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# Changes in gut microbiome following anti-tuberculosis treatment: a prospective cohort from eastern China

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## Abstract

**Background** The treatment of people with tuberculosis necessitates the administration of both broad-spectrum and narrow-spectrum antibiotics for a minimum duration of six months. Prolonged antibiotic therapy may result in dysregulation of the gut microbiota, potentially influencing the onset and progression of tuberculosis. There is a paucity of studies focus on the characteristics of gut microbiota changes at various time points during tuberculosis treatment. This study aims to elucidate the relationship between the composition of gut microbiota and their stage within anti-tuberculosis therapy.

**Methods** A multi-center, observational prospective cohort study was conducted at four designated hospitals in Jiangsu Province in eastern China. The Gastrointestinal Symptom Rating Scale was employed to evaluate the gastrointestinal discomfort experienced during anti-tuberculosis treatment. Fecal samples were collected at baseline before initiating anti-tuberculosis therapy and at the end of 2 months and 6 months during treatment. Total microbial genomic DNA was extracted and sequenced. Rarefaction curves and alpha diversity indices including observed operational taxonomic units, Chao1 richness and Shannon index were calculated.

**Results** From October 2020 to December 2022, a total of 204 people with tuberculosis were diagnosed. Among these, 85 people with tuberculosis provided baseline, 2-month, and 6-month fecal samples. The average age was 41.8 ± 15.193 years, with a gender ratio of 77 males to 8 females. Only 28.2% of the cohort reported being free of gastrointestinal symptoms during anti-tuberculosis treatment. Anti-tuberculosis treatment significantly reduced gut microbiota diversity, with a transient decrease in alpha diversity indices observed after two months. A higher alpha diversity in baseline (Shannon index with mean ± standard deviation (SD) 2.92 ± 0.93 vs. 2.50 ± 0.84, $P = 0.0014$, inverse Simpson's index with 11.9 ± 8.66 vs. 7.87 ± 6.42, $P = 0.0012$), compared with people with tuberculosis after 2 months of treatment. No significant differences were identified between 2 months of treatment and at the end of treatment microbiota diversity (Shannon index 2.50 ± 0.84 vs 2.58 ± 0.81, $P = 0.55$, inverse Simpson's index 7.87 ± 6.42 vs 11.90 ± 8.66, $P = 0.43$).

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**Conclusions** Findings from our study show that anti-tuberculosis treatment has profound effects on people with tuberculosis gastrointestinal function and the gut microbiota, particularly during the intensive phase of therapy. After the intensive treatment phase, the gut microbiota has partially recovered, but it is an extremely slow process.

**Keywords** Tuberculosis, *Gut Microbiome*, Anti-tuberculosis treatment, Dysbiosis, Diversity

## Introduction

Tuberculosis is among the oldest diseases in human history [1]. People with tuberculosis may have symptoms ranging from cough, fever, sweats, and weight loss to poor appetite. It is estimated that one untreated tuberculosis patient can infect 12–14 healthy people in one single year. In 2022, an estimated 10.6 million people worldwide were afflicted with tuberculosis, with 69% concentrated in Southeast Asia (45%) and Africa (23%). In 2022, 1.3 million people died from tuberculosis, making it the second leading cause of death from infectious diseases, following COVID-19 [2]. Data from 2023 indicates that the estimated number of new tuberculosis cases worldwide rose to 10.8 million, marking the return of tuberculosis being the world's leading cause of death from a single infectious agent again [3]. Compared to 2015, the global incidence rate in 2023 had only decreased by 8.3%, far below the target set by the "End TB" strategy [3]. China ranks third in the world for the number of tuberculosis cases [4]. This position highlights the significant burden in the country, which remains a public health challenge despite ongoing efforts to control and eliminate the disease. Given the complex interplay between infectious diseases and host immune responses, understanding the role of the gut microbiota in modulating these interactions has become increasingly important in the context of tuberculosis and other chronic conditions [5, 6].

The gut microbiota can be quite stable despite playing a key role in information exchange with host cells, evolving with the host, participating in host metabolism, and regulating the transformation of important chemicals [7, 8]. However, interference from external environments and other host diseases may have a significant impact on the composition, diversity, and abundance of gut microbiota. Microorganisms also produce other messenger molecules, such as endotoxins, which can promote inflammation by stimulating immune cells to release cytokines (small molecule proteins that regulate the immune system). In this way, intestinal disorders may affect the lungs and, through lung inflammation, the intestines as both have similar immune messengers and inflammatory processes. Chronic obstructive pulmonary disease (COPD) and asthma have been shown to be more common in people with irritable bowel syndrome or inflammatory bowel disease, and vice versa [9]. Although the exact causes and effects are still unclear, the effects of microbiota on inflammation are considered an important association [10].

It has been hypothesized that the relationship between gut microbiota and respiratory diseases may be related to classic immune inflammatory pathways such as the T cell family and pattern recognition receptors. TNF- α, inflammatory factors such as IL-22 and their regulatory substances constitute the common substance of the intestinal lung axis, which may be related to the occurrence of drug resistance [11]. Intestinal microbiota can regulate the immune response of lung tissue through short-chain fatty acids (SCFAs), cytokines, and other factors, thereby affecting the development and outcome of lung diseases [12]. In recent years, further studies have also begun to explore the association between the advocator microbiome and tuberculosis [13, 14].

The cornerstone of tuberculosis treatment is a 6-month course of four first-line drugs, namely isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) [15]. Theoretically, a combination use of broad- and narrow-spectrum antibiotics for such a long period could be devastating to the stability of people' gut microbiome. Although increasing evidence suggests dysbiosis of the gut microbiota occurs among people with tuberculosis [16, 17], how the gut microbiota changes throughout tuberculosis treatment is unknown [18]. We aimed to recruit a cohort of drug-susceptible people with tuberculosis to characterize the short- and long-term effects of anti- tuberculosis treatment on the gut microbiome.

