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Study on the Distribution of Respiratory Bacterial Communities in Patients with Pulmonary Tuberculosis

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Abstract: *Objective:* This study aims to reveal the changes in the lower respiratory tract bacterial community of tuberculosis patients compared to healthy individuals. It initially explores the correlation between the lower respiratory tract microbiota and the occurrence and development of pulmonary tuberculosis, aiming to provide new reference ideas for the treatment of pulmonary tuberculosis. *Methods:* This research methodology is based on second-generation sequencing, targeting the V3-V4 variable region of bacterial 16S rDNA for high-throughput sequencing. The study collected bronchial epithelial brush-off samples from 11 healthy volunteers and 20 confirmed pulmonary tuberculosis patients in Yunnan Province for sequencing. Subsequently, a comparative analysis of the sequenced bacterial community results was conducted to explore the association between tuberculosis and human microecology. *Results:* In this experiment, 40 samples of bronchial epithelial brush-off and throat swab were collected from 20 smear-positive pulmonary tuberculosis patients. For the healthy control group, 55 samples of bronchial epithelial brush-offs were collected from five different lung segments of 11 healthy volunteers in Yunnan Province. High-throughput sequencing was performed on these samples. The results showed that the colonized flora present in the lower respiratory tract of healthy people in our province mainly includes Bacillus, Lactococcus, Enterococcus, and Streptococcus at the genus level. The results from 20 tuberculosis patients revealed that the number of lower respiratory tract flora in tuberculosis patients is richer than that of healthy people. Besides *Mycobacterium tuberculosis*, the dominant bacteria mainly include Prevotella, Veillonella, Pseudomonas, and Streptococcus. Although Prevotella is the dominant flora in the samples of tuberculosis patients, its abundance varies significantly among samples. *Conclusion:* Comparing the respiratory flora of healthy people and tuberculosis patients revealed significant differences. Not only have the types and abundance of bacteria increased significantly, but there are also unique bacterial species in the patient's respiratory tract. This suggests that these unique bacteria are likely closely related to the occurrence and development of pulmonary tuberculosis. Furthermore, bacterial abundance may also be related to antibiotic use, providing a new direction for further research.

Keywords: Respiratory flora; Pulmonary tuberculosis; 16SrDNA; Sequencing technology

1. Introduction

According to the World Health Organization (WHO) estimates, there were approximately 10.4 million new tuberculosis cases globally in 2016, with 1.67 million deaths attributed to the disease [1]. Pulmonary tuberculosis has become one of the significant public issues constraining China's socio-economic development and progress [2]. The prevention and control of tuberculosis face unprecedented challenges [3,4]. Currently, researchers' investigations into the pathogenesis of tuberculosis primarily focus on two main directions: one centers around Th1 and Th2 cytokines. When the balance between the two is disrupted in the body, it can lead to the latent development of tuberculosis [5]. The second focus is on Mycobacterium tuberculosis (MTB), an intracellular parasite that alters the host's energy and protein metabolism through host-pathogen interactions [6,7], thereby triggering disease or inflammation. Additionally, there are studies exploring diagnosis, treatment, and pathogenesis at the proteomic level. This article takes a new approach, delving deeply into the development process of pulmonary tuberculosis from a fresh perspective, aiming to provide new directions for the treatment of tuberculosis.

The human body harbors a vast number of microorganisms. It is estimated that the human microbiota contains up to 10^{14} bacterial cells, which is ten times the number of human cells [9]. Skin commensal bacteria can protect the body from pathogens [10], while the intestine also hosts a significant amount of colonized microorganisms, accounting for approximately two-thirds of all microorganisms in the human body. Thus, the intestine is often regarded as the primary gathering place for human microorganisms, exerting a crucial influence on human health [11,12]. Due to difficulties in obtaining sterile lung samples and limitations of traditional culturing techniques, the lower respiratory tract has long been considered a sterile environment without colonized microorganisms [13–15]. However, in recent years, with the widespread application of fiberoptic bronchoscopy and rapid advancements in molecular detection techniques for microorganisms, it has become possible to investigate bacterial composition without the need for culturing individual microorganisms, greatly improving detection efficiency and accuracy [16,17]. Numerous studies have indicated that an increased proportion of neutrophils in the airways is a key factor in the progression and exacerbation of inflammation in Chronic Obstructive Pulmonary Disease (COPD) [18,19].

