January 2013

The Role Of Antibiotics In Microbial Interactions Of Copd

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A Thesis Presented to the Faculty of the Yale School of Public Health

Yale University

In Candidacy for the Degree of Master of Public Health

2013

Abstract

Background: Chronic Obstructive Pulmonary Disease **(**COPD) exacerbations caused by bacterial infections account for more than 50% of all exacerbation episodes, with *Haemophilus influenzae* as the most frequently isolated pathogen. The role of antibiotics in treating COPD bacterial exacerbations remains unclear.

Objective: The current analysis evaluates the interaction of *H. influenzae* with other pathogens while controlling for the use of antibiotics.

Methods: Data were from a longitudinal study of COPD patients conducted at a VA medical center from 1994-2010. Analysis was restricted to sputum samples collected over the course of one year in a total of 130 patients. Presence of *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and antibiotic use within the past 21 days were examined to predict the colonization by *H. influenzae* using repeated measures logistic regression.

Results: The cohort was primarily male (96.9), white (89.9%), with an average age of 66.7 (SD 9.6) years and with moderate to severe airway obstruction. *H. influenzae* was detected 20% at any given time in the 130 patients. There was a trend for the presence of *S. aureus* and use of antimicrobial therapy in the past 21 days to be associated with decreasing the likelihood of colonization by *H. influenzae*.

Conclusion: Future research of the underlying mechanisms of these complex polymicrobial interactions will require further investigation of the environment of the lung microbiome, the host immune response and the impact of the medication on these host-pathogen and pathogen-pathogen interactions. Management of COPD and prevention of exacerbations of COPD would reduce both the rapid decline in lung function associated with bacterial infections, and the associated healthcare costs.

Acknowledgements

First and foremost, I would like to thank Drs. Melinda Pettigrew and Janneane Gent for providing mentorship, support, and guidance with this project. Tim Murphy and Sanjay Sethi for letting me use the data. Special thanks the professors who have influenced my life and who have provided guidance in my endeavors. Lastly, I am grateful to my friends and family for their support through the course of my education at Yale.

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Introduction

Chronic Obstructive Pulmonary Disease (COPD) is as a set of heterogeneous disorders that restrict the airway flow and is not completely reversible. It ranks among the top ten leading diseases in mortality rates in the U.S. with high rates of hospitalization and is projected to rise in ranks for both morbidity and mortality. The heterogeneity of disease is due to an array of environmental and genetic factors as well as a range in clinical presentation and other comorbidities that reflects the variability in the management of disease. COPD is further complicated by exacerbations that require hospitalization and worsen quality of life. Acute exacerbations of COPD (AECOPD) due to bacterial infections are of concern because of higher relapse rate and the additional damage to the airway. However, conflicting results in role of bacteria in these exacerbations, their persistence during stable disease and efficacy of various antibiotics for treatment has contributed to increased research efforts to determine the key factors in this multifactorial disease and identify the best methods of control. With high rates of hospitalization and high cost for management of disease, identifying sources for increased risk for hospitalization and impaired airway function will improve management of the disease.

Drawing upon the current methods in managing COPD and knowledge of the emerging role of bacteria in the airways of patients with COPD, this paper first aims to describe the disease over the course of one year among COPD individuals at a Veteran Affairs Hospital. This description will focus in on bacteria identified from sputum samples and the use of antibiotics. Secondly, it will evaluate and attempt to explain whether antibiotic use is associated with isolation of specific bacteria. The primary outcome of interest is colonization of *H. influenza*e and whether its presence can be determined by other commonly isolated respiratory pathogens and antimicrobial therapy. Assessments of such an outcome have public health implications in the management of COPD and COPD exacerbations.