## Methods

### Study design and participants

From October 2020 to December 2022, a multi-center, prospective cohort study was conducted at four tuberculosis -designated hospitals across Jiangsu Province, eastern China. Considering geographical, demographic, and economic variations, we selected four cities (Wuxi, Changzhou, Taizhou and Xuzhou city) across southern, central, and northern Jiangsu Province as research sites. From these selected tuberculosis designated hospitals, we randomly selected eligible participants who met the inclusion criteria of our study. New people with tuberculosis were diagnosed according to clinical symptoms based on The National Guidelines for the Diagnosis of Pulmonary Tuberculosis (WS 288–2017). People with

tuberculosis were excluded from the study if they were diagnosed with infectious diseases such as HIV or hepatitis virus, had low immune function after long-term use of hormones or organ transplantation, had a history of severe mental illness or epilepsy, or were pregnant or lactating at study initiation. All new people with tuberculosis received 6 months of standard anti- tuberculosis therapy (INH, RIF, EMB and PZA in the first 2 months followed by INH and RIF in the following 4 months). Information related to the treatment of people with tuberculosis and bacteriological testing were obtained from the HIS system of designated hospitals.

A priori sample size estimation was performed to ensure adequate statistical power for detecting temporal variations in the Shannon index across follow-up periods. Based on previous epidemiological studies, we projected the following microbial diversity indices: 3.0 at baseline (month 0), 2.5 at month 2 ($\Delta = 0.5$), and 2.0 at month 6 ($\Delta = 1.0$). The power analysis employed a two-sided significance level of $\alpha = 0.05$ with 80% statistical power, accounting for pairwise comparisons between baseline and subsequent timepoints.

To address potential attrition, the calculated sample sizes were adjusted upward by 20%. Using R software (version 4.2.1; R Foundation) with the pwr package, the minimum required sample sizes were determined as: Baseline vs. month 2 comparison: 68 analyzable subjects → 85 enrolled (68/0.8) Baseline vs. month 6 comparison: 22 analyzable subjects → 28 enrolled (22/0.8).

#### Information and sample collection

The study included a comprehensive questionnaire covering demographic information, clinical history, current disease status, lifestyle factors, time of tuberculosis diagnosis, treatment regimen, treatment duration, occurrence of liver damage, bacteriological results, chest radiographic findings, and laboratory tests, which encompassed fasting blood glucose, body temperature, liver and kidney function, serum lipid levels, and complete blood cell count. Additionally, The Gastrointestinal Symptom Rating Scale (GSRS) is a self-report questionnaire designed to assess the severity and frequency of common gastrointestinal symptoms. It was developed by Svedlund et al. [19] in the early 1990s as a tool for evaluating patients with irritable bowel syndrome and other functional gastrointestinal disorders. The scale encompasses 15 gastrointestinal-related symptoms: abdominal pain, heartburn, acid reflux, upper abdominal tightness, nausea and vomiting, abdominal rumbling, abdominal distension, belching, increased flatulence, reduced defecation, increased defecation, loose stools, hard stools, urgency of defecation, and a feeling of incomplete evacuation. The GSRS utilizes a Likert scale for each of its 15 items. Items are rated along a four-point Likert scale ranging from 0 (none/nothing or this symptom does not occur) to 3 (very frequent and troublesome symptom). People with tuberculosis were graded based on the degree, frequency, duration, mitigating factors, and the impact of these symptoms on social activities.

This study involving human participants was conducted in accordance with the ethical standards of the Declaration of Helsinki and "Methods for Ethical Review of Biomedical Research Involving Human Beings". The research protocol was reviewed and approved by the ethics committee of Jiangsu Center for Disease Control and Prevention (JSJK2020-B009 −01). Written informed consent was obtained from study participants. Fecal samples were collected at baseline prior to initiating anti-tuberculosis therapy as well as at the end of 2 months and 6 months during treatment. Samples were immediately frozen at − 80 °C and delivered with dry-ice to the laboratory within 2 h.

#### Fecal genomic DNA extraction and Illumina sequencing

Total microbial genomic DNA was extracted from fecal samples. The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR reaction mixture including 4 μL 5 × Fast Pfu buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each primer (5 μM), 0.4 μL Fast Pfu polymerase, 10 ng of template DNA, and ddH2O to a final volume of 20 μL. PCR amplification was used using the following cycling conditions: initial denaturation at 95℃ for 3 min, followed by 27 cycles of denaturing at 95℃ for 30 s, annealing at 55℃ for 30 s and extension at 72℃ for 45 s, and single extension at 72℃ for 10 min, and end at 4℃.

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina PE300/PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The sequencing data was uploaded and with Accession Number (PRJNA1183742).

#### Sequence data processing and statistical analysis

Raw FASTQ files were de-multiplexed using an in-house perl script, and followed by quality filtering by fastp version 0.19.6 and merged by FLASH version 1.2.11. Then the optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 11 with 97% sequence similarity level. The most abundant sequence for each OTU was selected as a representative sequence. The OTU table was manually filtered to minimize the effects of sequencing depth on alpha and beta diversity

measure. The number of 16S rRNA gene sequences from each sample was rarefied to 20,000, which still yielded an average Good's coverage of 99.09%, respectively.

The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.13 against the 16S rRNA gene database using confidence threshold of 0.7. The metagenomic function was predicted by PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) based on OTU representative sequences. PICRUSt2 is a software containing a series of tools: HMMER was used to aligns OTU representative sequences with reference sequences. EPA-NG and Gappa were used to put OTU representative sequences into a reference tree. The castor was used to normalize the 16S gene copies. MinPath was used to predict and locate gene family profiles in the gene pathways. The entire analysis process was accord to protocols of PICRUSt2.