Other literature suggests a close relationship between Potentially Pathogenic Micro-organisms (PPMs) and airway inflammation during the transition from stable COPD to acute exacerbations [20]. In respiratory samples from patients with bronchial asthma, the proportion of various respiratory pathogens, such as *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* is higher than in the normal population [21,22]. Additionally, the diversity of bacterial communities in the lungs of patients with cystic fibrosis is significantly lower compared to healthy individuals [23]. These studies demonstrate that numerous respiratory diseases are closely related to changes in the respiratory microbiota. However, there are few reports on the respiratory microbiota of tuberculosis patients. This article focuses on patients with open pulmonary tuberculosis, revealing their respiratory microbiota status and initially exploring the correlation between lower respiratory tract microorganisms and the development of tuberculosis. It is hoped that this can provide a new reference for the clinical treatment of tuberculosis.

2. Materials and methods

In this experiment, two sets of samples were collected. For the healthy control group, bronchial mucosal scrapings from five lung segments on both sides were collected via fiberoptic bronchoscopy. For the tuberculosis group, bronchial mucosal scrapings were collected through fiberoptic bronchoscopy, along with throat swab samples. Patients in the tuberculosis group were required to have a positive sputum smear and a positive Xpert-MTB test.

2.1. Selection of research subjects

The health status of healthy volunteers and patients was evaluated through medical history inquiries (recorded in medical records), physical examinations, and necessary laboratory tests. After evaluation and screening, healthy volunteers were excluded if they met any of the following criteria:

(1) Used antibiotics or probiotics outside the hospital in the month before the visit;

(2) Had a history or current pulmonary infection, including cured tuberculosis, acute cardiac insufficiency, or diseases causing acute cough;

(3) Had chronic lung diseases such as chronic bronchitis, COPD, cystic fibrosis (CF), pulmonary interstitial fibrosis, bronchial asthma, chronic cardiac insufficiency, or diseases causing chronic cough;

(4) Had genetic metabolic diseases such as diabetes, hyperthyroidism, or congenital muscular dystrophy.

Exclusion criteria for tuberculosis patients were:

(1) Used antibiotics or probiotics outside the hospital in the month before the visit;

(2) Had other lung infections besides tuberculosis;

(3) Had tuberculosis combined with chronic lung diseases;

(4) Had tuberculosis combined with other genetic metabolic diseases.

After strict screening based on the above exclusion criteria, the study finally selected 11 healthy volunteers, including 8 males and 3 females, aged between 30 and 70 years old, and 20 tuberculosis patients, including 8 males and 12 females, aged between 16 and 75 years old. After analysis, the age groups of the two sets of subjects were relatively close, and there were basically no other diseases that could affect the microecology.

2.2. Methods

This study relied on second-generation sequencing technology to perform high-throughput sequencing targeting the V3–V4 variable region of bacterial 16S rDNA. The two sets of samples were analyzed, and then a comparative analysis of the bacterial community results obtained from sequencing was conducted to explore the relationship between tuberculosis and human microecology.

2.3. Statistical analysis

In this experiment, QIIME software was used to adjust the distance difference between samples based on importance, detect the beta diversity of respiratory bacteria, and analyze differences between groups. Two-dimensional or three-dimensional coordinates were created to identify important biomarkers. Special data was estimated using bias-corrected unimodal embedding, and hierarchical clustering analysis, principal coordinate analysis, and other analytical charts were obtained through R language analysis. Differences were analyzed using the *t*-test and nonparametric tests. A P-value < 0.05 indicated a significant difference, and the false discovery rate q-value was calculated for the P-value.

