META-TAXONOMIC PROFILING OF MICROBIAL CO-OCCURRENCE PATTERNS IN THE LUNGS OF TUBERCULOSIS PATIENTS

ADRAJ, S. A.[1] – MAKKI, R. M.[2] – AL-SHAHRANI, M. S.[3] – ALZAHRANI, A.[4] – ASIRI, K. A.[5] – TASHKANDY, N. R.[6*]

[1]Department of Biological Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
(e-mail: sadraj@stu.kau.edu.sa)

[2]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
(e-mail: rmakki@kau.edu.sa)

[3]Department of Medical Laboratories of Tuberculosis, King Fahad Armed Force Hospital, Jeddah, Saudi Arabia
(e-mail: MAlShahrani@kfafh.med.sa)

[4]Abdulwahab Alzahrani, Department of Molecular Biology, King Fahad Armed Force Hospital, Jeddah, Saudi Arabia
(e-mail: Abdulwahab.alzahrani@kfafh.med.sa)

[5]Department of Medical Laboratories of Tuberculosis, King Fahad Armed Force Hospital, Jeddah, Saudi Arabia
(e-mail: KASIRI@kfafh.med.sa)

[6]Department of Biological Sciences, Faculty of Science, King Abdul Aziz University, Jeddah 21598, Saudi Arabia
(phone: +966-543-353-535)

[*]Corresponding author
e-mail: ntashkandy@kau.edu.sa

(Received 5th Mar 2024; accepted 8th Jul 2024)

Abstract. Few studies have characterized the respiratory microbiota of tuberculosis disease, with inconclusive results. Our aim was to compare the microbial ecosystem of the sputum between Tuberculosis patients and patients with moderate lung disease of asthma, Chronic Obstructive Pulmonary Disease, bronchiectasis, and lung fibrosis using metagenomic profiling techniques. In particular, we assessed the common, overlapping communities of Tuberculosis patients. The Bacterial communities in the sputum of pulmonary tuberculosis patients within a cohort of patients in Saudi Arabia were identified using amplicon sequencing. Forty sputum samples were collected from patients admitted to King Fahad Armed Forces Hospital from (2019-2020). 16S rRNA V3-V4 hypervariable regions were sequenced using Illumina technology and analyzed using a pipeline of standard bioinformatics techniques to identify unique and common patterns of microbial communities. Our results demonstrated that the microbiota of the sputum of pulmonary tuberculosis patients was similar to that of the sputum of control participants at the phylum and genus levels. Additionally, we observed heterogeneity of taxa at the individual level. At the family level, the abundance of Mycobacteriaceae did not differ between anti-tuberculosis-treated and untreated cases. In contrast, the Corynebacteriaceae and Lactobacillaceae families differed significantly under the antibiotic and anti-TB regimens, respectively. This study provides insights into the microbial communities found in sputum samples from individuals with tuberculosis (TB) and the potential alterations that may occur as a result of antituberculosis treatment.

Keywords: *microbial communities, 16S rRNA gene sequencing, anti- tuberculosis regimens, antibiotics, Mycobacteriaceae family*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

Introduction

Tuberculosis (TB) epidemics are highly heterogeneous, and the World Health Organization (WHO) has listed high-burden countries in three categories: TB, multidrug-resistant TB and TB/HIV (WHO 2018). In western Saudi Arabia, the incidence of tuberculosis disease was 10.1/100,000 in 2018, decreasing to 8.7/100,000 in 2019, according to the Saudi Ministry of Health. TB is a major infectious disease in which it is challenging to obtain a deeper understanding of pathogen coinfections and antibiotic resistance patterns (Langbang et al., 2016). Interestingly, drug-resistant TB isolates did not appear to cluster or spread more broadly than drug-sensitive isolates, implying that poor treatment, rather than increased virulence or persistence, is the primary driver of the formation of drug resistance (Alyamani et al., 2019). Other factors also play a role in TB pathogenicity, as it has been shown that hypoxia, a challenging condition for *M. tuberculosis*, leads to the generation of a surviving population with drug resistance-conferring mutations (Smith et al., 2013).

The lung is believed to be the human organ with the lowest degree of microbial colonization, yet culture-independent methodologies have identified up to five hundred species in the lung core microbiota, including members of Firmicutes, Bacteroidetes and Proteobacteria (Shukla et al., 2017). Proteobacteria has been proposed to provide a "microbial signature" of diseases (Rizzatti et al., 2017). The homeostasis of the lung microbiota can be seriously disrupted under infections, which results in extensive waves of colonization (Namasivayam et al., 2019). Despite recent advances in microbiome profiling via next-generation sequencing (NGS), there are limited studies that have reported the lung microbiota under TB infection (Cui et al., 2012; Cheung et al., 2013; Wu et al., 2013; Botero et al., 2014; Vázquez-Pérez et al., 2020).

Two meta-analyses of TB data from Asia and Colombia revealed that the respiratory microbiota composition was complex and that the recorded diversity varied from study to study due to sample size, sequencing depth, data analysis methods (Majaneva et al., 2015), subject variability, and control selection. However, in pulmonary cases, the predominant detected bacterial clusters were Actinomyces and Rothia (Hong et al., 2018), whereas in another study (Eshetie and van Soolingen, 2019) found in addition to Rothia, Veillonella and Lactobacillus were revealed to be specific to the TB samples. The predominant family Burkholderiaceae was identified for the first time in TB sputum cases (Ueckermann et al., 2022). In contrast, in animal studies using a macaque model (Cadena et al., 2018), Leptotrichia, Prevotella, and Campylobacter were highly enriched in the TB group. Whether these microbiotas represent a continuum with the upper respiratory tract, or a distinct TB lung community remains an open question.

