**Supplementary Table 1.** Summary of studies investigating the microbiome in sputum samples of COPD participants

<table>
<thead>
<tr>
<th>Author et al. (Year)</th>
<th>Sequencing technique</th>
<th>Participants characteristics</th>
<th>Summary findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabrera-Rubio, et al. (2012)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16S rRNA</td>
<td>8 participants in stable phase</td>
<td>- Microbiome profiling in sputum, bronchial aspirate, BAL and bronchial mucosal biopsy revealed lower diversity in sputum samples compared to the other 3 sample types</td>
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<tr>
<td>Molyneaux, et al. (2013)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>16S rRNA, qPCR</td>
<td>14 participants in stable phase, 17 healthy controls</td>
<td>- After rhinovirus infection, there was a rise in bacterial burden and outgrowth of <em>Haemophilus influenzae</em> from pre-existing microbiota in COPD participants. This was not observed in healthy controls.</td>
</tr>
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</table>
| Garcia-Nunez, et al. (2014)<sup>3</sup> | 16S rRNA | 17 participants in stable phase | - Most prevalent phyla were Proteobacteria, Firmicutes, and Actinobacteria  
- Patients with moderate disease showed greater microbial diversity than patients with advanced disease  
- Alpha diversity was decreased in patients with advanced disease |
| Galiana, et al. (2014)<sup>4</sup> | 16S rRNA, qPCR | 19 participants in stable phase | - Bacterial diversity was higher in patients with moderate COPD than with severe COPD  
- Bacterial load was higher in severe COPD  
- In severe COPD patients, the composition of bacterial genera differed more among themselves, than samples from the mild/moderate group |
| Huang, et al. (2014)<sup>5</sup> | 16S rRNA, qPCR | 12 participants; sample collection before, during and after AECOPD | - Abundance of several bacterial phyla differed between stable phase and AECOPD, where Proteobacteria abundance was increased during AECOPD  
- Steroid and antibiotic therapy showed opposite effects on the microbiome, whereby steroid therapy increased Proteobacteria abundance  
- *Haemophilus influenzae* abundance was positively correlated to the abundance of phylogenetically related bacteria, whereas the abundance of other bacteria was negatively correlated |
| Barker, et al. (2015)<sup>6</sup> | 16S rRNA, qPCR | 120 participants in stable phase, 55 paired stable and AECOPD data | - *Haemophilus influenzae* bacterial load was an independent predictor of sputum TNF-alpha and IL-1beta levels in stable state  
- at AECOPD, change of *Moraxella catarrhalis* bacterial load compared to... |
<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Methodology</th>
<th>Participants</th>
<th>Findings</th>
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| Millares, et al. (2015) | 16S rRNA, metagenomics | 8 participants with severe COPD; sample collection during stable phase and AECOPD | - The relative abundance of bacterial phyla and genera were consistent between stable phase and AECOPD  
- Functional pathways changed at AECOPD events, indicating a shift of the metabolic functionality of the microbiome towards AECOPD |
| Brill, et al. (2015) | Sputum culture; 16S rRNA, qPCR | 99 participants in stable phase, 86 completed follow up, | - A randomized controlled trial investigating different antibiotic classes (moxifloxacin, doxycycline, azithromycin, placebo)  
- Total airway bacterial load did not decrease after 3 months of antibiotic therapy  
- Increases in antibiotic resistance in all treatment groups |
| Wang, et al. (2016) | 16S rRNA, qPCR | 87 participants; sample collection during stable state, AECOPD, 2 weeks post-therapy and 6 weeks recovery | - Sputum microbiome profiles were dynamic from stable state towards AECOPD, involving the change of microbial diversities, abundances of bacterial communities and the outgrowth of “keystone bacteria”, such as Haemophilus or Moraxella spp.  
- Microbiome structure and diversity were correlated with serum and sputum biomarkers  
- Steroid and antibiotic therapy showed opposite effects on the microbiome with respect to diversity and outgrowth of individual bacteria and bacterial communities |