3. Results

3.1. Detection results of lower respiratory tract bacteria at the genus level in healthy populations

The dominant colonizing bacteria in the lower respiratory tract of healthy populations in our province include Bacillus, Lactococcus, Enterococcus, and Streptococcus. The composition of the bacterial flora in the left and right lungs, as well as the upper and lower lobes of the lungs, of healthy individuals in our study indicated that there were no significant differences among all lung segments of the left and right lungs in healthy individuals from the same region (**Figure 1**).

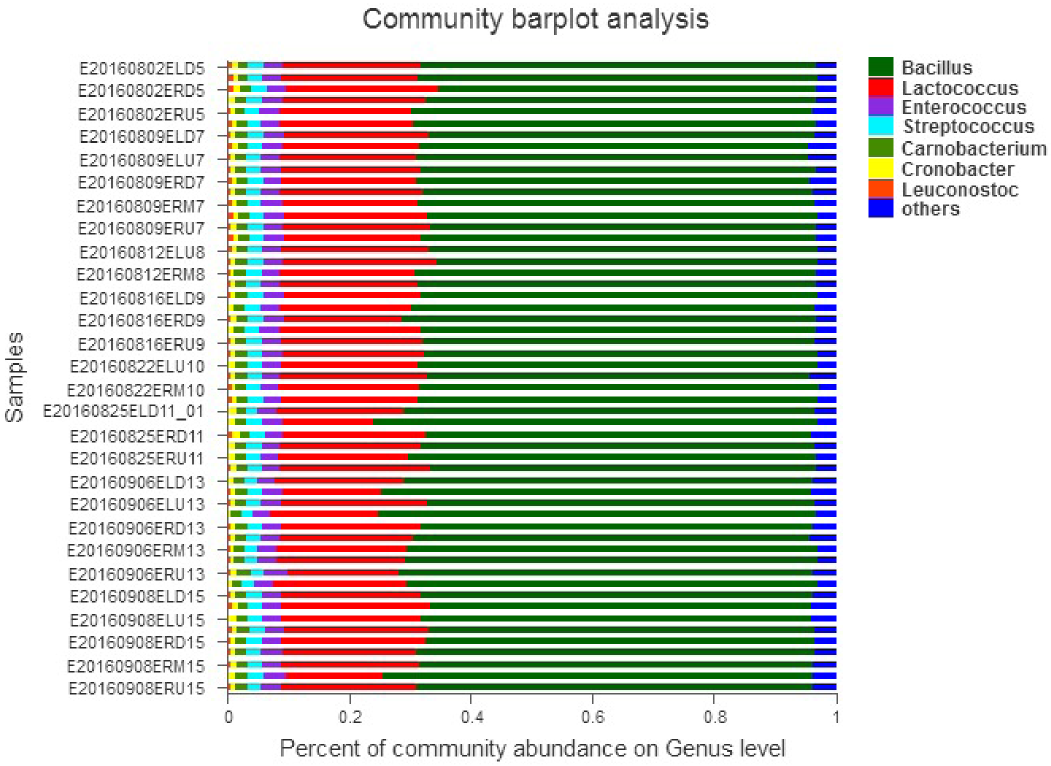

Figure 1. Bar chart showing the abundance of bacterial flora at the genus level in the lower respiratory tract of healthy individuals.

3.2. Detection results of lower respiratory tract bacteria at the genus level in tuberculosis patients

By comparing the composition of respiratory flora between healthy individuals and tuberculosis patients, it was found that the species and abundance of respiratory bacteria in tuberculosis patients increased significantly. Moreover, the respiratory tract of tuberculosis patients had its unique dominant bacterium, Prevotella, which is an anaerobic conditional pathogen that is difficult to cultivate effectively using traditional methods. Additionally, the abundance of other anaerobic bacteria in the respiratory tract of tuberculosis patients also increased, suggesting that the growth of anaerobic bacteria, predominantly Prevotella, may be closely related to the occurrence and development of tuberculosis.