Defining the Global Burden of COPD

Chronic Obstructive Pulmonary Disease (COPD) is the fifth leading cause of morbidity and mortality worldwide according to the 2001 report from the WHO Global Burden of Disease and Risk

Factors project[1] with rates that continue to rise. In the United States, it is the fourth leading cause of death with a high hospitalization prevalence and in-hospital mortality attributed to sequelae or other comorbidities.[2] Although definitions of COPD vary among organizations, it is generally classified as a slow progressing and heterogeneous disorder defined by irreversible airflow obstruction and impaired pulmonary function with increased inflammation and mucus production.[3] Given the heterogeneity of the disease and clinical presentation among patients, this also proves difficult in understanding the myriad of risk factors and management of COPD. The primary risk factor for COPD is tobacco smoke, however, other key risk factors include genetics, age, gender, indoor and outdoor pollutants, asthma, and respiratory infections.[1]

Additionally, morbidity and mortality rates vary globally and within regions in countries, especially in developing nations, where detection and treatment methods are limited.[4–6] In the United States, the disease is predominantly found in Caucasian males ages 45 and older and tends to increase in severity with age. Clinical symptoms range from cough, sputum production, and dyspnea to more severe disease of weight loss, anorexia, and hemoptysis due to respiratory infections.[7] Although COPD is a preventable and treatable disease, it has a slow progression that causes obstruction to airflow, limiting the quality of life. Statistics from the late 1990s report that COPD in the US is the leading cause of hospitalization in adults, especially among the elderly.[3] Additional statistics from the last decade also report COPD affects more than 10.2 million adults and is responsible for 14.2 million ambulatory visits.[3] This translates into direct and indirect costs of $32.1 billion as of 2003.[8] With a wide range of risk factors and complications, further investigation in the pathogenesis of COPD will help to improve management of medications and reduce morbidity and mortality.

To add to the complexity, the burden of COPD continues to be underestimated due to the varying and evolving definitions of the disease, the variation and severity in clinical presentation, and limited diagnostic methods or standards used for detection that contribute to the limited available information on COPD worldwide. Groups such as the Burden of Obstructive Lung Disease (BOLD) Initiative was developed to standardize methods for estimating COPD prevalence and obtain data on risk factors

globally across all levels of development.[9] The standard diagnostic method defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) is measured through spirometric testing of FEV_1/FVC to determine degree of airflow limitation and is classified into four stages from mild (stage 1) to severe (stage 4).[10] Although smoking is the predominant risk factor for COPD, globally, additional exposure to biomass fuel smoke in indoor cooking and air pollution also contribute to 35% of COPD cases in low and middle income countries.[1]

The difficulty in detecting and diagnosing COPD is further confounded, especially in other countries, by attribution of symptoms such as cough to smoking, aging, or other respiratory disease such as tuberculosis, or using the term "COPD" to refer to functional disorders of several lung diseases characterized by chronic airflow limitation.[4] Furthermore, in parts of Asia, COPD is often referred to by its symptoms and often is not diagnosed as COPD.[11] Only recently was the term COPD introduced to Japan.[12] Despite these barriers to estimating the true burden of COPD worldwide, the best estimates by WHO for mortality and Disability Adjusted Life Years (DALYs), a measurement of years lost to a 'healthy life', place COPD rates in China as the highest among the 25 most populous nations and in Asia.[1]

In the Western-Pacific region, China has the highest incidence of disease with 130.5 /100,000 age-adjusted deaths and 622 /100,000 age-adjusted DALYs.[1] Other studies report that Hong Kong has the highest mortality rates of 7.3/10,000.[13] The prevalence of COPD varies among provinces and cities throughout China with rates of COPD ranging from 5%-13% and contributes to 1.6% of all hospitalizations.[14] Like the United States, rates of COPD in China are found to be significantly higher among the aging population, and is more prevalent among men than women with a range of 8.3%-18.9% compared to 3.8%-7%.[1,15] Rates are higher in rural areas compared to urban areas, especially among non-smoking women.[14] The primary causes of COPD in China are tobacco exposure and indoor and outdoor air pollution from biomass fuel for cooking.[16,17] A study in Hong Kong demonstrated that exacerbations are most commonly caused by infections and air pollution which may also be present on mainland China. Bacteria isolated from the sputum during exacerbations were identified as *Haemophilus influenzae* (13%),