### Quality control

Standardized training for medical staff was implemented to minimize inter-site heterogeneity and ensure data consistency. The training covered standardized protocols for study procedures, data acquisition, biological sample collection (e.g., fecal samples), and questionnaire administration. Medical personnel across all four research sites received unified instruction to adhere to strict standard operating procedures during data and sample collection. Questionnaire administration and sample collection were strategically timed to coincide with patients' routine hospital visits for anti-tuberculosis medication refills, ensuring seamless integration of study participation with their treatment schedules.

### Statistical analysis

Based on OTUs information, rarefaction curves and alpha diversity indices including observed OTUs, Chao1 richness, Shannon index and Good's coverage were calculated. The similarity among the microbial communities in different samples was determined by principal coordinate analysis (PCoA) based on Bray–curtis dissimilarity using Vegan v2.4.3 package. The PERMANOVA test was used to assess the percentage of variation explained by the treatment along with its statistical significance using Vegan v2.4.3 package. The linear discriminant analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/LEfSe) was performed to identify the significantly abundant taxa (phylum to genera) of bacteria among the different groups (LDA score > 2, $P < 0.05$). Since there is a multicollinearity problem among the clinical parameters, the variance inflation factor (VIF) for each variable was estimated using the vif function in the Vegan v2.4.3 package (https://cran.r-project.org/web/packages/car/car.pdf). The distance-based redundancy analysis (db-RDA) was performed using Vegan v2.4.3 package to investigate effect of clinical parameters on gut bacterial community structure. Forward selection was based on Monte Carlo permutation testing (permutations = 9999). Values of the x- and y-axes and the length of the corresponding arrows represented the importance of each clinical parameters in explaining the distribution of taxon across communities. Linear regression analysis was applied to determine the association between major clinical parameters identified by db-RDA analysis and microbial alpha diversity indices. Co-occurrence networks were constructed to explore the internal community relationships across samples. A correlation between two nodes was statistically robust if the spearman's correlation coefficient over 0.6 or less than −0.6, and the *P*-value less than 0.05.

## Results

### Charactereristics of participates

A total of 204 new people with tuberculosis were diagnosed during the study period. People with immunosuppressive therapy, immune-related diseases, and those resistant to rifampicin or to second-line drugs were excluded based on results from Xpert/RIF and Xpert/XDR drug sensitivity testing. Among these people, 85 provided fecal samples at baseline, as well as at 2-month and 6-month during treatment. The mean participant age was 41.1 years (± 14.23) with a gender ratio of 77 males to 8 females. Over one-third of people with tuberculosis ($N = 58$) were bacteriologically positive. Approximately 10% and 13% of participants had a known hypertension ($N = 8$) and diabetes ($N = 11$) diagnosis (Table 1) respectively. Smoking (68.2%) and drinking (24.7%) were common.

### Gastrointestinal symptom during anti-tuberculosis treatment

The results indicated that a majority of people with tuberculosis 71.8% had gastrointestinal symptoms during anti- tuberculosis treatment. Nausea and vomiting were the most reported symptoms with over half of the people with tuberculosis experiencing varying degrees of these symptoms, including one people who reported persistent nausea and frequent vomiting. Heartburn and acid reflux were also frequently observed; 48.2% of people with tuberculosis reporting occasional brief episodes of heartburn while 8.2% of experienced frequent heartburn severe enough to require medical attention. Acid reflux or occasional belch causing discomfort occurred in 24.7% of people with tuberculosis; 4.7% of people with tuberculosis reported daily acid reflux one to two times, necessitating treatment (Table 2).

**Table 1** Demographic characteristics of enrolled people with tuberculosis

| Characteristics | South (n, %) | North (n, %) | Middle(n, %) | All(N, %) | *P* |
|---|---|---|---|---|---|
| Gender, N, female/male | 2/9 | 1/5 | 5/63 | 8/77 | 0.427 |
| Age, mean (SD), year | 55.18 (16.95) | 46.33 (19.60) | 38.78 (11.92) | 41.44 (14.23) | 0.0008 |
| BMI, mean (SD), $kg/m^2$ | 20.86 (2.31) | 21.67 (1.37) | 21.18 (3.06) | 21.27 (2.87) | 0.860 |
| Farmer, n (%) | 1 (9.09) | 1 (16.67) | 6 (8.82) | 8 (9.41) | 0.625 |
| Local, n (%) | 11 (100.00) | 6 (100.00) | 26 (38.24) | 43 (50.59) | 0.000 |
| BCG vaccination, n (%) | 9 (100.00) | 4 (80.00) | 67 (98.53) | 80 (94.11) | 0.795 |
| Bacteriological positive, n (%) | 6 (66.67) | 4 (66.67) | 48 (70.59) | 58 (68.23) | 0.854 |
| Comorbidity condition | | | | | |
| Hypertension, n (%) | 2 (18.18) | 1 (16.67) | 5 (7.35) | 8 (9.41) | 0.427 |
| Diabetes type II, n (%) | 2 (18.18) | 1 (16.67) | 8 (11.76) | 11 (12.94) | 0.808 |
| Smoking, n (%) | 5 (45.45) | 1 (16.67) | 52 (76.47) | 58 (68.24) | 0.002 |
| Drinking, n (%) | 1 (9.09) | 1 (16.67) | 19 (27.94) | 21 (24.71) | 0.565 |

