Cystic fibrosis microbiome: analysis of nasal middle meatus and sputum in different lung disease stages*

Abstract

**Background:** Culture independent methods of molecular detection of microbiome have shown the polymicrobial nature of respiratory infections in cystic fibrosis, with pathogenic agents undetectable in conventional culture methods. Composition and diversity of the airway microbiome are still poorly understood.

**Methodology:** This study evaluated the airway microbiome in 31 adult cystic fibrosis patients via the analysis of 16S rRNA sequences by next generation sequencing.

**Results:** Staphylococcus, Streptococcus and Corynebacterium were the most abundant genera in the middle meatus, and Pseudomonas, Haemophilus and Prevotella were the most abundant in sputum. In patients with advanced disease (FEV1< 50%), there was an increase in the prevalence of Pseudomonas in both sample types when studied separately. In each patient, in a paired analysis, the sputum and middle meatus showed similar microbiome composition in mild or moderate disease (FEV1≥ 50%). In patients with severe lung disease, the relative abundance of Pseudomonas had a positive correlation in both collection sites.

**Conclusions:** This is the first Brazilian study to evaluate the airway microbiome in cystic fibrosis patients. Our findings agree with those in the international literature and indicate the role of Pseudomonas in the sputum and middle meatus in patients with advanced disease.

**Key words:** cystic fibrosis; microbiology, 16S rRNA, high-throughput nucleotide sequencing, forced expiratory volume.

Introduction

In Cystic fibrosis (CF), the mucosal surface of the airways exhibits increased mucus viscosity and reduced mucociliary clearance. There is a predisposition toward infection by Staphylococcus aureus and Pseudomonas aeruginosa, which is associated with a decline in pulmonary function, the main cause of mortality. Chronic colonization by P. aeruginosa occurs most often up until the third decade of life. During the intermittent colonization phase, approximately 25% of cases of recolonization occur due to a bacterium of the same genotype, indicating a persistent source of environmental contamination or an unidentified reservoir in the patient. Furthermore, when comparing bacteria such as S. aureus and P. aeruginosa, present in the nasal lavage and sputum of CF patients, there is a high concordance of genotypes, with evidence of cross-infection between the upper and lower airways. CF patients subject to lung transplantation exhibit colonization by Pseudomonas with mutations and gene expression, similar to that found before transplantation. CF patients subject to endonasal endoscopic surgery followed by antibiotic therapy attained a reduction in the occurrence of positive cultures for CF-related pathogens in the first year after surgery. These and other studies suggest that the paranasal sinuses function as reservoirs of pathogenic microorganisms. Genetic sequencing platforms allow for more comprehensive analysis of the composition and relative abundances of microorganisms.
organisms in a given community. Such tools draw attention to the complexity of the microbial communities present in the airways of patients with chronic inflammatory diseases (10,11).

Extensive knowledge of the composition and diversity of the nasosinusal microbiome of CF patients is important in understanding the pathophysiology of the disease when considering the likely role of the paranasal sinuses as a reservoir of bacteria for lung infections and also considering that the interactions between species may influence the virulence of a bacteria in a polymicrobial community (12).

The objectives of this study were to compare the composition and diversity of the microbiome between the nasal middle meatus and the sputum of adult patients with cystic fibrosis and between groups with mild/moderate lung disease (forced expiratory volume in one second (FEV1 ≥ 50%) and severe lung disease (FEV1 < 50%).

Materials and methods
Study population
For this exploratory cross-sectional study, patients from the Clinics Hospital of São Paulo aged 18 years and older with a diagnosis of cystic fibrosis, confirmed by a compatible clinical condition, a sweat chloride above 60 mEq/l and genetic tests that identified at least 2 known mutations were recruited. The exclusion criteria were a) the occurrence of pulmonary exacerbation (5) and or the use of a systemic antibiotic in the last 4 weeks, or b) previous lung transplantation. There were 31 patients (17 patients with a forced expiratory volume in one second (FEV1) ≥ 50 and 14 patients with FEV1 < 50). A total of 22 middle meatus samples and 26 sputum samples were collected. Questionnaires validated in the Portuguese language were used, including the Sino-Nasal Outcome Test-20 (SNOT-20) (13) and the Nose Obstruction Symptom Evaluation (NOSE) (14). A pulmonary function test was performed to determine the FEV1 and a nasal endoscopy was performed for Lund-Kennedy Endoscopic Classification (15). This study was approved by the Ethics Committee for Analysis of Research Projects - CAPPesq (no. 1.453.561). All participants read and signed the informed consent form.

