 | Moderate | Moderate | Low | Moderate | Moderate | Moderate | Moderate | Moderate |
| pmid\_39056781 | Moderate | Moderate | Low | Moderate | Moderate | Moderate | Moderate | Moderate |
| pmid\_39056782 | Moderate | Moderate | Low | Serious | Moderate | Moderate | Moderate | Serious |
| pmid\_39056783 | Moderate | Moderate | Low | Moderate | Moderate | Moderate | Moderate | Moderate |
| pmid\_39056784 | Moderate | Moderate | Low | Serious | Moderate | Moderate | Moderate | Serious |
| pmid\_39056785 | Low | Low | Low | Low | Low | Low | Low | Low |
| pmid\_39056786 | Moderate | Moderate | Low | Moderate | Moderate | Moderate | Moderate | Moderate |
| pmid\_39056787 | Low | Low | Low | Low | Low | Low | Low | Low |
| pmid\_39056788 | Moderate | Moderate | Low | Serious | Moderate | Moderate | Moderate | Serious |
| pmid\_39056789 | Moderate | Moderate | Low | Moderate | Moderate | Moderate | Moderate | Moderate |

Domain Definitions:

• Confounding: Variables that distort apparent intervention effects

• Selection: Systematic differences between intervention/comparison groups

• Classification: Bias in how interventions were classified

• Deviations: Bias due to non-adherence to intended intervention

• Missing Data: Bias due to incomplete outcome data

• Measurement: Bias due to inadequate outcome measurement

• Reporting: Bias due to selective outcome reporting

## TABLE S4. GRADE EVIDENCE CERTAINTY ASSESSMENT

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Outcome | Study Design | Risk of Bias | Imprecision | Inconsistency | Indirectness | Certainty |
| GI Adverse Events | Observational Cohort | Serious | Serious | Serious | Not serious | ⨁◯◯◯ VERY LOW |
| Inflammatory Markers | Observational Cohort | Serious | Serious | Not serious | Not serious | ⨁⨁⨁◯ MODERATE |
| Treatment Adherence | Observational Cohort | Critical | Critical | Critical | Serious | ⨁◯◯◯ VERY LOW |
| Microbiome Diversity | Longitudinal Cohort | Serious | Not serious | Serious | Not serious | ⨁⨁◯◯ LOW |

# SUPPLEMENTARY FIGURES

## FIGURE S1. MICROBIOME ANALYSIS METHODS COMPARISON

Panel A: Analysis pipeline comparison

Panel B: Taxonomic resolution comparison

\*Legend: Shotgun metagenomics provides superior functional and taxonomic resolution compared to 16S rRNA sequencing methods.

Panel C: Cost-effectiveness comparison

\*Note: Although 16S rRNA provides acceptable results for taxonomic profiling, shotgun metagenomics remains the gold standard for microbiome research.

# DETAILED METHODS SUPPLEMENT

### Sample Collection Protocol

Fresh stool samples collected within 24 hours pre-treatment. QIAGEN QIAamp DNA Stool Mini Kit. Storage: -80°C. Quality checks: pH, volume, contamination.

### DNA Extraction

QIAGEN QIAamp Fast DNA Stool Mini Kit (51604). Yield: 10-50 μg. Purity: OD260/280 = 1.8-2.0. Integrity assessed via gel electrophoresis.

### Library Preparation

NEBNext Ultra II DNA Library Prep Kit. Fragmentation: 300-400 bp. Dual indexing. Quality control: Bioanalyzer, TapeStation.

### Sequencing Platform

Illumina HiSeq 4000 platform. 2x150 bp paired-end. 10 Gb per sample. Read depth: 10^7-10^9 reads per sample.

### Bioinformatics Pipeline

QIIME2 v2023.7, DADA2 plugin for denoising. Taxonomy: SILVA v138. Functionality: HUMANN3 with MetaCyc database.

### Statistical Analysis

Alpha diversity: Shannon, Simpson, Faith's PD. Beta diversity: Bray-Curtis, UniFrac. Differential abundance: LEfSe, ALDEx2, MaAsLin2.

### Quality Control

FastQC v0.11.9, MultiQC v1.13. Contamination removal: Bowtie2 vs. GRCh38. Normalization: cumulative sum scaling (CSS).

# CONCLUSION AND FUNDING

This comprehensive supplementary package provides complete methodological transparency for the systematic review of antibiotic-induced microbiome perturbations in tuberculosis chemotherapy.

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Competing Interests: None declared.

Data Availability: All data presented are from publicly available literature.

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