The effect of a long antituberculosis regime on microbiota diversity in the lower respiratory tract and the gut was discussed in a recent review (Barbosa-Amezcua et al., 2022), indicating an increased spread of TB infection under microbiota dysbiosis. A study showed a correlation between high Pseudomonas abundance and the persistence of Microbacterium in the recurrent TB infection experimental group. In addition, two recent studies (Lin et al., 2021; Valdez-Palomares et al., 2021) identified differential abundance of the family Sphingomonadaceae and increased abundance of the genera Leptotrichia and Campylobacter Granulicatella, respectively, in the drug-resistant tuberculosis groups compared to the drug-sensitive TB cases.

The overall aim of this work was to develop a better understanding of the human lung microbiome associated with tuberculosis infection, as individuals diagnosed with tuberculosis (TB) frequently exhibit an increased vulnerability to other infections and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

comorbidities. Investigating the involvement of the lung microbiota in these interactions and tailoring treatment regimens based on the microbiota profile of an individual might optimize treatment outcomes.

Materials and methods

Study design and sample collection

The study protocol was approved by the institutional review boards of the University of King Abdulaziz University and King Fahad Armed Forces Hospital from (2019-2020). Forty sputum samples were collected from patients clinically diagnosed as positive or negative for pulmonary tuberculosis and were sent to microbiology laboratories for routine examination. The remaining portion of the sputum samples was preserved at -80 °C for subsequent DNA extraction. The inclusion criteria for positive TB sputum samples were based on the polymerase chain reaction (PCR) laboratory results, and controls were collected based on coughing symptoms, sputum expectoration, and negativity for TB by PCR for comparison. The clinical data on antibiotic class usage for the controls were insufficient. In the TB sputum group, 10 cases were new TB infections, and 7 patients received standard first line antitubercular medications (isoniazid, pyrazinamide, ethambutol and rifampicin) for four months. *Table 1* presents the metadata of the 36 sputum samples included in this study from which high-quality DNA sequences were generated.

Table 1. *Differentially abundant species between sample types. Red marks species with increased and green with decreased abundance. Across comparisons common species are recorded in bold*

Variables	positive TB sputum samples (n=17)	negative TB sputum samples (n=19)
Age	14 – 61 (median 42)	23 – 87 (median 54)
Sex		
Male	12	13
Female	5	6
Comorbidities		
Diabetes	6	7
Colon cancer	1	0
HIV	0	3
Asthma	0	2
COPD	0	1
Lung fibrosis	0	2
Ischemic heart disease	2	2
Smoking	7	6
Treatments	Anti-TB 7	Antibiotic 4

DNA extraction and quantification

DNA sputum samples was extracted from 40 sputum samples using an automated system (MagNA Pure Compact System, Roche Life Science) and MagNA Pure Compact Nucleic Acid Isolation Kit I — Large Volume, according to the manufacturer's protocol. The extracted nucleic acids were used for the PCR amplification of 16S rRNA for

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

microbiota identification with the Illumina MiSeq system. The quantity of the DNA extracted from each sample was measured with a Quant-iT™ PicoGreen® dsDNA Assay kit and a Quantus™ Fluorometer and by gel electrophoresis.

PCR amplification and Illumina MiSeq sequencing

High-throughput 16S rRNA gene paired-end sequencing was performed, and index reads were used to multiplex a large number of samples in a single run according to the Illumina MiSeq sequencing protocol. Sequencing of the 16S rRNA V3-V4 hypervariable regions was performed using Bakt_341F CCTACGGGNGGCWGCAG as the forward primer and Bakt_805RG ACTACHVGGGTATCTAATCC as the reverse primer. For NGS library quality assessment, the template size distribution was checked on an Agilent Technologies 2100 Bioanalyzer using a DNA 1000 chip according to the manufacturer's protocol. To quantify the prepared libraries, qPCR was performed according to the Illumina qPCR Quantification Protocol Guide. To generate a standard curve of fluorescence readings and calculate the library sample concentration, Roche's Rapid Library Standard Quantification Solution and Calculator were used.

Data analysis, annotation and visualization

QC & clustering: Raw sequence read data, trimmed to remove long tails, were preprocessed using CD-HIT (Li and Godzik, 2006) to remove redundancy (at 97% sequence identity). This step resulted in 1,296,528 reads with a gamma diversity index (absolute number of OTUs) of 634, and processing was performed with UClust (Edgar, 2010). Among these sequences, 35 were identified as nearly identical to human sequences and were thus excluded from downstream analysis. The total number of reads that were included in the analysis after the quality control steps was 599. We define this reference set at the 97% redundancy level as R97. To proceed to consistent and reliable assignment to taxonomic categories (OTUs) in R97 and eliminate uncertainty related to overspecifying genus- and/or species-level sequence matches, we further applied CD-HIT to generate a reliable set of markers (at 97% sequence identity).

Taxonomic assignment and annotation

Taxon assignment: The 599 sequences in the R95 set were used to search GenBank and assign them to the most likely taxonomic level using RDP Classifier (Wang et al., 2007) in both Mothur (Schloss et al., 2009) and Silva (Quast et al., 2013). Chimera removal: All sequences were used for de novo and subsequent SILVA-based chimera detection using the uchime3_denovo and uchime_ref functionalities, respectively, as implemented in the vsearch package (v.2.21.1). No chimeras were detected in de novo mode, while 212 chimeras and 4 borderline sequences were identified by the reference-based module. Chimeric and borderline sequences were removed prior to downstream analyses.

Diversity statistics and relative abundance calculation

Within-sample (alpha) diversity was assessed using the observed taxa and Shannon index variation and tested with Wilcoxon (for two conditions) or Kruskal–Wallis and Dunn post hoc tests (for multiple conditions) (Costello et al., 2009; Turnbaugh et al., 2010). Across-sample (beta) diversity measurements were performed to examine sample dissimilarity by using the Aitchison distance. As described elsewhere (Gloor et al., 2017),

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

the use of the aforementioned distance metric is highly recommended, as it takes into consideration the compositional nature of 16S rRNA sequencing data. To assess the effect of each measured environmental factor on sample differences regarding the microbial composition, PERMANOVA was used. Distance matrices were con-structed based on an index of similarity of community membership and structure, clustered based on Aitchison distance (Gloor et al., 2017) to reveal major groups. The accepted paired-end, primer-trimmed reads were deposited in the National Center for Biotechnology Information database under accession numbers OL905961-OL906057 and OM311297-OM311574. Per sample relative abundance was calculated as the ration of each OTU's abundance to the total OTU abundance of the sample.