Sample collection
During endoscopic visualization, the swab (FLOQSwabs; COPAN Diagnostics, Inc., Murrieta, CA, USA) was inserted, covered by a sterile catheter in the middle meatus, the swab was uncovered for sample collection with at least 3 complete rotations within the meatus. The swab was covered again and pulled out of the nose. The tip of the swab was cut and stored in a sterile cryotube that was free of DNase and RNase. The sputum samples were collected in a sterile vial and transferred to a cryotube and both samples were stored at -80 °C.

Table 1. Demographic and clinical characteristics of subjects in both groups classified according to pulmonary disease severity.

|                         | Total | Mild/ moderate FEV1 ≥ 50 | Severe FEV1 <50 | p*       |
|-------------------------|-------|-------------------------|-----------------|----------|
| Patients (n)            | 31    | 17                      | 14              | -        |
| Age (mean, DP)          | 30.2 (14,8) | 29.5 (14,8)           | 31.1 (15,4)    | 0.33     |
| Gender (m=male, f=female)| 13 m, 18 f | 8 m, 9 f               | 5 m, 9 f       | 0.7      |
| Alpha dornase           | 58,10% | 47,10%                  | 71,40%          | 0.27     |
| Inhaled antibiotic      | 41,90% | 17,60%                  | 71,40%          | <0.01    |
| Oral antibiotic         | 71,00% | 64,70%                  | 78,60%          | 0.46     |
| Lund-Kennedy**          | 3,9   | 4,0 (2,6)               | 3,9 (3,1)       | 0.95     |
| SNOT-20***              | 31,6  | 32,0 (21,3)             | 31,6 (17,2)     | 0.98     |
| NOSE****                | 7     | 7,1 (6,1)               | 7,2 (5,4)       | 0.87     |

p* p-value; ** Endoscopic classification of Lund-Kennedy; *** Sino-Nasal Outcome Test-20; **** Nose Obstruction Symptom Evaluation.

Bacterial DNA extraction
The sputum samples were treated with a 0.1% dithiothreitol (DTT, Invitrogen)-sputolysin solution prior to the DNA extraction step (16,17). The total DNA was extracted from the collected samples with the QIAamp DNA Blood kit (Qiagen) and stored at -20°C.

Microbiome sequencing
The amplification of the V3-V4 region of the 16S rRNA gene was performed with 25 cycles of PCR using primers and conditions that were previously described (18). The amplicons were pooled and loaded into an Illumina MiSeq clasmshell style cartridge kit (v2) for 500 cycles for 250 paired-end sequencing at a final concentration of 12 pM. The library was clustered at a density of approximately 80,000 k/mm^2. The raw read files were demultiplexed and then bioinformatics analysis were conducted with QIIME software (19), as previously described (20). Then, using the Microbiome Analyst (21), principal coordinate analysis (PCoAs) were performed to determine the differences in beta diversity between the different sites and groups (22). Nucleic acid sequences are available at the Sequence Read Archive (SRA) under accession PRJNA548026. Species classification was performed using SPINGO software (23) using default parameters.