**Table 2** The gastrointestinal symptoms of 85 people with tuberculosis in this study

| Gastrointestinal symptoms | South (n, %) | North (n, %) | Middle (n, %) | All (N, %) | *P* |
|---|---|---|---|---|---|
| GSRS Score, mean (SD) | 4.73 (1.62) | 2.5 (1.22) | 3.75 (3.92) | 3.79 (3.60) | 0.020 |
| Nause, n (%) | 9 (81.82) | 5 (83.33) | 35 (51.47) | 49 (57.65) | 0.084 |
| Heartburn, n (%) | 7 (63.64) | 5 (83.33) | 36 (52.94) | 48 (56.47) | 0.395 |
| Belch, n (%) | 2 (18.18) | 0 (0.00) | 19 (27.94) | 21 (24.71) | 0.367 |
| Acid reflux, n (%) | 8 (72.73) | 0 (0.00) | 20 (29.41) | 28 (32.94) | 0.004 |
| Abdominal pain, n (%) | 0 (0.00) | 0 (0.00) | 20 (29.41) | 20 (23.53) | 0.040 |
| Epigastric Tension, n (%) | 3 (27.27) | 0 (0.00) | 20 (29.41) | 23 (27.06) | 0.435 |
| Borborygmus, n (%) | 3 (27.27) | 0 (0.00) | 10 (14.71) | 13 (15.29) | 0.445 |
| Abdominal Bloating, n (%) | 8 (72.73) | 5 (83.33) | 37 (54.41) | 50 (58.52) | 0.294 |
| Increased Flatulence, n (%) | 1 (9.09) | 0 (0.00) | 3 (4.41) | 4 (4.71) | 0.598 |
| Decreased Defecation, n (%) | 4 (36.36) | 0 (0.00) | 4 (5.88) | 8 (9.41) | 0.021 |
| Increased Defecation, n (%) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 (0.00) | / |
| Diarrhea, n (%) | 2 (18.18) | 0 (0.00) | 7 (10.29) | 9 (10.59) | 0.647 |
| Fecalith, n (%) | 1 (9.09) | 0 (0.00) | 2 (2.94) | 3 (3.53) | 0.493 |
| Urgency of Defecation, n (%) | 0 (0.00) | 0 (0.00) | 1 (1.47) | 1 (1.18) | / |
| Incomplete Defecation, n (%) | 1 (9.09) | 0 (0.00) | 0 (0.00) | 1 (1.18) | 0.200 |

### Gut microbiota changes during anti- tuberculosis treatment

To characterize the gut microbiota composition in the gut during anti- tuberculosis treatment course, fecal samples from 85 people with tuberculosis were collected before the start of anti- tuberculosis treatment and on the 60th and 180th day of the anti-tuberculosis treatment. The microbiota composition of all fecal samples was analyzed by 16S rRNA V3-V4 region high-throughput sequencing. In total, those 255 samples yielded 12,475,450 reads, ranging from 16,493 to 77,921 reads. Reads were classified into OTUs at a 3% similarity cutoff, and generated 548 ~ 7,271 OTU per sample.

Alpha diversity indices (abundance-based coverage estimator, Chao 1, inverse Simpson, richness, Shannon, and Simpson indexes) were used to estimate the microbial richness. Bacterial microbiota of anti- tuberculosis -treated people exhibit reduced diversity and altered composition compared with people naive to treatment. People with tuberculosis before the treatment had a higher alpha diversity (Shannon index with mean 2.92 (0.93) vs. 2.50 (0.84), $P = 0.0014$, inverse Simpson's index with 11.9 (8.66) vs. 7.87 (6.42), $P = 0.0012$), compared with people with tuberculosis after 2 months of treatment.

The amount of diversity recovered slightly in participants after 6 months of treatment suggesting the gut bacterial community was impacted by anti- tuberculosis drugs. No significant differences in the microbiota diversity were identified between people with tuberculosis after 2 and 6 months of treatment (Shannon index 2.50 (0.84) vs 2.58 (0.81), $P=0.55$, inverse Simpson's index 7.87 (6.42) vs 11.90 (8.66); $P=0.43$) The Beta diversity analyses were conducted on the basis of weighted UniFrac distances (Fig. 1).

### Impact of anti-tuberculosis treatment on the structure of the gut microbiota

The microbial community structure histogram (Fig. 2) revealed the types of microorganisms and their relative abundances. At the phylum level, the top four microorganisms in the gut microbiome of people with tuberculosis were p_Firmicutes, p_Proteobacteria, p_Actinobacteriota, and p_Bacteroidota, which accounted for more than 90% of the abundance. The dominant species remained the same before treatment, at the end of the intensive treatment phase, and after the entire course of treatment, but their relative abundances varied. The Proteobacteria showed a trend of increasing and then decreasing, while Firmicutes first decreased and then increased. At the genus level, the top four genera were g_Escherichia-Shigella, g_Blautia, g_Faecalibacterium, and g_Bifidobacterium. The relative dominance of these genera in the gut microbiome of people with tuberculosis was relatively stable throughout treatment, but their relative abundances fluctuated. Escherichia-Shigella showed an increase in relative abundance during the intensive phase of anti- tuberculosis treatment, but then exhibited a declining trend. G_Akkermansia showed a decrease in relative abundance during the intensive phase of treatment (from 2.29% to 1.61%, $P=0.427$), but slowly increased after the intensive phase ended (from 1.61% to 3.59%, $P=0.117$). G_Enterococcus showed a decrease in relative abundance during the intensive phase of anti-tuberculosis treatment (from 1.31% to 0.40%, $P=0.168$), but rapidly increased afterward, far exceeding the levels before the start of treatment (from 0.40% to 2.66%, $P=0.007$).