| Wang, et al. (2017) | 16S rRNA | 281 participants; sample collection at baseline and AECOPD | - Microbiome composition shifted from highly diverse to less diverse during AECOPD, where few bacterial genera become predominant abundant  
- Bacterial dysbiosis was associated with increased exacerbation severity, indicated by higher CAT scores and decrease of lung capacity  
- Microbial dysbiosis, in concert with eosinophilic inflammation, was associated with even higher exacerbation severity |
| Leitao Filho, et al. (2018) | 16S rRNA | 102 participants hospitalized due to AECOPD, followed for one year after discharge | - Microbiome profile in hospitalized AECOPD patients is significantly associated with 1-year mortality  
- Reduced microbial diversity indicated poorer survival prognosis  
- The combined absence of Veillonella/presence of Staphylococcus |
<table>
<thead>
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<th>Methods</th>
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<th>Findings</th>
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| Ghebre, et al. (2018) | 16S rRNA, RT-PCR panel for common respiratory viruses | 73 participants with AECOPD, 32 asthmatic patients with exacerbation | Asthma and COPD patients share 3 exacerbation biological clusters after integrating microbiome profiles and host-inflammatory profiles
- Cluster 1: increased pro-inflammatory mediators, evidence of neutrophilic inflammation, bacteria-associated with increased proportions of Proteobacteria and Proteobacteria/Firmicutes ratio
- Cluster 2: increased blood and sputum eosinophils, type 2 mediators and increased proportions of Bacteroidetes
- Cluster 3: increased type 1 mediators and proportions of Actinobacteria and Firmicutes |
| Sinha, et al. (2018) | 16S rRNA | 4 participants; sample collection during stable phase (two-days, 2-9 months) | Alpha diversity is similar over a two-day period
- Microbiome variability was increased over a 9-months period
- Firmicutes was the most prevalent phylum, followed by Bacteroidetes |
| Mayhew et al. (2018) | 16S rRNA | 101 participants, 584 (spontaneous and induced) sputum samples from stable and exacerbation time points over 1 year | Subtypes of COPD have distinct bacterial compositions and stabilities over time
- Microbiome profiles show less variation within an individual than between individuals, however, some individuals exhibited high variability over time
- With increasing disease severity, the abundance of Proteobacteria increases, whereas diversity overall decreases
- No significant changes of diversity or taxa relative abundance between stable and exacerbation phase (with the exception of Moraxella spp.)
- COPD patients with higher exacerbation frequencies exhibit less stable lung microbiome over time
- Microbiome composition of bacterial exacerbations differs from viral and eosinophilic |
| Wang et al. (2019) | 16S rRNA, qPCR, host RNA microarray, Proteomic assay | 16 healthy controls, 43 participants with COPD, sample collection during stable state, AECOPD, 2 weeks post-therapy and 6 weeks recovery, 6 months from stable state | Stable COPD patients showed a significantly increased relative abundance of the genera Moraxella, Streptococcus and Actinobacteria, as well as decreased alpha diversity, compared to healthy controls
- The relative abundance of Moraxella was increased at stable state in GOLD III versus II patients and in ICS versus non-ICS exposed patients
- During AECOPD, the relative abundance of Moraxella was increased and alpha |
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<th>the first stable visit</th>
<th>diversity decreased compared to the stable state, along with significantly increased neutrophil and decreased macrophage percentage.</th>
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<td>- the trend of increased <em>Moraxella</em> and decreased alpha diversity was reversed at post- exacerbation time points</td>
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<td></td>
<td>- significant associations between <em>Moraxella</em> and <em>Haemophilus</em> with host transcriptome and proteome profiles of host interferon and pro-inflammatory signaling pathways and neutrophilic inflammation</td>
</tr>
</tbody>
</table>

$q$PCR$=$quantitative PCR; AECOPD$=$ acute exacerbations of COPD

**REFERENCES:**


11. Leitao Filho FS, Alotaibi NM, Ngan D, et al. Sputum microbiome is associated with 1-