Pseudomonas aeruginosa (6%), and *Streptococcus pneumoniae* (5.5%). China also has a high prevalence of smoking, with 63% of adult men using tobacco. It is also the greatest producer and consumer of cigarettes in the world.[14] Among non-smokers, prevalence studies have demonstrated that there is an increased risk for COPD among older males with low BMI, low education level, and environmental exposure to tobacco smoke, coal and/or biomass smoke, poorly ventilated kitchens and family history of respiratory disease and recurrent childhood cough.[18] A nested case-control study indicated that tobacco consumption was associated with an increased risk of COPD.[19] Although the array of studies conducted vary in methodologies and standards for assessment of COPD and risks, COPD remains a burden to the health system in China as the fourth leading cause of death in urban areas and third in rural areas.[14]

Exacerbations

Quality of life with COPD is further complicated by episodes of acute exacerbations (AECOPD) that increase the rate of airway obstruction and lung function and is likely underreported by patients.[20] AECOPD usually occur later on in moderate to severe forms of the disease and is a leading cause of morbidity and mortality of COPD. [21] A general working definition of COPD exacerbation has been established as " *a sustained worsening of the patient's condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD*".[22] Despite this working definition, there is no general consensus on what the underlying etiologies of exacerbation are because there are a variety of outcomes and potential causes for exacerbations. Exacerbations are also associated with significant increases in health care costs, and hospitalization rates.[23]

During an exacerbation, there is an increased inflammatory response in the airways that may contribute to the decline in FEV.[24] In addition to an increased pro-inflammatory response, mucus secretion increases leading to an impairment of airflow and gas exchange resulting in impaired breathing requiring intervention and medication. This further inflammation of the airway above baseline [25] can lead to a systematic inflammation and further complications and decline in lung function in patients with

COPD. Although exacerbations may be caused by an environmental trigger, there is evidence demonstrating the importance of viral and bacterial infections. Episodes of exacerbation in association with bacterial infections are of great interest because of high hospitalization and frequency of episodes that reduce the quality of life.[20]

The Role of Bacteria in COPD and AECOPD

Findings on role of bacterial infections in the pathogenesis of exacerbations have been inconsistent, but it is generally accepted that bacterial infections can cause up to 50% of COPD exacerbations.[26–29] COPD exacerbation patients with bacterial infections have more frequent exacerbation episodes, longer hospitalizations, and increased sputum.[25] Patel et al. also demonstrated that occurrence of bacterial colonization of the lower respiratory tract in stable COPD patients also increases the frequency of AECOPD.[28] The presence of bacterial colonization may lead to more severe exacerbations and thus lead to a rapid decline in lung function.[25,30]

Exacerbations caused by bacterial infections are characterized by an increase in purulent sputum, increased sputum volume and increase in dyspnea. The most commonly identified bacterial pathogens isolated from patients' sputum with COPD exacerbations, from highest to lowest prevalence, include: *H. influenzae*, *Moraxella catarrhalis*, *S. pneumoniae*, and *P. aeruginosa*. [27,28,31–33] *H. influenzae* has also been identified from sputum in patients with stable disease, which has implications for theories on pathogenesis, efficacy of medication, evasion from the host immune response, and consequent and persisting exacerbations.[34] There also appears to a linear relationship between the number of pathogen colony forming units and inflammatory markers in sputum samples.[35] *H. influenzae* is has been shown to persistently colonize the airways of patients with COPD and may contribute to progressive impairment of lung function.[31] Furthermore, non-typeable *H. influenzae* has been found to induce COPD-like changes in mice, including presentation of progressive airway wall fibrosis, increased airway wall collagen, and increases in specific inflammatory markers and cytokines, which are similar to changes observed in patients with COPD.[36]

More importantly, it has been proposed that the detection of bacteria in the respiratory tract is not the only factor promoting exacerbations, but rather it is the acquisition of new bacterial strains that are essential to the pathogenesis of exacerbations.[34] Associations have been found between frequency of exacerbations and acquisition of new strains of *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, and *P. aeruginosa*.[27,34] The process of clearance of bacterial strains by the host immune system in COPD exacerbations is not fully understood; however, studies on the ecology of the lung microbiome has contributed to our understanding of the interactions that occur between the normal flora and bacterial pathogens and the competition that occurs between pathogens.[37,38] Understanding these interactions is essential to understanding how subsequent bacterial infections are acquired and are the key components involved in triggering an exacerbation.