Results

Description of microbial sequencing results

Among a total of 40 sputum samples, only 36 samples generated high-quality reads, including 17 TB-positive samples and 19 controls (Supplementary *Table 1*). There was a higher percentage of male subjects in both groups due to the sample properties. The raw sequences were generated as FASTA files, with 634 sequences covering a total length of 261,498 bp. Subsequent quality filtration resulted in 599 sequences ranging from 402 bp to 466 bp (average 418 bp; std. deviation 46.995 bp) and an average GC content of 53.326% (std. deviation 3.881). All of these sequences have been assigned to unique taxonomic-level identifiers. After chimera identification and removal, 383 sequences remained for down-stream analysis.

Diversity of microbial communities within and between sample phenotypes

Chimera-free sequences were employed to assess alpha diversity using both the number of observed taxa and the Shannon index. *Figure 1a-b* shows that sample comparisons according to infection status yielded no significant difference regarding within-sample diversity. The same outcome was obtained when comparing within-sample diversity between the different treatment groups (*Figure 1c-d*). Finally, the same levels of alpha diversity emerged when assessing sample-type groups (simultaneously taking into con-sideration treatment regimens and infection status) (Supplementary *Figure 1*).

Subsequently, we examined between-sample diversity using the Aitchinson distance to take into consideration the compositional nature of our data. More specifically, our samples were clustered into three major groups, with no clinical phenotype dominating the different categories (*Figure 2*). Nevertheless, treatment and infection status variables seemed to affect the final clustering, and the former even separated most of the untreated samples in a PCA (Supplementary *Figure 2*).

To assess which of the environmental factors affected the microbial communities to the greatest extent, we used PERMANOVA (*Table 2*). Although sample type and age explained the greatest percentages of the observed variability, treatment was the only environmental variable reaching statistical significance and accounted for the third greatest percentage. More specifically, significant sample variability was established between the group receiving antibiotics and untreated samples (adjusted p value: 0.042) in the comparison of a pair of conditions within the group that did not present variance heterogeneity according to ANOVA and permutation tests.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