After drawing the research conclusions, a review of patient case data revealed significant differences in the abundance of dominant bacteria among patients who received antibiotic treatment before bronchoscopy. Meanwhile, gender factors may also affect the distribution of dominant bacteria. Comparing the differences in bacterial colonies among various tuberculosis patients, it was discovered that some patients had a significantly higher proportion of *Mycobacterium tuberculosis*, resulting in a substantial decrease in the abundance of newly emerging dominant bacteria. This could be caused by the amount of tuberculous infection or by certain factors inhibiting the growth of dominant bacteria, thereby promoting the proliferation of *Mycobacterium tuberculosis* (**Figure 2**).

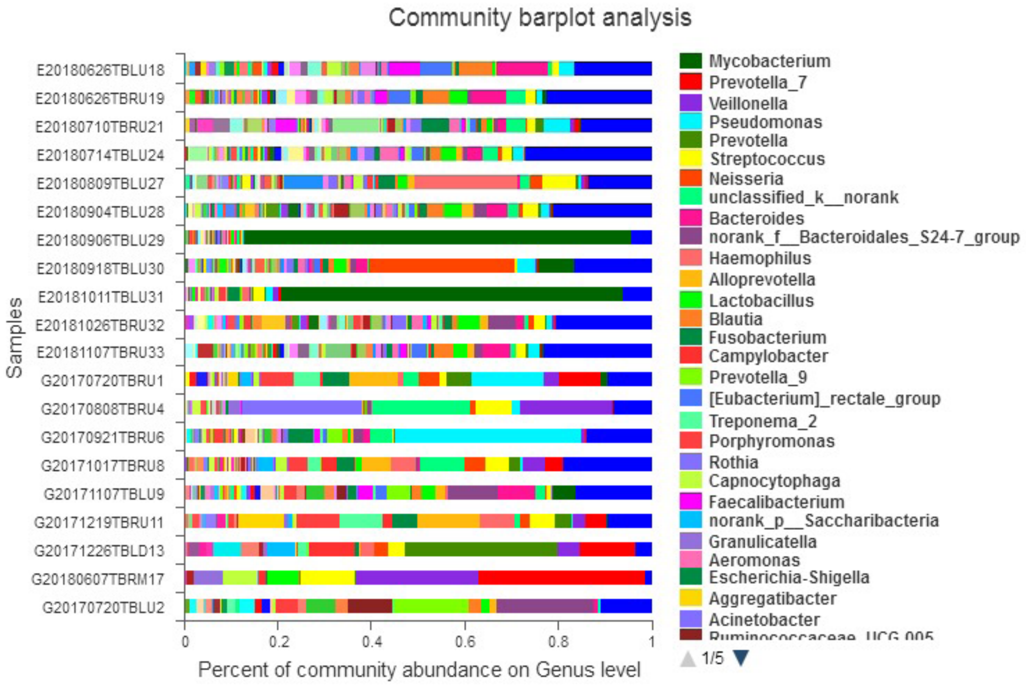

Figure 2. Bar chart showing the abundance of bacterial flora at the genus level in the lower respiratory tract of tuberculosis patients.

3.3. Detection results of upper respiratory tract bacteria at the genus level in tuberculosis patients

The dominant bacteria in the upper respiratory tract flora of tuberculosis patients include Prevotella, Streptococcaceae, Fusobacteriaceae, and Veillonellaceae. Among them, Streptococcaceae and Veillonellaceae are common colonizing bacteria in the upper respiratory tract. The abundance of Streptococci and Fusobacterium is relatively high. Prevotella is represented by four genera. Although the proportion of each genus varies, their overall proportion is still significantly higher than other bacteria (**Figure 3**).