Microbial Interactions

The human airway microbiota is colonized by a diverse community of organisms that is altered in individuals with COPD, leading to an increase in opportunistic infections and impairment of the host defense mechanisms. Bacterial infections and the interaction among different pathogens have been studied extensively in patients with Cystic Fibrosis (CF).[39,40] *H. influenzae* has been well studied in CF patients and hypermutable strains of *H. influenzae* have been shown to colonize the airway of CF patients for long periods of time increasing the risk for antibiotic resistance.[41] The interaction of the multiple pathogens in the already compromised lungs of CF consequentially leads to a dynamic immune-pathogen response that increases the chance for hypermutability and degradation of the lung tissue through new methods of evading host response and resistance to antibiotics.[42]

Similarly, there is evidence that suggests an increase in microbial diversity with moderate and severe COPD[43] that impacts the progression of disease and can play into the management of COPD through antibiotics and other management medication. Hilty et al. determined that airway microbiota of patients with asthma and COPD had higher frequencies of certain bacteria, especially of the genus *Haemophilus* and compared to controls.[44] Although the microbiota were diverse, the sample size was

small (N=5). Furthermore, Huang et al. used a culture-independent microarray (16S rRNA PhyloCHIP) to classify bacteria and discovered that among their small cohort of COPD patients, there was a diverse community of bacteria present during exacerbations that persisted in the lung.[45] Other experiments investigating the role of competition and interaction of these organisms in the nasal passage of rats demonstrated that acquisition of new strains and new species is a dynamic process .[37] The diversity of microbial communities and the alterations that occur with infections greatly influences and potentially challenges the current standards for treating diseases of the respiratory tract like COPD.

Treatment and Management: Antimicrobial Therapy

Symptoms of COPD are usually treated with bronchodilators, oral corticosteroids while antibiotics are used for bacterial AECOPD. In general, less severe exacerbations can be controlled with just bronchodilators and a short course of corticosteroids, however, with more severe exacerbations, especially in advanced COPD stages, antibiotic therapy is considered mandatory.[46] The GOLD standards indicate that antibiotics should only be given under the following guidelines: 1) the three cardinal symptoms of increased dyspnea, increased sputum volume, and increased sputum purulence 2) increased sputum purulence and one other cardinal symptom, and 3) patients who require mechanical ventilation.[10] Despite these standards, the ability for antimicrobial therapy to effectively treat AECOPD is dependent on an array of factors, including the bacterial species, its susceptibility to antibiotic resistance, recent treatment with antibiotics, and the individual being treated.

There have been several studies conducted in the use of antibiotics from randomized control trials, meta-analyses and observational studies.[47–52] The conflicting data in the use of antibiotics as a COPD therapeutic is a result of the variability in the type of antibiotic used, duration of therapy, outcome of interest, definition of exacerbation and study design limitations. The most common antibiotic classes used to treat bacterial exacerbations include penicillin (amoxicillin), cephalosporin, fluoroquinolone, macrolide, and tetracycline. The most well studied antibiotic class for COPD belong to the macrolide

class where the immunomodulatory and anti-inflammatory properties have been shown to improve COPD outcomes.[53,54]

In acute exacerbations, a meta-analysis in 1995 by Saint et al. concluded that compared to placebo, antibiotic therapy was slightly better at improving exacerbations,[47] whereas later studies and reviews conclude that administration of intravenous or oral antibiotics is not efficacious.[26,51] Other studies have demonstrated that antibiotic use lowered rates of relapse among outpatients and improved outcomes among hospitalized patients.[55] The range of findings in regards to the impact of antibiotic use in reducing COPD exacerbations are in part due to the multiple bacterial species and strains involved, wide spectrum of antibiotic activity to target the types of bacteria, and differences in study design, methodology, and participant demographics. In another study at a VA Medical Center, a retrospective cohort analysis of visits for AECOPD found a 22% higher rate of relapse among patients who were not given antibiotics.[55]