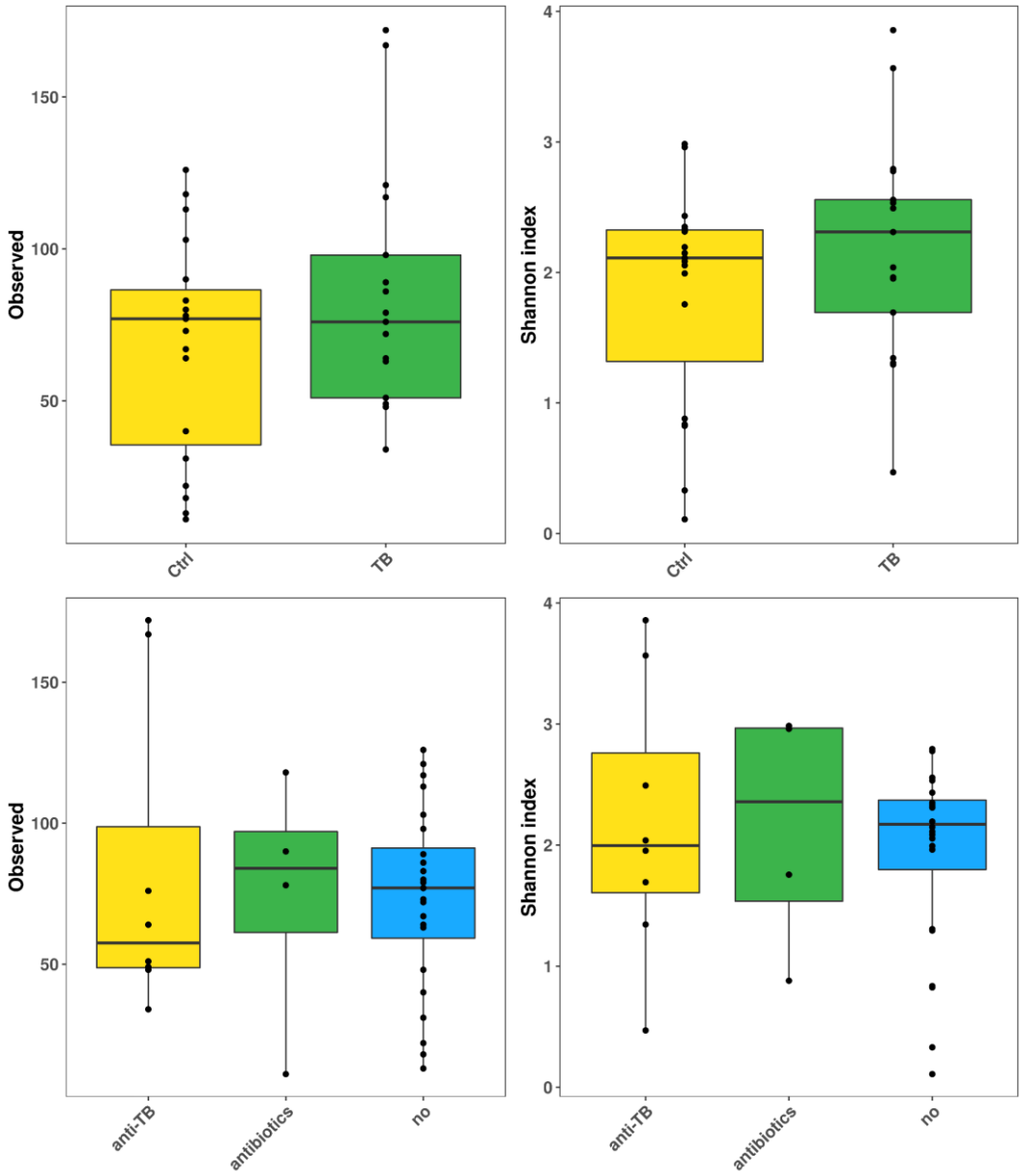

Figure 1. *Boxplots based on observed species and Shannon indices showing the alpha-diversity among microbial communities. (a) Comparison of observed species between the Tb and control groups. (b) Comparison of Shannon indices between the Tb and control groups. (c) Comparison of ob-served species between antibiotic-treated and control samples. (d) Comparison of Shannon indi-ces between antibiotic-treated and control samples. Within-sample diversity was compared be-tween Tb-based groups of samples using the Kruskal–Wallis test followed by Dunn's post hoc test. Comparisons between antibiotic groups were performed using the Wilcoxon test. An ad-justed or nominal p value threshold of 0.05 was used to define statistical significance. * (adjusted) p value < 0.05; ** (adjusted) p value < 0.01; *** (adjusted) p value < 0.001; NS non-significant*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

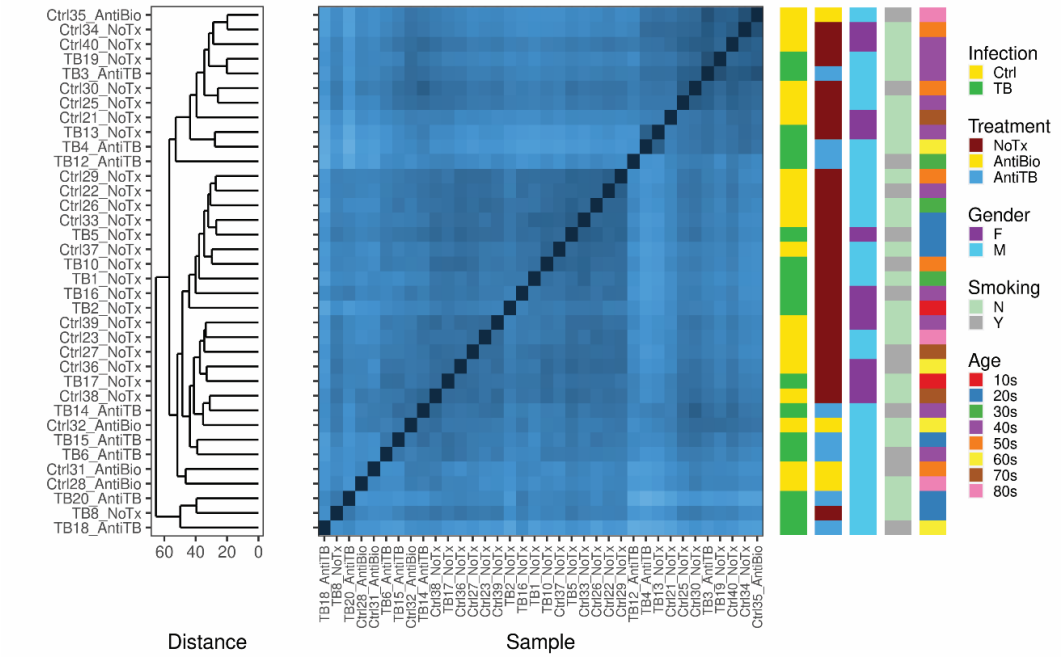

Figure 2. *Beta diversity heatmap with hierarchical clustering of individual samples. Aitchison distance was used to hierarchically cluster individual samples using complete linkage*

Differences in sputum microbiome taxonomy between samples determined using 16S rRNA gene sequencing

To evaluate the differences between microbial communities, we compared the relative abundance of taxa at different taxonomic levels between the sample-type, treatment, and infection status sample groups. Beginning at the phylum level, Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria were the dominant taxa in almost all of our samples (*Figure 3a*). Nevertheless, no differences at the phylum level were detected between any of the sample groups (Supplementary *Figure 3*), leading us to examine lower taxonomic levels. Interestingly, each patient was characterized by a different profile reflected by the taxonomic assignments; for example, lower bacterial taxa showed a high abundance of Proteobacteria in the TB sputum and control samples (*Figure 3a*), and this group might out-compete other taxa when unrestricted by the absence of antibiotics (Huang et al., 2011).

At the family level, several differences were identified between sample categories (*Figure 3b-d*). As far as different clinical phenotypes are concerned (*Figure 3b*), Clostridiales Incertae Sedis XIII, Corynebacteriaceae, Lactobacillaceae and Mycobacteriaceae were those families supporting significant differences. More specifically, Clostridiales Incertae Sedis XIII showed an increased abundance in TB samples not receiving any treatment compared to those treated with anti-TB medication, while Corynebacteriaceae exhibited higher abundance in control samples treated with antibiotics versus their untreated counterparts. Lactobacillaceae showed increased abundance after TB patients were treated with anti-TB drugs compared to untreated patients. Finally, Mycobacteriaceae presented decreased abundance in both untreated and antibiotic-treated control samples compared to TB and anti-TB-treated samples.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