Statistical analysis
For the demographic and clinical characteristics of the patients, the continuous variables were represented as the means and
their respective standard deviations (SD), and the categorical
variables were represented as percentages. After the verification
of normality using the Shapiro-Wilk test, the ages, Lund-Ken-
nedy scores, SNOT-20 scores and NOSE scores were compared
between the groups using a nonparametric Wilcoxon-Mann-
Whitney test. The comparisons based on sex and the use of alp-
pha dornase and antibiotics were performed using Fisher’s exact
test. The statistical analysis of the microbiome was performed by
using SPSS version 22. For all the analysis, the level of signifi-
cance was p≤0.05. Analysis of group similarities (ANOSIM) were
performed also using Microbiome Analyst
with Bray-Curtis
distances, to observe the differences in beta diversity based on
the sample type and the disease severity.

Results
Characterization of the sample group
The demographic and clinical data are summarized in Table 1. The
groups did not differ in relation to age, sex, endoscopic
scores (Lund-Kennedy), SNOT-20 and NOSE questionnaires, use
of prophylactic oral antibiotics (azithromycin, 3 times a week)
or alpha dornase (p>0.05). The group with severe lung disease
used proportionally more inhaled antibiotics (tobramycin or
colistin) (p <0.01).

Characterization of the microbiome
The main genera found were Streptococcus (22.30%), Staphy-
lococcus (20.90%), Corynebacterium (8.60%) and Pseudomonas
(6.88%) in the middle meatus and Pseudomonas (44.10%),
Haemophilus (11.22%), Veillonella (6.85%) and Prevotella (6.40%)
in the sputum samples.

The individual patient results that refer to the relative abun-
dance of the genera in the middle meatus and sputum samples
(Figure 1) showed a difference in the microbial profiles between
the collected materials, with a prevalence of Streptococcus and
Staphylococcus in the middle meatus samples and Pseudomonas
and Prevotella in the sputum samples.

Microbiome and pulmonary function - Severity of lung
disease
Middle meatus
It was observed that the nasal middle meatus of patients with
severe lung disease exhibited, on average, a greater abundance
of Staphylococcus, Streptococcus and Stenotrophomonas, as well
as a smaller abundance of Pseudomonas, Haemophilus and Co-
rynebacterium, in comparison with those with mild to moderate
lung disease (Figure S1). However, none of the results showed
statistical significance (Table S1).

On the other hand, when we evaluated the relative abundance
of the genera in each individual, we found a different micro-
biome profile. Staphylococcus and Streptococcus were present in
both groups with similar abundances. In the mild to moderate
lung disease group, only one patient exhibited colonization by
Pseudomonas, but the abundance was greater than 90%. In the
severe lung disease group, five patients were colonized with
Pseudomonas but in smaller abundances.

Sputum
On average, the sputum of the patients with severe lung disease
had a higher proportion of *Pseudomonas, Prevotella, Streptococcus* and *Veillonella* and a smaller proportion of *Haemophilus, Neisseria* and *Burkholderia-Paraburkholderia* compared to those with mild/moderate lung disease (Figure S2). The difference in the abundance of *Haemophilus* (p=0.05) was statistically significant. In the sputum analysis, the comparison of the mean abundance of each of the bacterial genera in each group (Table S1) was similar to the profile found in the individual analysis, which showed an increase in the abundance of *Pseudomonas* in patients with severe lung disease, as well as an increase in the relative abundances of *Prevotella, Veillonella* and *Fusobacterium*.

**Paired samples (same patient comparison)**

It was possible to make paired analysis only in those patients who had both samples available, which were 17 patients (10 patients with mild/moderate disease and 7 with severe disease). The relative abundances of the bacterial genera were compared in the paired samples from each patient according to pulmonary function (Figure 2). It was noted that the middle meatus and sputum samples from almost all patients with mild/moderate lung disease.
lung disease shared certain bacterial genera, mainly *Streptococcus*, *Staphylococcus* and *Prevotella*. In patients with severe lung disease there was a very distinct profile in terms of relative abundance when comparing the two sites studied, which showed a higher prevalence of *Staphylococcus* in the samples from the middle meatus and of *Pseudomonas* in the sputum samples. In this case, only one patient (# 32) exhibited relative similarity in the microbiome profiles obtained from the samples. In terms of clinical information, this patient showed the highest SNOT-20 and NOSE scores among those with severe disease.

