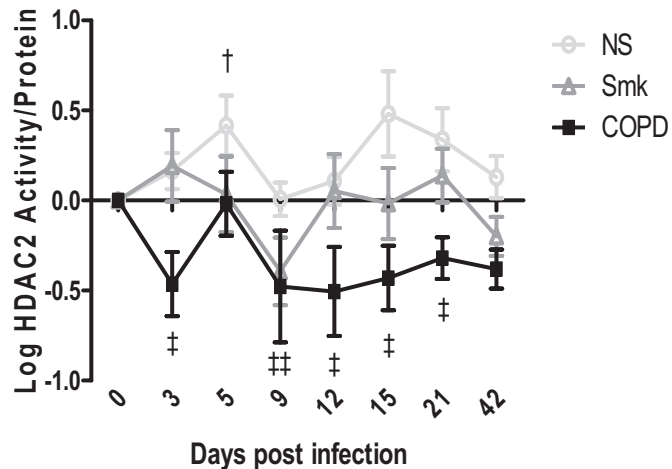exacerbation HDAC2 activity fell in COPD subjects, this represents a potential mechanism of excessive inflammation. The same pattern is not seen in control subjects.

Abstract S14 figure 1 Time course of sputum macrophage HDAC2 activity in individual groups normalised for baseline. Data presented mean±SEM. (Paired t test between day 0 and post infection day † in NS p<0.05; ‡ in COPD p<0.05, ‡‡ in COPD p<0.01).

S15 | DETECTION OF BACTERIA IN SPUTUM FOLLOWING EXPERIMENTAL RHINOVIRUS INFECTION IS MORE COMMON IN COPD THAN CONTROLS SUBJECTS

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Introduction and Objectives There is increasing evidence that the majority of acute exacerbations of COPD (AECOPD) are caused by virus infection, and rhinoviruses are the most frequently identified species. Bacteria are also responsible for AECOPD but the relationship between these two is poorly understood. To investigate this further bacterial culture was performed in sputum samples collected from GOLD stage II COPD subjects (n=9) and non-obstructed smoking (n=10) and non-smoking (n=11) controls enrolled in a rhinovirus challenge study.
Methods Rhinovirus infection was confirmed with quantitative PCR performed on nasal lavage and sputum samples collected at baseline and days 3, 5, 9, 12, 15, 21 and 42 post virus inoculation. Semi-quantitative bacterial detection was performed in sputum samples by a CPA-accredited microbiological laboratory. Any subject that had bacteria detected on or after day 9 was defined as "bacteria positive" and those with none detected were defined "bacteria negative". Species frequently associated with respiratory illness were defined as pathogenic (S pneumoniae, H influenzae, M catarrhalis and S aureus) and any others as non-pathogenic.
Results No bacteria were detected in baseline sputum samples. Peak virus load occurred on day 9 with maximum bacterial colonies identified on day 15. There were significantly more bacteria positive subjects in the COPD group (67%) with the majority of control subjects (81%) being classified as bacteria negative, (Abstract S15 figure 1, χ^2 p=0.04). COPD subjects with bacteria detected at any time point in the study had significantly more pathogenic species in their sputum samples (n=8/8) compared to controls (n=1/9), (χ^2 p=0.0001). No non-pathogenic bacteria were detected in COPD subjects.
Conclusions Detection of bacteria is common after rhinovirus infection, with the peak occurring 6 days after maximum virus load.

COPD subjects are more likely to have pathogenic bacteria detected than controls following virus infection. Mechanisms responsible for this phenomenon merit further investigation.
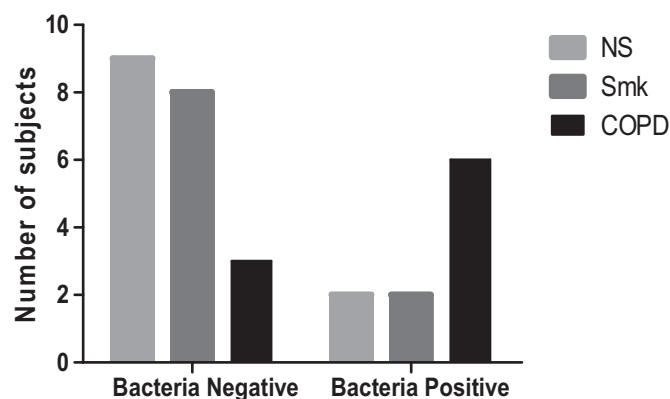
Abstract S15 Figure 1 Number of subjects in each study group with bacteria negative or bacteria positive sputum samples on or following day 9, (χ^2 p=0.04).