In general, antibiotic selection and dosing for treatment of acute exacerbations of COPD depends on several factors. Choice of antibiotic varies depending on its ability to penetrate tissues to target intracellular bacteria and consider its cost-effectiveness.[50] In a hospital setting, early antibiotic treatment improved outcomes including lower rates of mortality and readmission for a secondary exacerbation episode.[56] Lower relapse rates are also associated with antibiotic treatments compared to non-antibiotic treated patients with amoxicillin treatment groups having the highest relapse rates.[55] Overall, these studies show that antibiotic treatment lowers mortality rates and reduces exacerbations.[50,55,56] Other studies, however, show little benefit to antibiotic therapy. For instance, a limited study at one hospital demonstrated the ineffectiveness of antibiotics, especially in dual therapy of macrolides with beta-lactams or fluoroquinolones.[51]

A Cochrane Review from 2009, reviewed several databases for randomized control trials on antibiotic use within 5 days compared to placebo to evaluate the impact of antibiotics on specific outcomes. The review discovered that antibiotic use does provide a reduction in short-term mortality (77%), risk of treatment failure (53%) and sputum purulence (44%), regardless of the type of antibiotic

administered.[57] This broad review was unable to provide more outcomes due to the variability in study design and patient selection. In depth analyses on the pharmacokinetics of these antibiotics may provide additional information in the effectiveness of antimicrobial therapy on specific outcomes.

It has been suggested that instead of a standardized decision tree for treatment of COPD bacterial exacerbations, that personalized treatment might be of use.[58] This is in part due to the types of bacterial infections that trigger an exacerbation episode, while taking into account the frequency of reduced exacerbations and long term effects of antibiotic resistance on the immune system. Sialer et al. have also suggested a stratification method for use of antibiotics dependent on the severity of COPD and exacerbations, isolated microorganisms, and other risk factors to provide the correct antibiotic and dosage to the patients [49].

In summary, there is increasing evidence that bacterial infections are strongly tied to COPD exacerbations and stable disease. The role of bacterial infections in exacerbations complicates antibiotic treatment decisions since patients with a history of frequent exacerbations have a worse quality of life, increased risk of hospital admission and duration, and greater risk of mortality compared to patients with fewer exacerbations.[8,20,59] In addition, the host inflammatory response to bacterial exacerbations differs compared to nonbacterial exacerbations and contributes to a more intense systemic inflammation in COPD exacerbations.[60] Furthermore, antimicrobial therapy may alter the microbial community in the respiratory tract as it has been shown in patients with CF.[61,62] Thus, the role of bacterial infections in COPD exacerbations warrants additional research in scope and depth in the mechanisms of pathogenesis. This paper aims to describe and evaluate the interaction of pathogens and prescription of antimicrobial therapy on the pattern of colonization in the respiratory tract of a COPD cohort.

Methods

Study population and design

The data used in this analysis is from a prospective, longitudinal study of COPD patients from the Veterans Affairs Hospital in Buffalo, NY from 1994-2010, as previously described.[27,31] Patients were

enrolled if they presented with bronchitis, without asthma and bronchiectasis based on clinical assessment. Additionally, patients were seen on a monthly basis and also when symptoms suggested an exacerbation episode. During each visit, clinical information and sputum and serum samples were collected. Methods of collection of additional data, preservation and analysis of sputum samples isolation and detection of bacteria are described elsewhere.[27,31]

For the purpose of this study, the population was selected from a subset of the original study participants with the criteria of one year of completed data. One-hundred thirty patients of the original 163 fit the criteria for inclusion in the proposed analysis in evaluating the role of antibiotics in the pattern of colonization of bacteria in the cohort.

To evaluate the role of antibiotics in the subset of patients, antibiotics were classified based on the major classes and the frequency of the classes in the cohort. The following groups were formed from this classification: penicillin, cephalosporin, fluoroquinolone, macrolide, sulfonamide-trimethoprim compounds, and other antibiotics, which include tetracycline, other beta-lactams and aminoglycosides. Antibiotics were then matched to visit dates with sputum samples for each patient and classified as to whether or not a patient took any of the antibiotic classes within 7, 14, or 21 days from each visit.