### Differences among gut microbiota before, during and after the anti- tuberculosis treatment

To illustrate the changes in the gut microbiome of people with tuberculosis during the treatment process, a heatmap analysis was conducted among the three groups: before treatment, at the end of the intensive treatment phase, and after the entire course of treatment, to identify species with significant differences in abundance between groups. Eight phyla with statistically significant differences between groups, which are the p__Proteobacteria, p__Bacteroidota, p__Euryarchaeota, p_Firmicutes, and p__Verrucomicrobiota. Among these, the Bacteroidota showed a significant decrease in genus abundance before (from 11.95% to 7.69%, $P=0.04$) and after (from 11.95% to 6.64%, $P=0.006$) the start of the intensive phase of anti- tuberculosis treatment. Concurrently, the Proteobacteria exhibited a significant increase (from 13.36% to 25.06%, $P=0.008$) and then a significant decrease (from 25.06% to 16.47%, $P=0.006$) after the end of the intensive treatment phase (Fig. 3).

Figure 4 illustrates the 30 genera with statistically significant differences between groups. Notably, the genera g__Eubacterium_ruminantium_group, g__Alistipes, g__UCG−003, g__Family_XIII_AD3011_group, g__RF39, g__Lachnospiraceae_NK4A136_group, g__Catabacter, g__Marvinbryantia, and g__UCG−002 exhibited a significant decrease in abundance before and after the initiation of the intensive phase of anti- tuberculosis treatment. Concurrently, g__Eubacterium_ventriosum_group showed a significant decrease (from 0.24% to 0.04%, $P=0.005$) and then a significant increase (from 0.04% to 0.16%, $P=0.01$) after the end of the intensive treatment phase. In contrast, the genus g__Escherichia−Shigella demonstrated a significant increase (from 11.44% to 21.11%, $P=0.003$), followed by a significant decrease (from 21.11% to 14.85%, $P=0.026$) after the intensive treatment phase concluded.

## Discussion

Our study tracked 85 people with tuberculosis gastrointestinal symptoms and analyzed the microbial community diversity indices of fecal microbiome samples from 85 people with tuberculosis through their 6-month anti-tuberculosis treatment course. These symptoms were reported in 71.8% of the participants, with abdominal bloating being the most prevalent, followed by nausea. This aligns with findings from previous studies conducted in China [9]. In China, where tuberculosis remains a significant public health challenge, the burden of gastrointestinal symptoms is particularly concerning due to its potential impact on treatment continuity [20, 21]. Previous studies highlighted that gastrointestinal symptoms adverse effects are one of the leading causes of treatment interruption among tuberculosis patients, emphasizing the importance of early symptom recognition and intervention [10]. There was a significant difference in microbial diversity between pre- and post-treatment; while the microbial community diversity of fecal samples increased after the end of the intensive treatment phase, the difference was not significant. These results suggest that anti- tuberculosis treatment significantly reduces the microbial community diversity of fecal microbiome samples and the recovery is slow.