S16 | MOLECULAR PROFILING OF THE AIRWAY MICROBIOME IN COPD

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Introduction It is hypothesised that bacteria are important in the pathogenesis of COPD exacerbations and clinical expression of disease. To date, most bacteriological research in COPD has been performed using culture based methods. However, novel molecular approaches offer more detailed evaluation of the airway microbiome that may better inform the role of bacteria in COPD.
Aims To characterise the microbial community in COPD and examine whether detectable changes occur with serial longitudinal assessment at stable, exacerbation, follow-up and recovery visits.
Methods 115 COPD patients that were part of a clinical trial had sputum samples collected at the four time points. Patients received antibiotics and / or oral corticosteroids after clinical assessment to treat exacerbations. Follow-up and recovery samples were collected 2 and 6 weeks after the exacerbation sample. Real-time quantitative PCR (qPCR) was performed on sputum DNA using universal 16S gene primers and specific gene targets to quantify total bacterial load and the specific pathogens Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis and Staphylococcus aureus. In a subgroup of 30 patients, 454 high-throughput pyrosequencing was performed at each of the 4 visits to examine changes to the global microbiome.
Results Quantitative PCR identified one or more pathogens in 94% of stable samples and 97% of exacerbation samples. There was no significant difference in the total bacterial load or any specific pathogen between longitudinal stable and exacerbation samples. 454 pyrosequencing identified Proteobacteria and Firmicutes to be the dominant groups contributing >80% of the sequence reads at phylum level. Haemophilus, Moraxella and Streptococcus were the dominant groups at genus level. Cluster analysis characterised three groups on the basis of the ratio of Proteobacteria to Firmicutes. No significant differences in patient characteristics were observed between microbiome clusters. There was no significant change across visits in the microbial community at either phylum or genus level. No treatment specific effects on the microbiome were observed.

Conclusions Molecular profiling identifies heterogeneity in the airway microbiome of COPD patients, with dominance of pathogens routinely identified at culture. However, a precise role for bacteria in COPD remains unclear.

S17 | IMPACT OF COPD SEVERITY AND SPUTUM PRODUCTION ON ANTIBIOTIC RESISTANCE

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Introduction Bacterial infections are a well-known trigger for exacerbations of COPD. A variety of antibiotics are regularly prescribed for this group of patients but the risk and frequency of antibiotic resistance in the COPD population is less understood. Routine culture data can be evaluated to establish resistance prevalence and patterns.

Methods Culture data were collected from the sputum samples of 293 patients in the London COPD cohort over a period of 5 years (01/01/2006–31/12/2010) mean ($\pm$SD) months in study 28.4 ($\pm$19.9); age 69.9 years ($\pm$8.9); predicted FEV_1 47.8% ($\pm$16.5); male gender 58%; exacerbation samples 48.9%; sputum producers 77.5%. Identification of bacterial presence was established and where clinically indicated drug sensitivity tests (DSTs) were performed. A resistant sample was reported as any bacterial isolate resistant to at least one antimicrobial agent.

Results 92/293 (31.4%) patients had at least one bacteria positive sample over the study period. 87/92 (94.6%) patients had samples where DSTs were performed on bacteria positive samples. Resistance was observed in 69/87 (79.3%) patients. 30/293 (10.2%) patients were resistant to all samples where DSTs were performed. 227/293 (77.5%) of patients were sputum producers. There was no significant relationship between predicted FEV_1 and antibiotic resistance frequency in this cohort (X^2-test; p=0.577). Patients who were classified as regular sputum producers were more likely to exhibit resistance in culture positive bacteria (p=0.048).

Conclusion Results from this analysis conclude that an estimated 23.5% of COPD patients will develop resistance to an antimicrobial agent within 28.4 months of follow-up with sputum producers being at a higher risk. This study highlights the importance of investigating sputum samples with determination of resistance patterns. Information on resistance patterns and transmission of resistance in COPD can allow more appropriate and targeted antibiotic therapy for COPD exacerbations with improved outcomes.