In the analysis of correlation between genera in middle meatus and sputum, using Pearson’s correlation, for some genera, the correlation reached statistical significance. In patients with mild/moderate disease, it was observed a positive correlation of *Prevotella* ($p=0.02$), *Veillonella* ($p=0.03$) and *Neisseria* ($p=0.03$) in the two collection sites. In patients with severe disease, it was found a positive correlation of *Pseudomonas* ($p=0.02$). In both groups, it was found a negative correlation of *Staphylococcus* ($p=0.03$ for mild/moderate disease and $p=0.04$ for severe disease) and *Corynebacterium* ($p<0.01$ for mild/moderate disease and $p=0.01$ for severe disease).

Species analysis
In the same patient, analysis of species revealed that *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Fusobacterium nucleatum* were present in both middle meatus and sputum in mild/moderate and severe lung disease patient’s samples. *Pseudomonas aeruginosa* were present in 80% of the sputum sample of mild/moderate lung disease and in 57% of sputum samples from patients with severe disease (Figure S3 and S4). (Note that inhaled colistin or tobramycin was not suspended for the study).

Alpha and beta diversity
The alpha diversity (Shannon and Simpson indexes) and richness (Chao1 richness estimator) were observed in the middle meatus and sputum samples according to pulmonary disease severity. Weighted analyses were plotted according to the more abundant genera (left) and unweighted analyses were plotted according to all bacteria genera (right): a) by sample site (weighted: $p<0.001$; unweighted: $p<0.001$); b) by disease severity in the middle meatus samples (weighted: $p = 0.99$; unweighted: $p = 0.82$); c) by disease severity in the sputum samples (weighted: $p = 0.28$; unweighted: $p = 0.92$); d) by sample site in mild/moderate disease (weighted: $p < 0.001$; unweighted: $p < 0.001$); and e) by sample site in severe disease (weighted: $p < 0.001$; unweighted: $p < 0.001$). This result expresses the microbial community structure in (a) each site, (b) and (c) sample site according to disease state, (d) and (e) disease state according to sample site. There were significant differences ($p<0.001$) in microbial community in each sample site (a), and in each sample site according to disease state (d and e).
severity (Figure 3). However, none of the results showed statistical significance.

To observe the differences in relation to beta diversity, PCoA chart was prepared based on the weighted and unweighted UniFrac distance matrices. In general, the spatial distribution of the sputum and middle meatus samples showed a significant difference between the samples in the weighted (p < 0.001, F = 4.90) and unweighted (p < 0.001, F = 7.45) analysis (Figure 4a). We observed no difference when comparing the mild/moderate lung disease group with the severe lung disease group (weighted: p = 0.44, F = 0.96; unweighted: p = 0.84, F = 0.55). The use of alpha dornase (weighted: p = 0.50, F = 0.91; unweighted: p = 0.72, F = 0.66) and the use of inhaled antibiotics (weighted: p = 0.35, F = 1.06; unweighted: p = 0.78, F = 0.60) were also factors that did not significantly alter the bacterial community structure in the groups studied. In relation to the use of prophylactic oral antibiotics, we observed a statistically significant difference between the patients who used them compared with those who did not in the weighted analysis (p = 0.03, F = 1.79), but no statistically significant difference in the unweighted analysis (p = 0.32, F = 1.08).

When we examined the middle meatus samples, we could not distinguish between the bacterial community profiles from patients with mild/moderate lung disease and those from patients with severe lung disease in the weighted (p = 0.99, F = 0.31) and in the unweighted analysis (p = 0.82, F = 0.66) (Figure 4b). When the sputum sample profile was analysed, there was no distinction statistically significant between groups with mild to moderate and severe lung disease (weighted: p = 0.28, F = 1.23; unweighted: p = 0.92, F = 0.32) (Figure 4c).

When comparing the profile results of bacterial communities from different collection sites (sputum and middle meatus) and the severity of the disease (Figure 4d and 4e), we were able to obtain interesting findings that corroborated the relative abundance results previously shown (Figure 2).