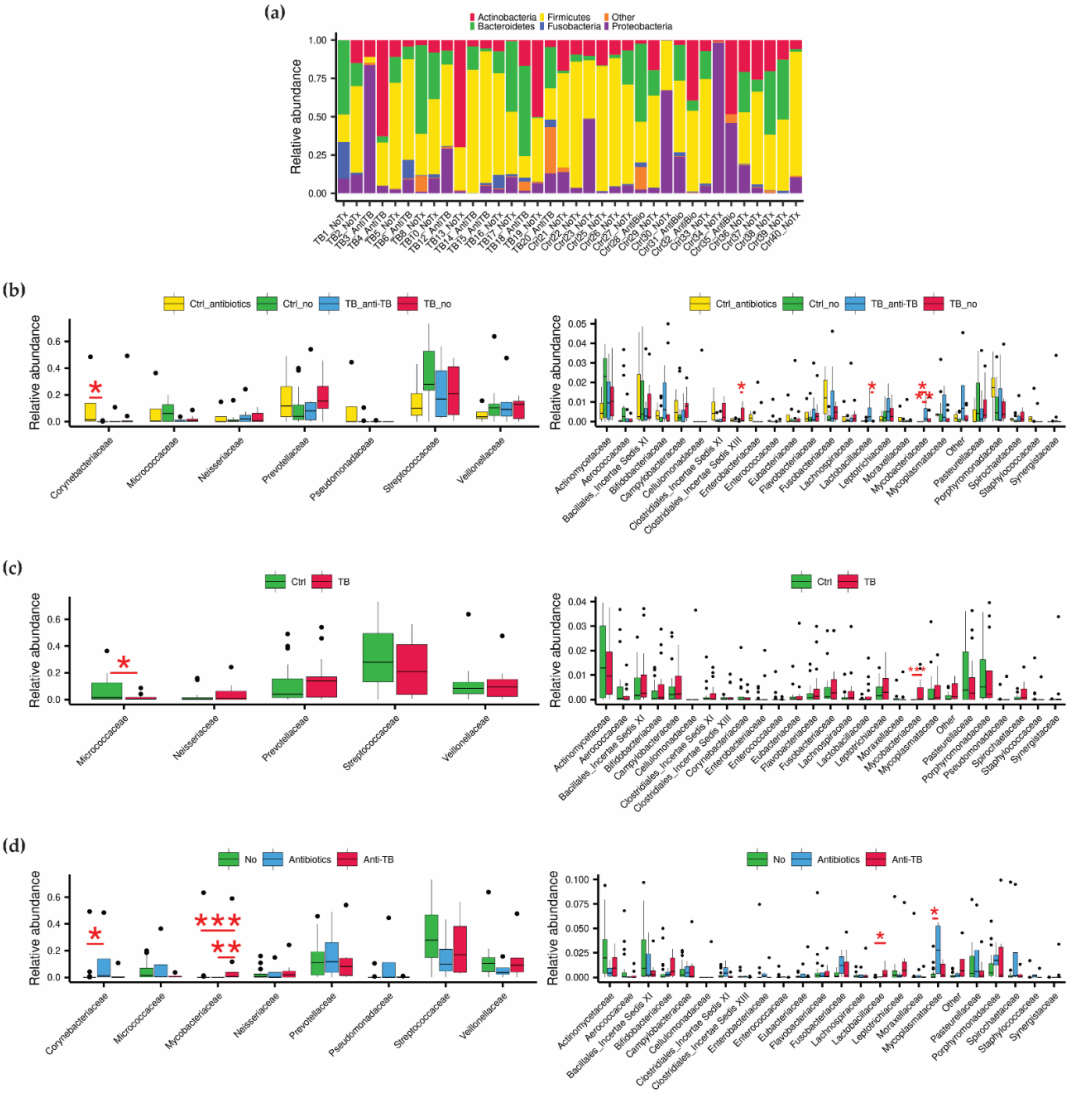

Figure 3. *Relative abundance of phyla in the TB and control groups. For better visualization, families are separated in high and low abundance at the left and right of each panel, respectively. (a) Relative abundance of each sample top phylum. All taxa with a gene copy number_ corrected relative abundance below the respective 3rd quartile were classified as "Other". TB sputum samples 1-20, control sputum samples 21-40. (b) Comparison of relative abundance between sample-type groups at the family level. (c) Comparison of relative abundance between phenotype groups at the family level. (d) Comparison of relative abundance between treatment groups at the family level*

When examining samples based on their infection status, Micrococcaceae and Mycobacteriaceae were the families that showed changes in abundance (*Figure 3c*), while Corynebacteriaceae, Lactobacillaceae, Mycobacteriaceae and Mycoplasmataceae were identified when comparing different treatment regimens (*Figure 3d*). More specifically, Micrococcaceae were more abundant in control samples than in TB-infected samples, whereas the opposite pattern was observed for Mycobacteriaceae. Regarding treatment conditions, Corynebacteriaceae and Mycoplasmataceae abundance increased in antibiotic-treated samples compared to untreated samples. Mycobacteriaceae were more abundant in the anti-TB group than in all the other treatment groups. Lactobacillaceae

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

were more abundant in anti-TB-treated samples than in untreated samples. Interestingly, none of these families were among the highly abundant families, such as Streptococcaceae or Prevotellaceae, suggesting the presence of subtle but potentially important differences be-tween the investigated microbial communities. Additionally, differential abundance analyses performed at the genus level revealed no significant differences in any of the within-sample-type, treatment regime or infection status comparisons.

In conclusion, according to the above findings, changes in the Corynebacteriaceae and Lactobacillaceae families seem to be due to antibiotic and anti-TB regimes, respectively. The observed effect of medication on Mycoplasmataceae does not suggest an important event, as it was not repeated when examining the family abundance between sample types. Similarly, TB infection might be the only variable affecting the Micrococcaceae family. On the other hand, the Mycobacteriaceae family consistently showed changes in all three sample groups.

Discussion

There are still uncertainties about the nature of the core microbiota of the gut-lung axis and whether we can establish a specific disease status of the more persistent microbial communities related to disease (Shukla et al., 2017). Shifts in community equilibrium indeed show that oral aspiration serves as a source of the lung microbiome (Pragman et al., 2012), as two studies (Yu et al., 2016; Bingula et al., 2020) have confirmed separated clusters of bacterial diversity and abundance between oral and lung samples. Previous studies investigating the core phyla in healthy airways have clearly demonstrated the presence of Firmicutes and Bacteroidetes at the highest abundances, followed by Proteobacteria and Actinobacteria (Bernasconi et al., 2016). These core phyla remain abundant COPD, asthma, pneumonia, and CF patients, with Proteobacteria dominating the total community (Bernasconi et al., 2016). Specifically, the dominant bacterial taxa are associated with the status and anatomical site of lung disease (Erb-Downward et al., 2011); in the case of lung tissue tumors, the population is dominated by Firmicutes as a substitute for Proteobacteria (Bingula et al., 2020).