Statistical Methods and Data Analysis

Statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). The primary analysis evaluated the relationship between pathogens, primarily *H. influenzae* and other major pathogens found in the respiratory tract, controlling for antibiotic use. The primary outcome of interest was presence of *H. influenzae*. Presence of the primary pathogens *M. catarrhalis*, *S. pneumoniae*, *S. aureus*, *P. aeruginosa* and antibiotic use were examined to predict *H. influenzae* using repeated measures logistic regression. This was done using PROC GENMOD (SAS Institute), to obtain generalized estimating equations and used an autoregressive correlation structure (AR1). Only the first visit in a given month was used in the model, and individual patients could contribute up to 12 visits for the year. Since COPD in the US is found primarily among older, white males and the heterogeneity of the disease and progression varies among patients, no demographic factors were included in the analysis.

Results

Patient baseline characteristics and demographics from time of enrollment are in Table 1. The mean age of the 130 patients was 66.7 (SD 9.6) with a median age of 68 years ranging from 45 to 85 years. The majority of the cohort was white (89.9%) and male (96.9%). At the time of enrollment, approximately two-thirds (65.9%) were former smokers and had COPD for an average of 10.2 years (SD 11.3). Most patients were classified as having moderate to severe airway obstruction based on post-bronchodilator FEV_1 % predicted values.

In the year of observation, patients contributed a total of 1,281 visits with sputum samples. Each patient only contributed one visit with sputum sample per month for a maximum of 12 visits. Table 2 describes the distribution of clinic visits with an average of approximately 10 visits with sputum samples (9.8 SD 1.5) and with a range of contributing visits from as few as 3 visits to as many as 12 visits in a year. Most visits (94.5%) were scheduled monthly visits according to study protocol. There were also a limited number of subjects that came in for additional unscheduled visits, an indicator for an exacerbation. These individuals contributed to 5.5% of the remaining visits. Patients that had additional unscheduled visits came in an average of 9.4 times (SD 3.0) compared to the average 9.8 visits for patients who only had scheduled visits.

Sputum collected from the patients were processed and cultured for identification of bacteria. Overall, in the 1,281 sputum samples, *H. influenzae* was present in 210 (16.4%), followed by *S. aureus* in 122 (9.5%) samples, *M. catarrhalis* was present in 67 (5.2%) samples, *S. pneumoniae* was present in 45 (3.5%), and *P. aeruginosa* was present in 35 (2.7%) samples (data not shown). In the analysis, 122 out of 130 patients (93.8%) had at any given time at least one species of bacteria detected in the sputum samples. Patients had anywhere from only one bacterium isolated from their sputum to as many as 11 different bacteria isolated. Thirty-one patients (23.9%) had at least one bacterium present, 54 (41.5%) patients had 2-3 different organisms, 32 individuals (24.6%) had between 4-6 different bacteria, and only 5 (3.8%) had more than 7 organisms at a given time (data not shown).

Table 3 demonstrates the distribution of bacteria in any of the patients' sputum samples. The rate at which any of these 5 pathogens were detected in any patient for the year was 44.6% (N=58). From sputum samples, the primary pathogens cultured and isolated from sputum samples in any given patient included *H. influenzae* (20%), *S. aureus* (14.6%), *M. catarrhalis* (5.4%), *P. aeruginosa* (3.1%), and S. *pneumoniae* (1.5%).

Patients were prescribed the use of antibiotics for both COPD and other diagnoses or procedures as shown in Table 4. Among the 130 patients, 69 (53.1%) patients were taking any antibiotic over the course of a year. Fifty-two individuals (40.0%) were on at least one antibiotic, while 17 (13.1%) were taking two to three antibiotics. Of the individuals taking antibiotics, 22 (16.9%) were on penicillins, 19 (14.6%) were taking sulfonamides, followed by 18 (13.8%) on fluoroquinolones, 16 (12.3%) on macrolides, 10 (7.7%) on cephalosporins, and 5 (3.8%) taking other antibiotics. Antibiotic use was further categorized into whether patients were on antibiotics within the past 7, 14, or 21 days. There were 1,198 (93.5%) visits not associated with administration of antibiotics. In the remaining visits, any antibiotic use was accounted for 29 visits (2.3%) within 7days, 56 visits (4.4%) within 14 or 21 days, and 83 visits (6.5%) within 21 days from each visit with sample sputum.

