Secondary Analysis of Host Lung Microbiome Interactions with Tuberculosis

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Abstract

In the past decade, multiple studies have examined the human lung microbiome in tuberculosis (Tb) patients, reflecting a growing interest in human microbiomes and their impact on health outcomes. This project involved a secondary analysis of microbiome diversity and bacterial abundances at the species- and genus-levels in individuals infected with Tb compared to healthy controls. The analysis used 16S rRNA sequencing data from four studies, published between 2013 and 2016 ,which took place in China, India, and Colombia. These data comprised 145 samples and were collected from different sample types including a respiratory secretion called sputum, nasal swabs, oropharynx (throat) swabs, and bronchoalveolar lavage fluid. The raw data was processed using the PathoScope processing pipeline. The data analysis was then conducted using the animalcules R package, a microbiome analysis tool that provides various data visualizations such as boxplots, heatmaps, and bar charts. Two of the most prominent patterns observed in our analysis were an increased abundance of *Streptococcus* pneumoniae in nasal samples and a concurrent decrease of *Prevotella* and *Veillonella* in oropharynx samples in Tb patients compared to controls. Neither of these abundance changes were observed in the sputum samples of the same individuals despite sputum being the predominant sample type among existing Tb/lung microbiome studies. Future research on the microbes identified in this study will determine if the observed patterns have connections to clinical Tb symptoms or can aid in the diagnosis of Tb. Additionally, the fact that these patterns were present in nasal and oropharynx samples suggests that airway samples beyond sputum should be collected in the future.

Background and Research Questions

We are interested in studying the way that the host lung microbiome interacts with tuberculosis (Tb) infection in hopes of applying that knowledge toward methods for tuberculosis diagnosis and/or treatment. The overarching goal of this work is to use computational tools to develop microbiome signatures that have clinical applications in tuberculosis treatment through diagnostics and predictions of disease progression, and investigation of existing microbiome data is the first step to understanding the associations between the human microbiome and Tb infection. After preliminary examination showed that there was a much greater presence of the genus *Streptococcus* in Tb patients in the 2014 Botero et al. data, we were interested in exploring three questions in this dataset:

1. Is this increased abundance of *Streptococcus* in Tb patients the result of novel infection or opportunistic growth?
2. Are there other bacterial taxa whose abundance is related to Tb infection status?
3. Are these microbial infection patterns associated with specific sample types?

Methods

Botero data (2014):
- 16S rRNA sequencing using the V1-V2 hyper-variable region

	Control	TB	Total
Nasal	6	6	12
Oropharynx	6	6	12
Sputum	N/A	6	6
Total	12	18	30

Raw Data → PathoScope → animalcules → Data Visualizations

Results
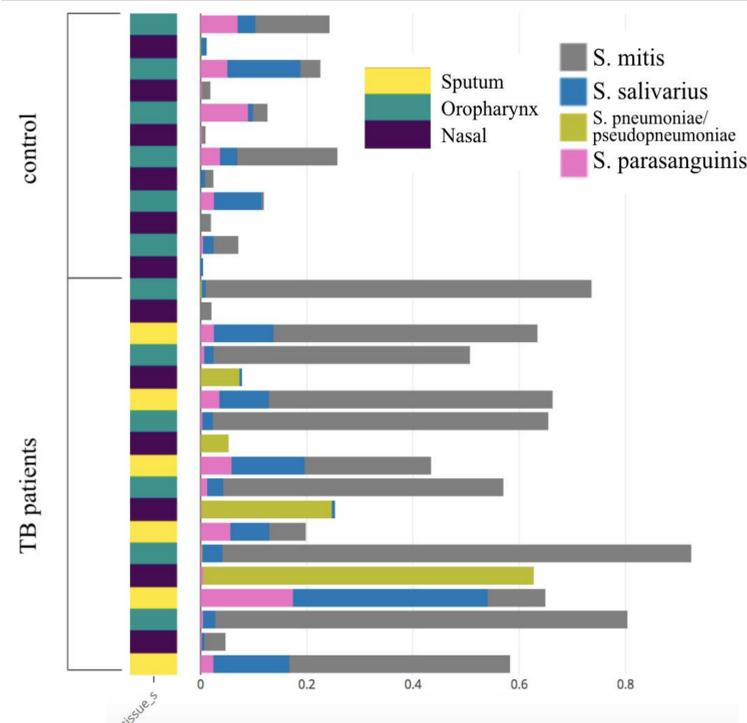
Figure 1. Relative abundances of *Streptococcus* species, colored by sample type