In the analysis of patients with severe lung disease (Figure 4e), the comparison of the spatial distribution of the sputum and middle meatus samples showed a difference between the collection sites that was larger than that seen in patients with mild to moderate lung disease (Figure 4d).

Using ANOSIM analysis, there were significant difference between sample types (R: 0.39773; p < 0.001 - Figure 5a), sample types related to pulmonary function, middle meatus and sputum samples from patients with mild to moderate disease (R: 0.32154; p < 0.001 - Figure 5d) and middle meatus and sputum samples from patients with severe disease (R: 0.4606; p < 0.001 - Figure 5e). There was no statistically significant difference in middle meatus samples from patients with mild to moderate and severe disease (R: 0.091768; p = 0.935 - Figure 5b), or in sputum samples from patients with mild to moderate and severe disease (R: 0.0028814; p = 0.425 - Figure 5c).

**Discussion**

A significant difference was found in the composition of the microbiome between the middle meatus and the sputum.

Goddard et al. found a difference between the compositions of the sputum and upper airway microbiomes; despite using an oropharyngeal swab instead of one from the middle meatus (26), the data were similar to that of the present study. Despite the differences in bacterial abundance in the sample according to the disease state (Figure 1 and 2, Table S1), and the differences in microbial community structure (Figure 4) there were no significative differences between alfa diversity indexes (Figure 3) in the present study.

In evaluating the relative abundances of the genera found in the middle meatus, we observed that 5 patients in the group with severe lung disease had concomitant contamination with *Staphylococcus* and *Pseudomonas*, whereas *Pseudomonas* was almost absent in patients with mild disease, apart from a single patient who presented a relative abundance of *Pseudomonas* greater than 90%. The observation of the coexistence of these two bacteria within the same sample in the severe lung disease group is compatible with data from the literature that shows that the association of these two bacteria is related to a decline in pulmonary function (179). Although we found higher mean alpha diversity in the middle meatus in patients with severe disease, that was not statistically significant, the interindividual difference was greater in the mild/moderate group. Some data from the literature indicates that lower diversity is related to worse pulmonary function (12,25–27). Like us, Paganin et al. also found no statistically significant difference between sputum samples from patients that were classified by lung disease severity, even within a sample of 78 patients (28).

In a study by Fodor et al., the most prevalent bacteria in the sputum of adult CF patients represented *Pseudomonas* and *Burkholderia* (27). In our sample, the main genera observed were *Pseudomonas*, *Haemophilus*, *Prevotella*, *Veillonella* and *Burkholderia*.

Pulmonary function is negatively influenced by the presence of *Pseudomonas* as the dominant genus, according to findings by Coburn et al. (29). We found 4 patients in the group with mild/moderate lung disease and 6 patients in the group with severe disease in whom *Pseudomonas* was the dominant genus in the sputum.

Several studies suggested that middle meatus may be a reservoir for some pathogens (16,29–31). In our findings, the microbial compositions of the middle meatus and the sputum were different in the study population, but certain genera colonized both of the evaluated sites, including pathogenic bacteria such as *Pseudomonas*, *Staphylococcus*, *Prevotella*, *Fusobacterium* and *Haemophilus*. However, these data are questionable when comparing the results from the middle meatus and sputum according to the severity of the lung disease. In patients with
mild/moderate lung disease, both sites presented with mainly Streptococcus, Staphylococcus and Prevotella. In the samples from patients with severe lung disease, it was observed that the patients presented a very distinct profile in terms of relative abundance when comparing the two sites. These findings suggest that patients with mild/moderate lung disease share more genera between the middle meatus and sputum than those patients with severe lung disease. This information is corroborated by species analysis, where it was also observed. Data published in the literature have shown that the middle meatus is a source of lung infection; however, no distinctions between the levels of lung disease severity were shown in the existing studies [6–9,29–33]. Indeed, Fletcher et al. showed a loss of niche specificity of CF airway microbial communities in relation to control population, but they also did not take into account the levels of lung disease severity in the analysis [34]. On the other hand, Lucas et al., found a different result from all these studies. They found distinct bacterial communities between upper and lower airway. The bacterial communities clustered more closely by sampling site, rather than by patient. Therefore, as in the other studies, they did not perform separate pulmonary function analysis [35].