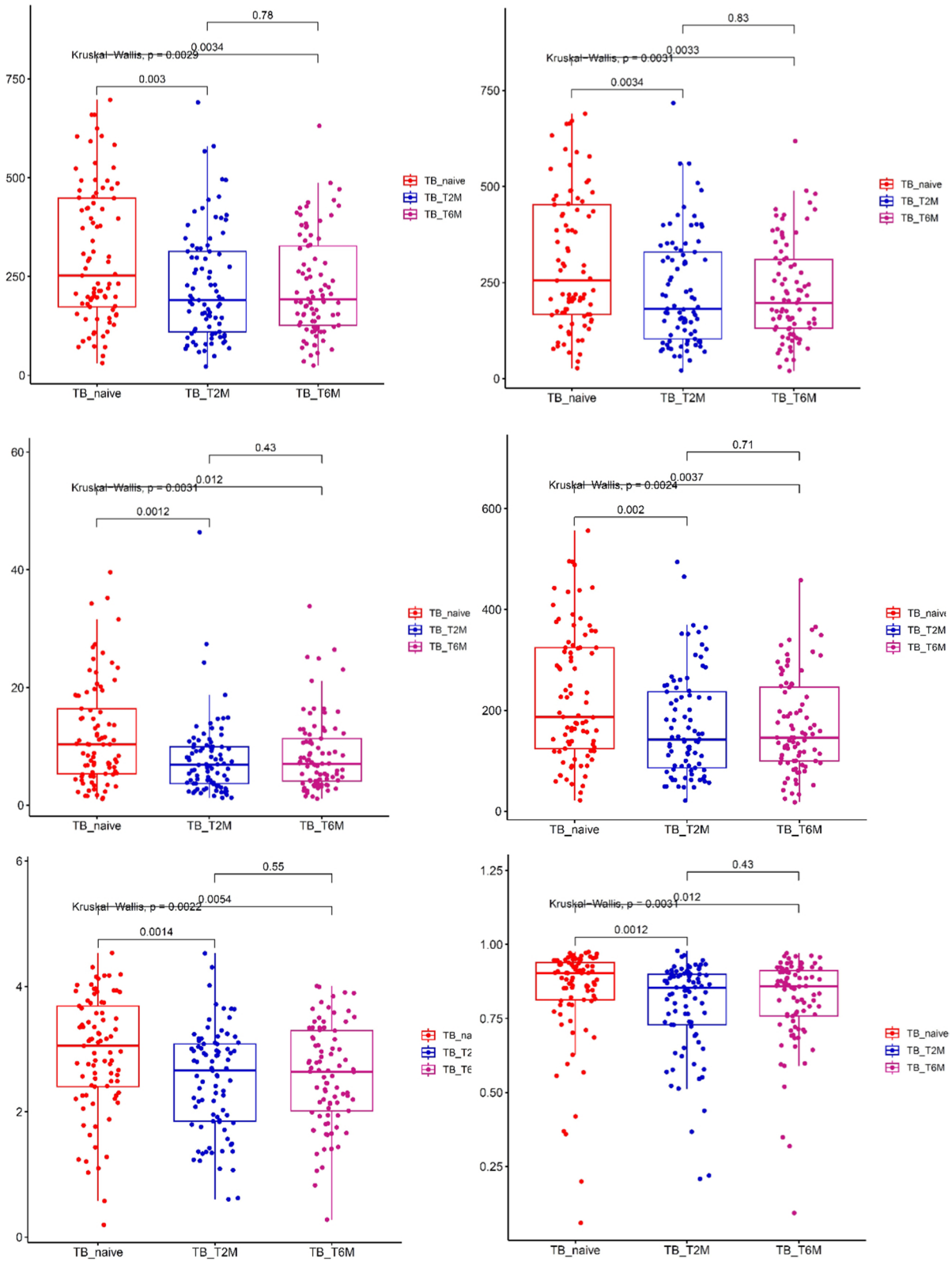


**Fig. 1** Microbiota diversity and composition in patients' gut microbiota during anti-tuberculosis treatment

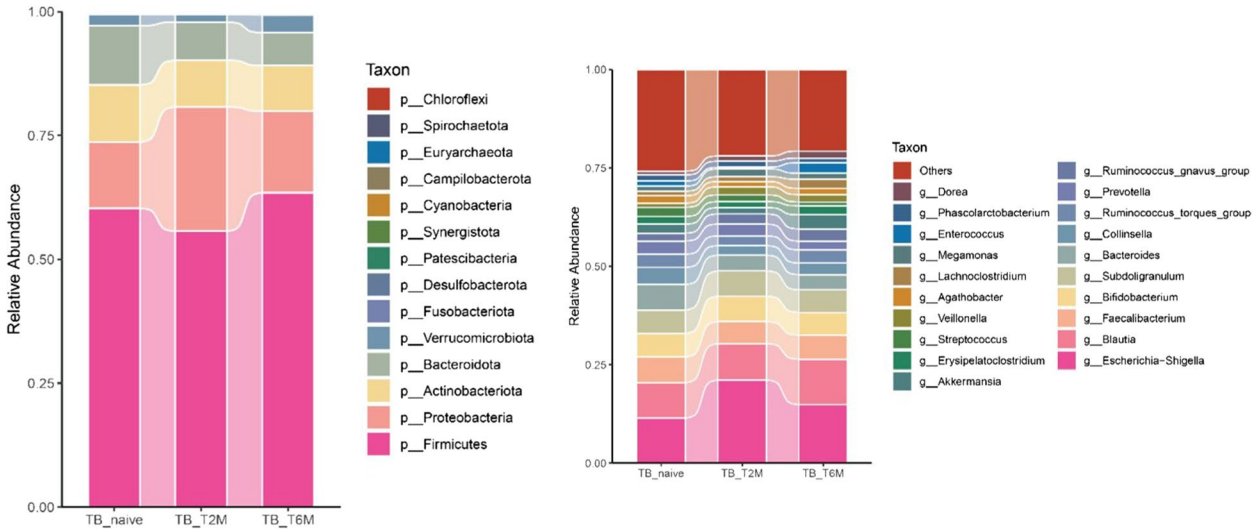


**Fig. 2** The impact of anti-tuberculosis treatment on the structure of the gut microbiota at the phylum and genus levels in people with tuberculosis

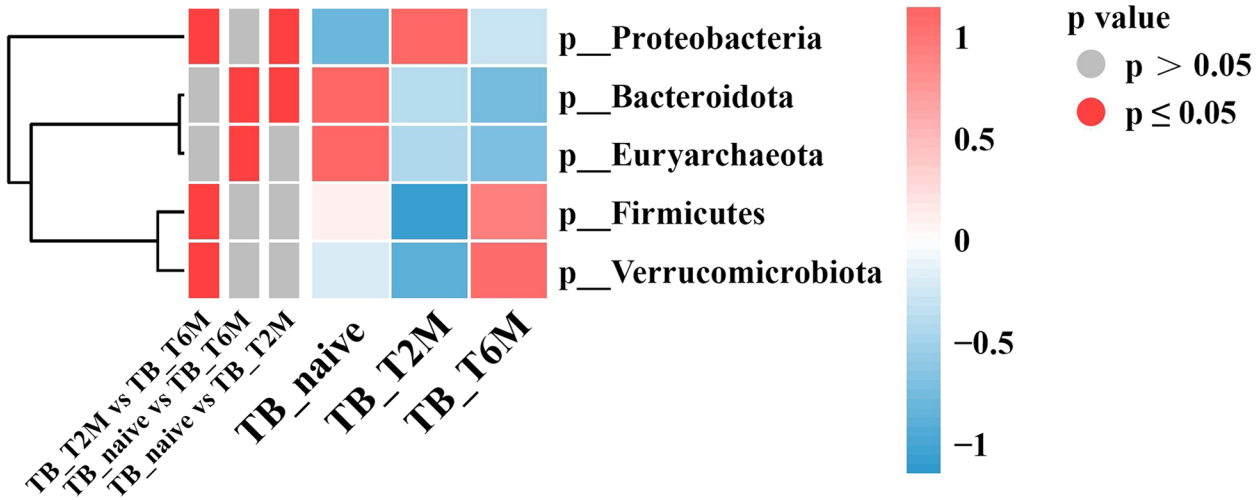


**Fig. 3** Differential species analysis at the phylum level

Studies on the dysbiosis in the gut microbiome of people with tuberculosis have drawn growing attention in recent years [10, 17, 22–38]. These studies have encompassed: (i) case control studies involving healthy controls and people with tuberculosis and (ii) cohort studies of people with tuberculosis before and after anti- tuberculosis treatment. Both designs have limitations. Case control studies often do not specify whether or not people start taking anti- tuberculosis drugs [23, 27]. Cohort studies that have been published have suffered from limited sample size and short follow-up time [30, 35]. This group of studies have yielded conflicting conclusions [24, 30] leading to debate in the tuberculosis research and clinical community. To our knowledge, our multi-center cohort study, focusing on the impact of anti-tuberculosis treatment on the structure and quantity of the gut microbiota, as well as on people' gastrointestinal function is amongst the largest and most followed-up of those currently published. Findings from our study suggest that anti-tuberculosis treatment has profound effects on gastrointestinal function and the gut microbiota, particularly during the intensive phase of therapy. After the intensive phase, there is some recovery of the gut microbiota, but this process is slow.