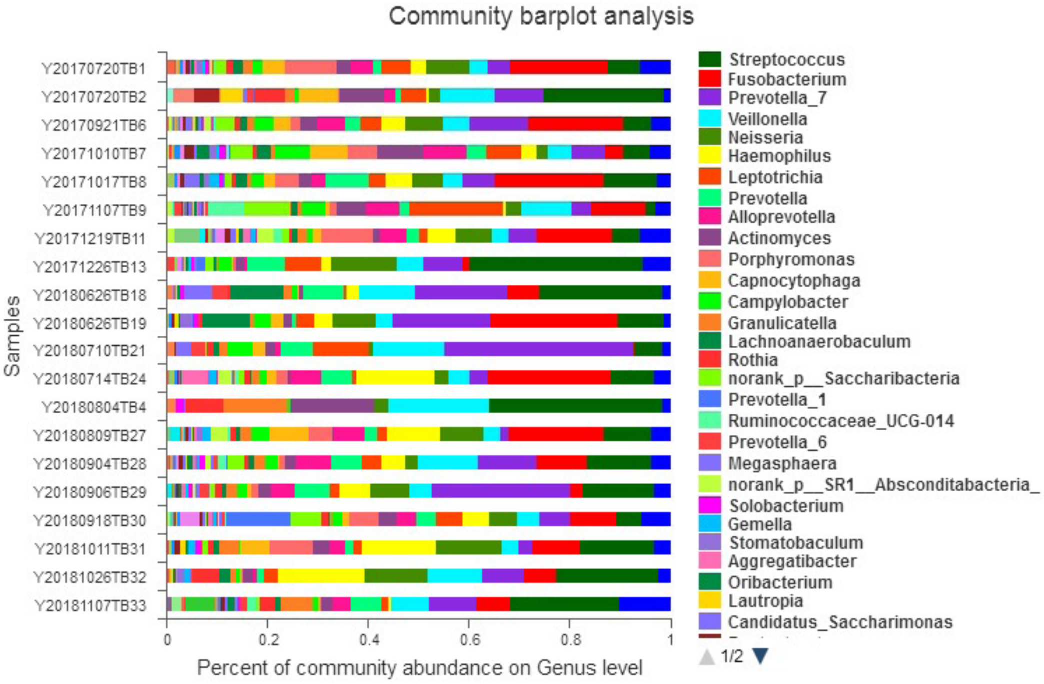

Figure 3. Bar chart showing the abundance of bacterial flora at the genus level in the upper respiratory tract of tuberculosis patients.

4. Discussion

The results of this study show that, when comparing samples from healthy volunteers with those from tuberculosis patients, there is a significant increase in the diversity and abundance of respiratory bacteria in tuberculosis patients. However, there are some overlapping bacteria in the two groups of samples, which the study believes are likely to exist as background bacteria and have a smaller impact on disease development. It is worth focusing on other bacteria in the samples of tuberculosis patients that are distinct from healthy individuals. These bacteria may rapidly proliferate based on the original foundation after the disease occurs, suggesting that when the human body is infected with tuberculosis, the respiratory microecology not only increases *Mycobacterium tuberculosis* but also breaks the original balance, leading to more active bacteria. During the occurrence, development, and prognosis of tuberculosis, various bacteria may have a significant or minor role, especially the dominant flora with higher abundance, which should be given extra attention as they are likely to play a key role in the disease process.

Tuberculosis is a respiratory infectious disease. However, there are significant differences in the prognosis of individuals infected with *Mycobacterium tuberculosis*. Some people do not develop the disease after infection and only discover a history of infection during physical examinations. This indicates that the onset of tuberculosis may be closely related to changes in the body's microecology, besides being associated with *Mycobacterium tuberculosis* infection. Therefore, if other treatment methods can be used to maintain the lung microecology of patients consistent with that of healthy individuals, it may be possible to improve the prognosis of tuberculosis patients. Of course, this requires continuous follow-up to observe changes in bacteria. If a bacterium is identified as a pathogenic bacterium causing the disease, a large amount of literature needs to be reviewed to explore effective and feasible countermeasures.

5. Conclusion

There are significant differences in respiratory microorganisms between tuberculosis patients and healthy individuals. These unique bacteria are likely to be closely related to the occurrence and development of tuberculosis. Additionally, bacterial abundance may also be associated with antibiotic use. In subsequent studies, the study will focus on analyzing the specific mechanisms of these unique bacteria in the process of tuberculosis, while exploring the intrinsic relationship between antibiotic dosage, duration, and changes in bacterial abundance. The study hope to provide a scientific basis for tuberculosis prevention and treatment strategies.

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Disclosure statement

The authors declare no conflict of interest.

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