In the middle meatus, Staphylococcus and Corynebacterium were among the most prevalent genera between all patients studied. In severe disease group, Corynebacterium had a smaller relative abundance, compared to the other group. Besides that, pathogenic bacteria such as, Prevotella, Veillonella, Neisseria and Pseudomonas had a positive correlation between both collection sites, that is, the greater the relative abundance in the middle meatus, the greater also in the sputum, with statistical significance. And commensal bacteria, previously described as composing a healthy airway microbiome [36], such as Staphylococcus and Corynebacterium, had a negative correlation between the two sites, with statistical significance.

The microbiome may promote or maintain inflammation that was previously induced by a failure of mucosal integrity or by local immunity. It is unclear whether chronic inflammation changes the composition of the microbiota, if the overgrowth of certain species affects the abundance of other species and then promotes inflammation, or if the two phenomena occur simultaneously [37]. According to the findings of Mika et al. [38], dysbiosis of the airway microbiome can be observed as early as the second month of life in CF patients.

Microbiome study is a relatively new field of investigation and we must consider the limitation of this study. The nasal middle meatus is the drainage site for the maxillary, ethmoid and frontal sinuses and may be a good representative of the sinus microbiota, as several studies with evidence Level IIb have shown [39]. Surgical access to these sites limits the size of the sample and selects for more symptomatic or more severe cases of the disease. Collection of middle meatus material is less invasive, which facilitates the reproducibility of the method in clinical practice. As an observational study, it was not possible to interfere with the treatment of these patients, and specially in severe lung disease patients group using inhaled antibiotics against Pseudomonas (71,4%), this medication could reduce the abundance of this specific bacteria in middle meatus.

Certain measures were taken to reduce possible bias as the identification of prophylactic antibiotics in the sample. The middle meatus samples from 10 patients were lost during DNA extraction because they did not present satisfactory DNA quality or quantity. Six of these patients used inhaled antibiotics, seven used azithromycin three times a week as a prophylactic oral antibiotic, and eight used alpha dornase.

Despite being the study with the largest sample and the first study to evaluate the airway microbiome in CF patients from Sao Paulo, it is still a small one. This is a pilot cross-sectional study, however with an important goal. It is necessary to first understand the composition and then formulate new hypothesis for future studies. Other studies must be done to enrich knowledge in airway microbiome in CF to enable comparison with other populations around the world.

Conclusions

Our findings agree with those of the international literature, which indicate the role of Pseudomonas in advanced disease, present in the sputum and middle meatus. Pseudomonas aeruginosa, Staphylococcus aureus and Fusobacterium nucleatum were present in both middle meatus and sputum in mild/moderate and severe disease patient’s samples. The results shown here indicate that the middle meatus and the sputum share some important genera involved in the pathophysiology of CF, in the mild or moderate stages of the disease. In patients with severe lung disease, there was a positive correlation for the relative abundance of Pseudomonas between the two sites. More studies need to be conducted to better understand the pathophysiology of colonization in these patients and to confirm the hypothesis of the middle meatus as a reservoir to lung infections.

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Authorship contribution

FGOM, RRMP, RA and RLV conceived the study design. FGOM and CRT drafted the manuscript. FGOM, RRMP and RA enrolled
patients. FGOM collected clinical data and samples and processed samples for sequencing. LGS and CRT sequenced samples, processed sequence data, and analysed and interpreted the data. RVC prepare the sequences and submitted them to GenBank. LGS created all the figures. All authors reviewed the manuscript.

**Conflict of interest**
The authors declare that there are no competing financial or nonfinancial interest and there are no personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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**Availability of data and materials**
Not applicable.