The human gut harbors a diverse array of microorganisms, collectively known as the gut microbiota, which comprises over 1000 species and has a bacterial cell count ten times that of the human body's total cells

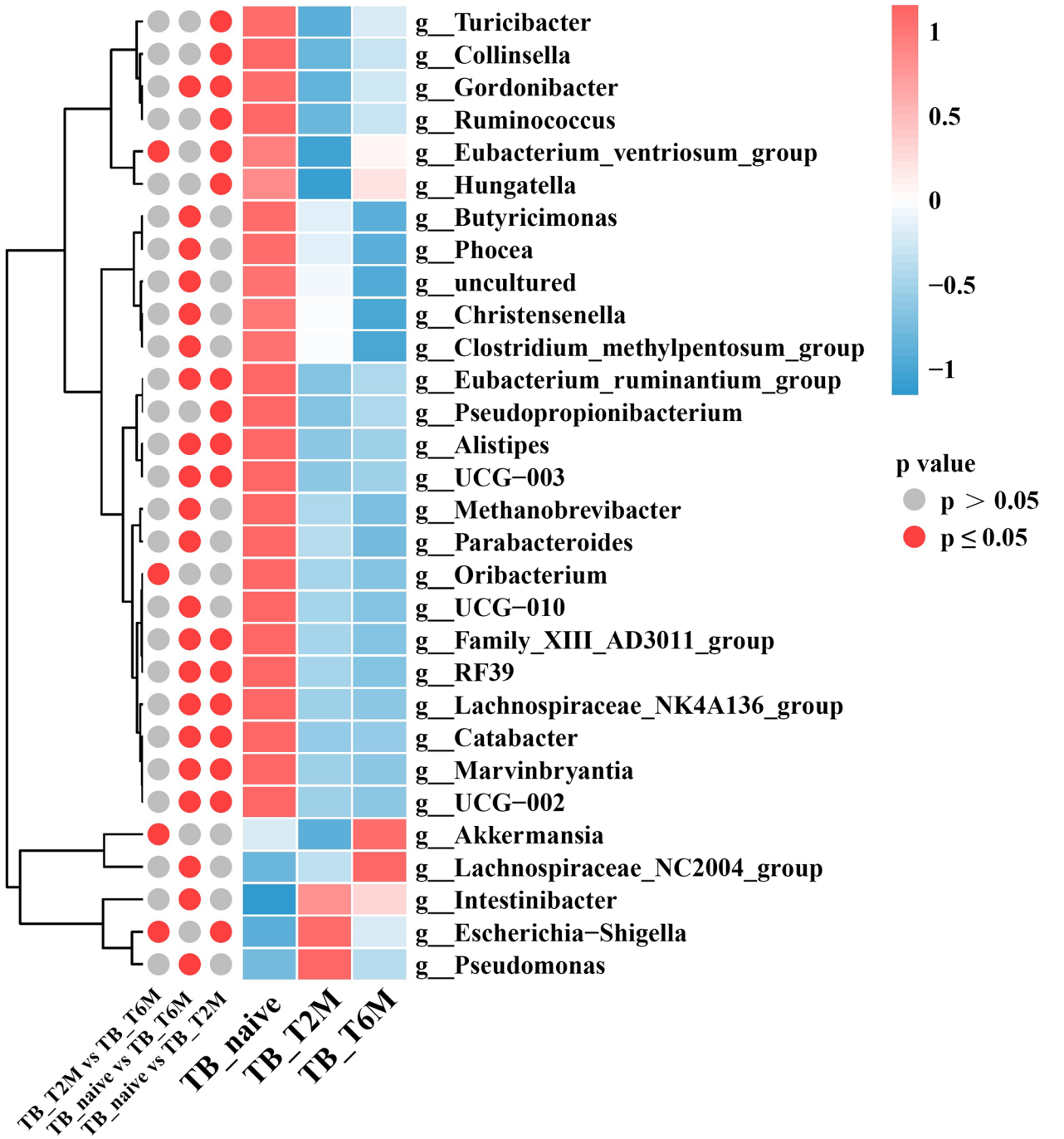


**Fig. 4** Differential species analysis at the genus level

[39]. Normally, the gut microbiota maintains a stable structure with probiotics and harmful bacteria each retaining a certain proportion. However, external environmental disturbances and other diseases of the host can significantly impact the composition, diversity, and abundance of the gut microbiota. Concurrently, the gut microbiota plays a considerably important role in human health. Recent research on gut microbiota has revealed that its influence on the human body extends far beyond a simple symbiotic relationship. The diverse gut microbiota not only engages in material and information exchange among themselves but also continuously communicates with host cells, co-evolves with the host, participates in host metabolism, and regulates

the transformation of important chemical substances, including drugs [7]. In theory, the prolonged and multifaceted use of broad-spectrum and narrow-spectrum antibiotics, as seen in anti-tuberculosis therapy, may exert significant impacts on the gut microbiota, both immediately and in the long term.