A key factor that could impact our findings is that the control group samples were not taken from healthy individuals due to the difficulty of expectorating sputum and the low density of bacterial communities in the lungs of healthy individuals. Our control group consisted of individuals who had coughing symptoms but showed negative TB results by PCR. These included three individuals with HIV-positive status, asthma, COPD, and lung fibrosis. Similar to the findings of Cheung and colleagues, sequences of the V1/V2 region of 16s RNA were applied, and the diversity of taxa was found to be similar between the TB and control samples, although the most dominant phyla were Proteobacteria and Bacteroidetes (Cheung et al., 2013). Also, using the V3 region (Cui et al., 2012) , the taxa identified in TB-positive sputum samples were Firmicutes, Bacteroidetes and Proteobacteria, but in healthy control samples, only Firmicutes was found. Our results obtained using the same hypervariable V3 region were similar, except that the three taxa were also found in the control group.

In addition to an overall similar alpha diversity between the two groups, we found no changes in the TB lung microbiota under the effect of the first-line antituberculosis treatment, except at the levels of two families, which was consistent with a study described in Sala et al. (2020). This study had a larger sample size collected from different

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

geographical regions, where alpha diversity was similar between the TB and the non-TB samples. It is well established that antibiotic therapy can alter the composition of commensal communities, which is associated with adverse health effects on patients (Hong et al., 2016). For example, Lactobacillus was found to be depleted in the gut of treated TB patients (Namasivayam et al., 2017) and was unique to the lunges of TB patients without treatment (Eshetie and van Soolingen, 2019). In the case of pneumonia, Lactobacillus was found to be an inflammatory marker in the pneumonia lung, alongside Rothia and Streptococcus (Buelow et al., 2017). Compared to our TB group, the family Lactobacillaceae showed significant differences under treatment relative to the controls involving the following genera: Lactobacillus, Leuconostoc, Enterococcus and Weissella. However, another member of the phylum Firmicutes, Clostridiales, which has not previously been reported as a component of the lung TB microbiome, was significantly decreased in the anti-TB-treated group. This was in line with a study on the guts of murine model mice infected with M. tuberculosis conducted in Namasivayam et al. (2017).

A more challenging situation emerges for Actinobacteria, a large and highly diverse group that includes the causative agent, M. tuberculosis. Previous studies of the lungs of TB patients have reported a low abundance of *M. tuberculosis* in the investigated specimens. This phenomenon was confirmed in Sulaiman et al. (2018), as the 16S rRNA gene was less sensitive for detecting Mycobacterium. In our study, Mycobacteriaceae showed significant differences relative to the control conditions, and the genus Mycobacterium was detected in eleven sputum samples. This contrasts with a recent study (Kateete et al., 2021), who found a decreased abundance of M. tuberculosis after the administration of rifampicin by months 2 and 5. This study also identified a high abundance of Neisseriaceae by month 5 after treatment. At this point, we cannot reliably interpret the persistence of Mycobacteria and whether the signal came from living/viable or dead cells without gene expression and metabolomic analyses (Hu et al., 2020). The abundance of Corynebacteriaceae in the same family significantly changed under antibiotic regimes in the control group. Coryne-bacterium spp. have been previously reported as a possible causative agent in the lungs of pneumonia patients in several reports, and it was recently confirmed to be predominant in bronchoalveolar lavage samples under treatment with different classes of antibiotics (Yang et al., 2018). Specifically, Rothia, which has been indicated to be an important genus in TB-positive samples, was not clearly detected, as the entire family of Micrococcaceae exhibited low abundance, represented by a single taxon of each Microbacterium, Kocuria, Ornithinimicrobium, Rothia and Tropheryma. The last taxon includes a well-known causative agent of acute pneumonia, “Tropheryma whipplei”, and is specific to the lung environment (Fenollar et al., 2012). The richness of Micrococcaceae in the controls included the same taxon, without Tropheryma.

In summary, our analysis demonstrates a wide range of similar microbial communities present in the sputum of TB-positive as well as TB-negative samples. Whether this is a cause of idiosyncratic TB infection or a result of such a course remains unknown at present. Regardless, the comparison of microbial flora in our samples revealed the presence of previously unknown taxa in TB patients and paves the way for the further examination of these interesting communities. This study has several limitations. The sample size of the examined sputum specimens was relatively small, lack of a control group of healthy subjects with no lung diseases which limits the interpretation of the obtained data. Additionally, the control comparison could have been improved by the consideration of fewer variables, for example, TB vs. COPD or pneumonia cases.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

Conclusion

Multiple studies have demonstrated that the lung microbiota has the capacity to modulate the immune response to Mycobacterium tuberculosis (Mtb) infection, hence influencing the course of the associated illness. The presence of dysbiosis in the lung microbiota has been associated with tuberculosis and various other respiratory ailments. Nevertheless, there was substantial variation in the microbial community makeup observed in the sputum samples of tuberculosis (TB) patients across different individuals.

Funding. The project was self-funded.

Declaration of competing interest. None declared.

REFERENCES

[1] Alyamani, E. J., Marcus, S. A., Ramirez-Busby, S. M., Hansen, C., Rashid, J., El-Kholy, A., Spalink, D., Valafar, F., Almehdar, H. A., Jiman-Fatani, A., Khiyami, M. A., Talaat, A. M. (2019): Genomic analysis of the emergence of drug-resistant strains of Mycobacterium tuberculosis in the Middle East. – Scientific Reports 9(1): 4474.

[2] Barbosa-Amezcua, M., Galeana-Cadena, D., Alvarado-Peña, N., Silva-Herzog, E. (2022): The Microbiome as Part of the Contemporary View of Tuberculosis Disease. – Pathogens (Basel, Switzerland) 11(5): 584.

[3] Bernasconi, E., Pattaroni, C., Koutsokera, A., Pison, C., Kessler, R., Benden, C., Soccal, P. M., Magnan, A., Aubert, J.-D., Marsland, B. J., Nicod, L. P. (2016): Airway Microbiota Determines Innate Cell Inflammatory or Tissue Remodeling Profiles in Lung Transplantation. – American Journal of Respiratory and Critical Care Medicine 194(10): 1252-1263.