**Consent for publication**
Not applicable.

**Availability of data and materials**
Not applicable.

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Supplementary Material

Table S1. Relative abundances of the principal genera in the middle meatus and sputum samples according to lung disease severity. The microbiome composition of middle meatus from patients with severe lung disease exhibited an increase of \textit{Burkholderia-Paraburkholderia}, in comparison with those with mild to moderate lung disease ($p=0.05$). The microbiome composition of the sputum from patients with severe lung disease had a decrease of \textit{Haemophilus}, compared to those with mild/moderate lung disease ($p=0.05$).

| Genera          | Middle meatus | Sputum          |
|-----------------|---------------|-----------------|
|                 | Severity of lung disease | Severity of lung disease |
|                 | Mild to moderate | Severe | Mild to moderate | Severe |
| Pseudomonas     | Range | 0 – 95.3 | 0 – 31.7 | 0 – 96.7 | 0 – 98.2 |
|                 | Mean   | 8,24 | 6,91 | 33,72 | 40,9 |
|                 | SD*    | 26,22 | 11,18 | 39,21 | 42,06 |
|                 | p a    | 0.76 | 0.63 |
| Porphyromonas   | Range | 0 – 2.0 | 0 – 0.2 | 0 – 2.0 | 0 – 11.10 |
|                 | Mean   | 0.31 | 0.04 | 1.03 | 2.13 |
|                 | SD*    | 0.68 | 0.07 | 1.87 | 3.6 |
|                 | p a    | 0.16 | 0.16 |
| Prevotella      | Range | 0 – 5.7 | 0 – 12.8 | 0.1 – 20.4 | 0.5 – 62.6 |
|                 | Mean   | 1.51 | 1.66 | 6.3 | 12.9 |
|                 | SD*    | 1.85 | 4.18 | 7.17 | 17.12 |
| Gemella         | Range | 0 – 3.90 | 0 – 3.0 | 0 – 4.70 | 0 – 5.90 |
|                 | Mean   | 0.9 | 0.88 | 0.72 | 1.51 |
|                 | SD*    | 1.22 | 1.12 | 1.29 | 1.96 |
|                 | p a    | 0.98 | 0.18 |
| Staphylococcus  | Range | 0 – 76.3 | 0.1 – 85.4 | 0 – 26.10 | 0 – 24.20 |
|                 | Mean   | 19.76 | 22.48 | 3.6 | 3.1 |
|                 | SD*    | 25.13 | 32.42 | 7.46 | 6.8 |
|                 | p a    | 0.81 | 0.85 |
| Streptococcus   | Range | 0 – 98.3 | 0.5 – 99.5 | 0.1 – 31.0 | 0 – 28.8 |
|                 | Mean   | 18.46 | 27.91 | 5.04 | 7.06 |
|                 | SD*    | 26.35 | 39.45 | 8.57 | 9.15 |
|                 | p a    | 0.47 | 0.54 |
| Veillonella     | Range | 0 – 6.0 | 0 – 14.8 | 0.1 – 16.40 | 0 – 41.2 |
|                 | Mean   | 1.59 | 2.36 | 5.5 | 10.2 |
|                 | SD*    | 1.8 | 4.77 | 5.86 | 12.57 |
|                 | p a    | 0.57 | 0.2 |
| Fusobacterium   | Range | 0 – 8.40 | 0 – 3.70 | 0 – 7.30 | 0 – 42.7 |
|                 | Mean   | 1.13 | 0.76 | 1.4 | 7.06 |
|                 | SD*    | 2.4 | 1.31 | 2.24 | 12.87 |
|                 | p a    | 0.65 | 0.1 |
| Burkholderia-Paraburkholderia | Range | 0 – 0.1 | 0 – 2.30 | 0 – 94.0 | 0 – 1.0 |
|                 | Mean   | 0.007 | 0.37 | 14.36 | 0.07 |
|                 | SD*    | 0.02 | 0.74 | 34.92 | 0.27 |
|                 | p a    | 0.05* | 0.12 |
### Table 1