S18 | A COMPARISON OF PREVALENCE AND LOAD OF AIRWAY BACTERIA IN COPD PATIENTS WITH PAIRED STABLE AND EXACERBATION STATE SAMPLES

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Introduction Airway bacterial infections are associated with COPD exacerbations. The most frequently identified bacteria in COPD are *Haemophilus influenzae* (HI), *Moraxella catarrhalis* (MC) and *Streptococcus pneumoniae* (SP) (Wilkinson *et al*, 2006), though studies have used culture techniques, with little data available on PCR methodology in airway infection. Using the London COPD cohort, we aimed to assess and quantify bacterial prevalence and load via quantitative PCR, in paired baseline and exacerbation sputum samples.

Methods Quantitative PCR was utilised, measuring prevalence and load on paired baseline and exacerbation samples, with baseline samples obtained within 1 year prior to its paired exacerbation. SP, HI and MC gene targets were Spn9082; *Haemophilus influenzae* P4 lipoprotein gene; copB outer-membrane-protein gene, respectively. The baseline state was defined as being at least 6 weeks after, and 2 weeks before, an exacerbation. Exacerbation was defined as two consecutive days of at least two increased symptoms (Anthonisen criteria), at least one of which is a major symptom (dyspnoea; sputum purulence; sputum volume).

Results Sixty-nine paired baseline and exacerbation sputum samples were obtained from 56 patients: mean ($\pm$SD) age 71.0 years ($\pm$8.4); predicted FEV_1 46.4% ($\pm$17.0); male gender 60.4%; current smoker 30.2%. Bacteria were detected at significantly higher rate at exacerbation, being seen in 36/69 (52.2%) exacerbations, and 19/69 (27.5%) baseline samples (χ^2-test; p=0.003). Mean bacterial load was significantly higher at exacerbation, with mean load of 8.3 ($\pm$1.1) $\log_{10}$ cfu/ml, compared with mean of 7.3 ($\pm$1.8) $\log_{10}$ cfu/ml at baseline (paired-samples t test; p<0.001), indicating a 10-fold overall-load increase at exacerbation. MC frequency increased significantly from 4.3% (3/69) at baseline to 17.4% (12/69) at exacerbation (p=0.014). Prevalence of HI (17.4% vs 26.1%) and SP (8.7% vs 20.3%) showed non-significant increases. Mean loads of SP and MC increased significantly from baseline to exacerbation (p=0.048; p=0.008, respectively).

Conclusion Prevalence and load of airway bacteria in COPD increases from baseline to exacerbation. This confirms that bacteria play an important role in exacerbation aetiology, implicating increasing bacterial load as a key underlying mechanism, and emphasises the importance of prompt antibiotic therapy at COPD exacerbation.
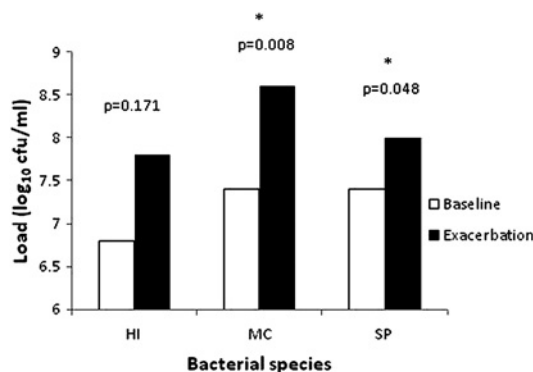
Abstract S18 Figure 1   Bacterial load at baseline and exacerbation of COPD as determined by quantitative PCR.

S19 | MOLECULAR FINGERPRINTING AND METAGENOMIC ANALYSIS REVEALS A POLYMICROBIAL ELEMENT IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Introduction and objectives Chronic obstructive pulmonary disease (COPD) patients commonly exhibit a multi-factorial pathology with neutrophilic inflammation and chronic obstructive bronchiolitis. COPD patients suffer episodes of pulmonary exacerbations. The role of bacteria in exacerbations has been investigated in COPD using culture-dependent techniques. Unlike cystic fibrosis (CF), there are few molecular studies describing the possibility of a