Legend: Sputum, Oropharynx, Nasal; S. mitis, S. salivarius, S. pneumoniae/pseudopneumoniae, S. parasanguinis

Differential analysis using DESeq2 yielded an adjusted p-value of 0.0314 on the condition of disease and 4.4e-15 on the condition of sample type (comparison of nasal and sputum)

Figure 2. Relative Abundances of *Prevotella* and *Veillonella* in Oropharynx samples, colored by disease condition

Legend: Prevotella, Veillonella

Differential analysis using DESeq2 yielded an adjusted p-value of 0.0357 for *Veillonella* and 0.0926 for *Prevotella* on the condition of disease.
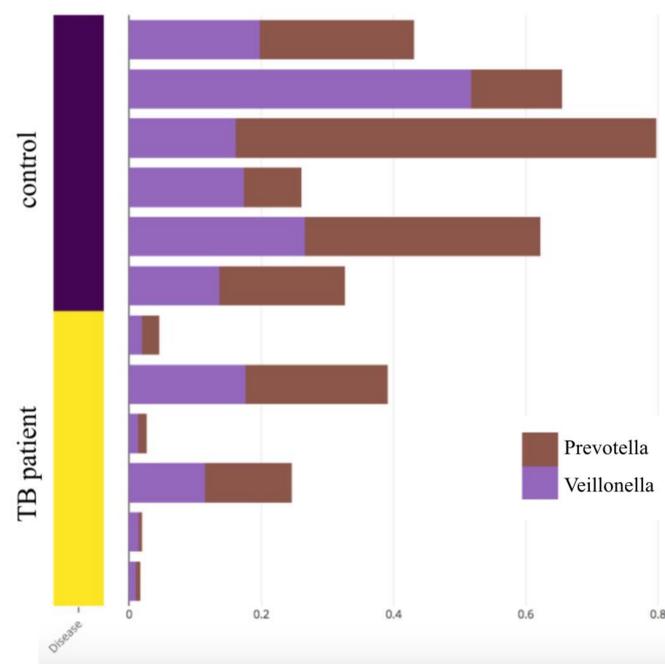
Results, Cont'd

1. Most *Streptococcus* species appear to be opportunistic growth because the populations exist in controls as well as patients, but the fact that *S. pneumoniae* and *S. pseudopneumoniae* are only present in Tb patients suggests novel infection.

2. In 4 of 6 Tb patients, the relative abundances of the genera *Prevotella* and *Veillonella* are substantially decreased compared to the control samples; further, this decrease occurs either in both genera or neither, suggesting a relationship between the two.

3. Presence of *S. pneumoniae/pseudopneumoniae* is only visible in nasal samples; *Prevotella/Veillonella* associated decrease is only visible in oropharynx samples.

Discussion and Future Directions

- *S. pneumoniae/pseudopneumoniae* are pathogenic species of *Streptococcus* causing pneumonia; *Prevotella* and *Veillonella* both have potential associations with digestive pathways
  - The observed patterns may be related to clinically observed tuberculosis symptoms

- Sputum is the preferred sample type in Tb/host lung microbiome research, but there is valuable information to be gained from taking other types of samples as well

- Further research is required to confirm results in larger datasets and to elucidate specific roles of the microbes in question

References and Acknowledgements

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