[4] Bingula, R., Filaire, E., Molnar, I., Delmas, E., Berthon, J.-Y., Vasson, M.-P., Bernalier-Donadille, A., Filaire, M. (2020): Characterisation of microbiota in saliva, bronchoalveolar lavage fluid, non-malignant, peritumoural and tumour tissue in non-small cell lung cancer patients: a cross-sectional clinical trial. – Respiratory Research 21(1): 129.

[5] Botero, L. E., Delgado-Serrano, L., Cepeda, M. L., Bustos, J. R., Anzola, J. M., Del Portillo, P., Robledo, J., Zambrano, M. M. (2014): Respiratory tract clinical sample selection for microbiota analysis in patients with pulmonary tuberculosis. – Microbiome 2(1): 29.

[6] Buelow, E., Bello González, T. D. J., Fuentes, S., de Steenhuijsen Piters, W. A. A., Lahti, L., Bayjanov, J. R., Majoor, E. A. M., Braat, J. C., van Mourik, M. S. M., Oostdijk, E. A. N., Willems, R. J. L., Bonten, M. J. M., van Passel, M. W. J., Smidt, H., van Schaik, W. (2017): Comparative gut microbiota and resistome profiling of intensive care patients receiving selective digestive tract decontamination and healthy subjects. – Microbiome 5(1): 88.

[7] Cadena, A. M., Ma, Y., Ding, T., Bryant, M., Maiello, P., Geber, A., Lin, P. L., Flynn, J. L., Ghedin, E. (2018): Profiling the airway in the macaque model of tuberculosis reveals variable microbial dysbiosis and alteration of community structure. – Microbiome 6(1): 180.

[8] Cheung, M. K., Lam, W. Y., Fung, W. Y. W., Law, P. T. W., Au, C. H., Nong, W., Kam, K. M., Kwan, H. S., Tsui, S. K. W. (2013): Sputum microbiota in tuberculosis as revealed by 16S rRNA pyrosequencing. – PloS One 8(1): e54574.

[9] Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., Knight, R. (2009): Bacterial community variation in human body habitats across space and time. – Science (New York, N.Y.) 326(5960): 1694-1697.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

[10] Cui, Z., Zhou, Y., Li, H., Zhang, Y., Zhang, S., Tang, S., Guo, X. (2012): Complex sputum microbial composition in patients with pulmonary tuberculosis. – BMC Microbiology 12(1): 276.
[11] Edgar, R. C. (2010): Search and clustering orders of magnitude faster than BLAST. – Bioinformatics (Oxford, England) 26(19): 2460-2461.
[12] Erb-Downward, J. R., Thompson, D. L., Han, M. K., Freeman, C. M., McCloskey, L., Schmidt, L. A., Young, V. B., Toews, G. B., Curtis, J. L., Sundaram, B., Martinez, F. J., Huffnagle, G. B. (2011): Analysis of the lung microbiome in the "healthy" smoker and in COPD. – PloS One 6(2): e16384.
[13] Eshetie, S., van Soolingen, D. (2019): The respiratory microbiota: new insights into pulmonary tuberculosis. – BMC Infectious Diseases 19(1): 92.
[14] Fenollar, F., Ponge, T., La Scola, B., Lagier, J.-C., Lefebvre, M., Raoult, D. (2012): First isolation of *Tropheryma whipplei* from bronchoalveolar fluid and clinical implications. – The Journal of Infection 65(3): 275-278.
[15] Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., Egozcue, J. J. (2017): Microbiome Datasets Are Compositional: And This Is Not Optional. – Frontiers in Microbiology 8.
[16] Hong, B.-Y., Maulén, N. P., Adami, A. J., Granados, H., Balcells, M. E., Cervantes, J. (2016): Microbiome Changes during Tuberculosis and Antituberculous Therapy. – Clinical Microbiology Reviews 29(4): 915-926.
[17] Hong, B.-Y., Paulson, J. N., Stine, O. C., Weinstock, G. M., Cervantes, J. L. (2018): Meta-analysis of the lung microbiota in pulmonary tuberculosis. – Tuberculosis (Edinburgh, Scotland) 109: 102-108.
[18] Hu, Y., Cheng, M., Liu, B., Dong, J., Sun, L., Yang, J., Yang, F., Chen, X., Jin, Q. (2020): Metagenomic analysis of the lung microbiome in pulmonary tuberculosis - a pilot study. – Emerging Microbes & Infections 9(1): 1444-1452.
[19] Huang, Y. J., Nelson, C. E., Brodie, E. L., Desantis, T. Z., Baek, M. S., Liu, J., Woyke, T., Allgaier, M., Bristow, J., Wiener-Kronish, J. P., Sutherland, E. R., King, T. S., Icitovic, N., Martin, R. J., Calhoun, W. J., Castro, M., Denlinger, L. C., Dimango, E., Kraft, M., Peters, S. P., Wasserman, S. I., Wechsler, M. E., Boushey, H. A., Lynch, S. V. (2011): Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. – The Journal of Allergy and Clinical Immunology 127(2): 372-381.e1-3.
[20] Kateete, D. P., Mbabazi, M. M., Nakazzi, F., Katabazi, F. A., Kigozi, E., Ssengooba, W., Nakiyingi, L., Namiiro, S., Okwera, A., Joloba, M. L., Muwonge, A. (2021): Sputum microbiota profiles of treatment-naïve TB patients in Uganda before and during first-line therapy. – Scientific Reports 11(1): 24486.
[21] Langbang, A., Deka, N., Rahman, H., Kalita, D. (2016): A Study on Bacterial Pathogens causing Secondary Infections in Patients Suffering from Tuberculosis and their Pattern of Antibiotic Sensitivity. – International Journal of Current Microbiology and Applied Sciences 5(8): 197-203.
[22] Li, W., Godzik, A. (2006): Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. – Bioinformatics (Oxford, England) 22(13): 1658-1659.
[23] Lin, D., Wang, X., Li, Y., Wang, W., Li, Y., Yu, X., Lin, B., Chen, Y., Lei, C., Zhang, X., Zhang, X., Huang, J., Lin, B., Yang, W., Zhou, J., Zeng, J., Liu, X. (2021): Sputum microbiota as a potential diagnostic marker for multidrug-resistant tuberculosis. – International Journal of Medical Sciences 18(9): 1935-1945.
[24] Majaneva, M., Hyytiäinen, K., Varvio, S. L., Nagai, S., Blomster, J. (2015): Bioinformatic Amplicon Read Processing Strategies Strongly Affect Eukaryotic Diversity and the Taxonomic Composition of Communities. – PLOS ONE 10(6): e0130035.
[25] Namasivayam, S., Maiga, M., Yuan, W., Thovarai, V., Costa, D. L., Mittereder, L. R., Wipperman, M. F., Glickman, M. S., Dzutsev, A., Trinchieri, G., Sher, A. (2017): Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. – Microbiome 5(1): 71.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