| Genera   | Middle meatus | Sputum |
|----------|---------------|--------|
|          | Severity of lung disease | Severity of lung disease |
|          | Mild to moderate | Severe | Mild to moderate | Severe |
| Neisseria | Range | 0 – 4,40 | 0 – 2,32 | 0 – 13,60 | 0 – 9,50 |
|          | Mean | 0,66  | 0,46  | 3,8  | 2,06  |
|          | SD*  | 1,2   | 0,72   | 4,98  | 3,07  |
|          | p    | 0,65  | 0,26  | 0,26  | 0,39  |
| Haemophilus | Range | 0 – 90,2 | 0 – 5,20 | 0,1 – 95,8 | 0,1 – 8,20 |
|          | Mean | 8     | 1,18   | 17,13 | 2,25  |
|          | SD*  | 24,74 | 1,75   | 28,37 | 2,49  |
|          | p    | 0,39  | 0,05*  | 0,05* | 0,05* |
| Moraxella | Range | 0 – 95,8 | 0 – 4,0  | 0 – 95,8 | 0 – 4,0  |
|          | Mean | 7,36  | 0,51   | 0     | 0,01  |
|          | SD*  | 26,57 | 1,32   | 0     | 0,03  |
|          | p    | 0,42  | 0,12  | 0,12  | 0,12  |
| Pseudomonas | Range | 0,1 – 34,8 | 0 – 24,9 | 0 – 0,1 | 0 – 0,1 |
|          | Mean | 10    | 6,65   | 0,01  | 0,007 |
|          | SD*  | 12,61 | 8,58   | 0,03  | 0,02  |
|          | p    | 0,46  | 0,53  | 0,53  | 0,53  |
| Stenotrophomonas | Range | 0 – 0,1 | 0 – 39,5 | 0 – 0 | 0 – 10,60 |
|          | Mean | 0,007 | 4,95   | 0     | 0,86  |
|          | SD*  | 0,02  | 13,03  | 0     | 2,92  |
|          | p    | 0,14  | 0,26  | 0,26  | 0,26  |

SD*: standard deviation; p*: p-value

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**Figure S1.** Heat map of bacterial genera in middle meatus according to lung disease severity. Patients with severe lung disease exhibited a greater abundance of *Staphylococcus*, *Streptococcus* and *Stenotrophomonas*, and a smaller abundance of *Pseudomonas*, *Haemophilus* and *Corynebacterium*, in comparison with those with mild to moderate lung disease.

**Figure S2.** Heat map of bacterial genera in sputum according to lung disease severity. Patients with severe lung disease had a higher proportion of *Pseudomonas*, *Prevotella*, *Streptococcus* and *Veillonella* and a smaller proportion of *Haemophilus*, *Neisseria* and *Burkholderia-Paraburkholderia* compared to those with mild/moderate lung disease.
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Figure S3. Main bacterial species found in middle meatus samples in each patient. Pseudomonas aeruginosa, Staphylococcus aureus and Fusobacterium nucleatum were present in both disease state.

Figure S4. Main bacterial species found in sputum samples in each patient. Pseudomonas aeruginosa were present in 80% of sample of mild/moderate lung disease and in 57% of samples of severe disease.
Figure S5. PCoA analyses of group similarities (ANOSIM) plots of Bray-Curtis distances between microbial communities from a) middle meatus and sputum samples ($R$: 0.39773; $p<0.001$), b) middle meatus samples from patients with mild to moderate and severe disease ($R$: -0.091768; $p=0.935$), c) sputum samples from patients with mild to moderate and severe disease ($R$: -0.0028814; $p=0.425$), d) middle meatus and sputum samples from patients with mild to moderate disease ($R$: 0.32154; $p<0.001$) and e) middle meatus and sputum samples from patients with severe disease ($R$: 0.4606; $p<0.001$). The two sites showed different community diversity (a). This was also observed when analysed separately by severity of lung disease (d and e).