Bacteroidetes and Firmicutes are the predominant phyla within the gut microbiota [40]. Bacteria within the phylum Bacteroidota are capable of utilizing dietary fibers, resulting in the production of SCFAs [41]. Also known as volatile fatty acids, SCFAs include acetate, propionate, and butyrate [21]. SCFAs are one of the energy sources for colonocytes and can regulate host fatty acid synthesis and lipid synthesis, maintaining the stability of the gut microbiota [42], elevating the levels of anti-inflammatory cytokines and reducing inflammation [43], regulating the immune system [12, 44], including immune response to *Mycobacterium tuberculosis* [42, 45]. Acting as signaling molecules, SCFAs may travel throughout the body via the bloodstream, aiding in the regulation of the immune system. Apart from producing SCFAs [46], firmicutes exert control over systemic immune responses through their cell wall glycoconjugates, notably by facilitating the release of IL-34, which activates macrophages and bolsters defensive capabilities against pulmonary inflammation [47]. The cell wall glycoconjugates of Firmicutes more readily traverse the intestinal barrier, gaining access to the host's systemic circulation and persisting within the body, thereby enhancing their ability to modulate systemic immunity [47]. These functions highlight the multifaceted role of Firmicutes in maintaining host homeostasis and immune defense. Based on the findings from our cohort study, we conclude that anti-tuberculosis therapy is associated with a reduction in the abundance of phylum Firmicutes and phylum Bacteroidota, potentially exerting consequential effects on the immune system.

Enterococcus can enhance gastrointestinal adhesion by producing extracellular polysaccharides, which facilitates the colonization of probiotics. However, in people with tuberculosis undergoing long-term antibiotic therapy or with compromised immunity, Enterococcus are prone to translocation, leading to enterococcal infections. Moreover, their ability to accumulate mutations and acquire exogenous genetic elements confers additional antibiotic resistance, rendering Enterococcus as one of the major nosocomial pathogens [48]. During our follow-up period, we observed a sharp change in the abundance of *Mycobacterium tuberculosis* within the anti-tuberculosis treatment in people with tuberculosis, suggesting potential drug resistance or pathogenic risk.

Apart from being opportunistic pathogens, Escherichia-Shigella has been found to dominate the gut microbiome dysbiosis in people with tuberculous meningitis [49]. Treatment for tuberculosis meningitis typically involves higher doses of anti-tuberculosis drugs and extended treatment durations [50]. Therefore, we can reasonably speculate that Escherichia-Shigella could serve as a strong indicator for forecasting microbiota dysbiosis triggered by anti-tuberculosis treatment. Conversely, in our study, the genus Akkermansia exhibited a significant decrease, which was subsequently followed by a gradual increase. Similar results are also reflected in animal models. Compared to the control group, the abundance of Akkermansia in rats administered isoniazid and rifampicin for two weeks significantly decreased [51]. Oral administration of Akkermansia muciniphila or its metabolite palmitoleic acid significantly suppresses *Mycobacterium tuberculosis* infection in rats [52]. Also, the genus Akkermansia has been identified as a risk factor for the development of tuberculosis in a two-sample Mendelian randomization analysis [53]. The health impact of Akkermansia, whether beneficial or detrimental, is likely influenced by the interplay of several factors [54]. Future research is warranted to elucidate the underlying mechanisms.

This study has several limitations: First, research on the health impacts of the gut microbiota is still evolving, and to date, a standardized method for evaluating microbiota function has not been established. Various factors, including distinct study populations, sample size, sampling times, regions of the 16S rRNA gene, sequencing techniques, and analytical methods, may all influence the evaluation of the gut microbial community. This wide array of variables that may cause heterogeneity may lead to difficulties and limitations in comparing results across different studies. Second, this study only included people with drug-susceptible tuberculosis who were receiving initial treatment. Studies with larger samples including distinct types and severities of people with tuberculosis, including drug-resistant people, would be beneficial. Third, during the follow-up period, the people' dietary intake and possible probiotic supplementation were not documented in detail, which may introduce either bias or confounding.

## Conclusions

In summary, this study investigated differences in species composition and abundance of gut microbiota in newly diagnosed people with tuberculosis before and after the initiation of anti-tuberculosis treatment. The results demonstrate that anti-tuberculosis treatment significantly reduces the diversity of the gut microbiome, suggesting a profound impact on the intestinal microbial community during therapy, particularly in the intensive phase. The recovery of gut microbiota diversity after

intensive anti-tuberculosis treatment is slow and potentially incomplete, highlighting the need for strategies to support microbiome restoration in tuberculosis post-treatment. Additionally, the dynamic changes observed in opportunistic pathogens, such as Escherichia-Shigella, and the potential role of Akkermansia as a biomarker for microbiome dysbiosis during anti-tuberculosis treatment underscore the complexity of host-microbe interactions and their implications for tuberculosis management.

The findings of this study may provide valuable insight for the adjunctive treatment of people with tuberculosis, suggesting that the disruption of gut microbiota homeostasis may emerge as a potential therapeutic target and a valuable biomarker for medical interventions.

#### Abbreviations

| | |
|---|---|
| PZA | Pyrazinamide |
| EMB | Ethambutol |
| INH | Isoniazid |
| RIF | Rifampin |
| GSRS | Gastrointestinal Symptom Rating Scale |
| DOTS | Directly Observed Treatment Short-course |
| OTUs | Operational taxonomic units |
| PCoA | Principal coordinates analysis |
| LEfSe | Linear discriminant analysis (LDA) effect size |
| FDR | False discovery rate |

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#### Authors' contributions

QL, JW and SZ conceived the study, analyzed the data and drafted the manuscript; ZX, ZW and ZL participated in the study design and, implemented the field investigation involved in data collection; LZ, XF and LM participated in the study design and helped draft the manuscript. All authors contributed to the study and have read and approved the final manuscript.

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#### Data availability

The datasets generated and/or analysed during the current study are available in the NIH biosample repository, Accession Number is PRJNA1183742 [https://www.ncbi.nlm.nih.gov/bioproject/1183742].

## Declarations

#### Ethics approval and consent to participate

This study was approved by the ethics committee of Jiangsu Center for Disease Control and Prevention (JSJK2020-B009 -01). Written informed consent was obtained from study participants.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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