[26] Namasivayam, S., Kauffman, K. D., McCulloch, J. A., Yuan, W., Thovarai, V., Mittereder, L. R., Trinchieri, G., Barber, D. L., Sher, A. (2019): Correlation between Disease Severity and the Intestinal Microbiome in *Mycobacterium tuberculosis*-Infected Rhesus Macaques. – mBio 10(3): e01018-19.
[27] Pragman, A. A., Kim, H. B., Reilly, C. S., Wendt, C., Isaacson, R. E. (2012): The lung microbiome in moderate and severe chronic obstructive pulmonary disease. – PloS One 7(10): e47305.
[28] Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2013): The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. – Nucleic Acids Research 41 (Database issue): D590-596.
[29] Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., Gasbarrini, A. (2017): Proteobacteria: A Common Factor in Human Diseases. – BioMed Research International 2017: 9351507.
[30] Sala, C., Benjak, A., Goletti, D., Banu, S., Mazza-Stadler, J., Jaton, K., Busso, P., Remm, S., Leleu, M., Rougemont, J., Palmieri, F., Cuzzi, G., Butera, O., Vanini, V., Kabir, S., Rahman, S. M. M., Nicod, L., Cole, S. T. (2020): Multicenter analysis of sputum microbiota in tuberculosis patients. – PLOS ONE 15(10): e0240250.
[31] Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., Weber, C. F. (2009): Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. – Applied and Environmental Microbiology 75(23): 7537-7541. Available at: https://doi.org/10.1128/AEM.01541-09.
[32] Shukla, S. D., Budden, K. F., Neal, R., Hansbro, P. M. (2017): Microbiome effects on immunity, health and disease in the lung. – Clinical & Translational Immunology 6(3): e133.
[33] Smith, T., Wolff, K. A., Nguyen, L. (2013): Molecular biology of drug resistance in Mycobacterium tuberculosis. – Current Topics in Microbiology and Immunology 374: 53-80.
[34] Sulaiman, I., Wu, B. G., Li, Y., Scott, A. S., Malecha, P., Scaglione, B., Wang, J., Basavaraj, A., Chung, S., Bantis, K., Carpenito, J., Clemente, J. C., Shen, N., Bessich, J., Rafeq, S., Michaud, G., Donington, J., Naidoo, C., Theron, G., Schattner, G., Garofano, S., Condos, R., Kamelhar, D., Addrizzo-Harris, D., Segal, L. N. (2018): Evaluation of the airway microbiome in nontuberculous mycobacteria disease. – The European Respiratory Journal 52(4): 1800810.
[35] Turnbaugh, P. J., Quince, C., Faith, J. J., McHardy, A. C., Yatsunenko, T., Niazi, F., Affourtit, J., Egholm, M., Henrissat, B., Knight, R., Gordon, J. I. (2010): Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. – Proceedings of the National Academy of Sciences 107(16): 7503-7508.
[36] Ueckermann, V., Lebre, P., Geldenhuys, J., Hoosien, E., Cowan, D., van Rensburg, L. J., Ehlers, M. (2022): The lung microbiome in HIV-positive patients with active pulmonary tuberculosis. – Scientific Reports 12(1): 8975.
[37] Valdez-Palomares, F., Muñoz Torrico, M., Palacios-González, B., Soberón, X., Silva-Herzog, E. (2021): Altered Microbial Composition of Drug-Sensitive and Drug-Resistant TB Patients Compared with Healthy Volunteers. – Microorganisms 9(8): 1762.
[38] Vázquez-Pérez, J. A., Carrillo, C. O., Iñiguez-García, M. A., Romero-Espinoza, I., Márquez-García, J. E., Falcón, L. I., Torres, M., Herrera, M. T. (2020): Alveolar microbiota profile in patients with human pulmonary tuberculosis and interstitial pneumonia. – Microbial Pathogenesis 139: 103851.
[39] Wang, Q., Garrity, G. M., Tiedje, J. M., Cole, J. R. (2007): Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. – Applied and Environmental Microbiology 73(16): 5261-5267.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240

[40] Wu, J., Liu, W., He, L., Huang, F., Chen, J., Cui, P., Shen, Y., Zhao, J., Wang, W., Zhang, Y., Zhu, M., Zhang, W., Zhang, Y. (2013): Sputum Microbiota Associated with New, Recurrent and Treatment Failure Tuberculosis. – PLOS ONE 8(12): e83445.
[41] Yang, K., Kruse, R. L., Lin, W. V., Musher, D. M. (2018): Corynebacteria as a cause of pulmonary infection: a case series and literature review. – Pneumonia (Nathan Qld.) 10: 10.
[42] Yu, G., Gail, M. H., Consonni, D., Carugno, M., Humphrys, M., Pesatori, A. C., Caporaso, N. E., Goedert, J. J., Ravel, J., Landi, M. T. (2016): Characterizing human lung tissue microbiota and its relationship to epidemiological and clinical features. – Genome Biology 17(1): 163.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2205_42274240