Results of repeated measures logistic regression performed to predict colonization of *H. influenzae* are shown in Table 5. Significant findings were interpreted as a positive association between *H. influenzae* and the dependent variables was indicated by an odds ratio (OR) $\geq$ to 1 and a negative association was indicated by an OR<1. Additionally, an OR of 1.0 or any 95% confidence interval (CI) that includes 1.0 indicated no significant association.

Colonization by *H. influenzae* was not significantly associated with *S. aureus*, *M. catarrhalis*, *P. aeruginosa S. pneumoniae* or antimicrobial therapy within the past 21 days. Odds ratios (ORs) indicated a negative association with *S. aureus*, *M. catarrhalis*, and *P. aeruginosa* and a positive association with *S. pneumoniae*. Antimicrobial drug therapy within the past 21 days was also negatively associated with predicting *H. influenzae* colonization. None of these associations were significant based on OR values,

95% CI and p-values. However, results for *S. aureus* and antimicrobial therapy approached significance (p=0.080 and 0.077 respectively) in decreasing the likelihood of *H. influenzae* colonization.

Discussion

This study aimed to describe the colonization of the respiratory tract among 130 patients with moderate to severe COPD with *H. influenzae* as the predicted outcome with the presence of four other major pathogens *S. aureus*, *S. pneumoniae*, *M. catarrhalis*, and *P. aeruginosa*, controlling for antimicrobial therapy. The model predicting *H. influenzae* colonization demonstrated no significant associations with other pathogens or antibiotic use. Although not statistically significant, there were trends of negative associations seen between *H. influenzae* and pathogens *S. aureus, M. catarrhalis*, and *P. aeruginosa* and a trend of a positive association with *S. pneumoniae* colonization. Additionally, antimicrobial use within the past 21 days was also negatively associated with *H. influenzae* colonization. Although the findings were not significant, presence of *S. aureus* and antimicrobial therapy use were approaching significance in decreasing the odds of *H. influenzae* colonization.

This lack of significance in the model may be a result of the limited number of patients and samples used in this analysis, thus diminishing the power of the analysis. Furthermore, the model was unable to differentiate whether the association between *H. influenzae* colonization occurred during stable COPD or during an exacerbation. It is well demonstrated that *H. influenzae* plays a major role in exacerbations, accounting for 20-30% of all exacerbations and in stable disease.[31,34,63,64] While the prevalence of *M. catarrhalis* and *S. pneumoniae* accounts only for 10-15% of all exacerbations and plays a minor role in stable disease.[34] The interaction between *M. catarrhalis* with *H. influenzae* has not been as well studied since *M. catarrhalis* has only in recent years been recognized as important in the pathogenesis of disease.[65,66] The low prevalence of *P. aeruginosa* is in agreement with other studies, and is most often found in advanced stages of COPD.[32,64] *S. aureus*, although frequently isolated in this group of patients, is usually infrequently isolated from sputa, and is an unlikely cause of exacerbations or stable disease.[34]

Competitive interactions between these specific bacteria have been well documented in other respiratory infections, in mouse models and in *in vitro* assays.[37,39,40,43,67–69] Colonization by bacteria is determined by location, density, competition between normal flora and pathogens, host immune response and antibiotic resistance genes. Unlike healthy lung microbiome, the COPD lung microbiome has a significant increase in microbial diversity as the disease progresses.[43] Thus, the difference in ecology of the lung microbiome may contribute to susceptibility to colonization by bacteria and increase the risk for infection among individuals with impaired lung function.

On a molecular level, in trying to elucidate the mechanism of these polymicrobial communities and host immune response in the respiratory tract, *in vitro* studies as well as *in vivo* studies in mice have identified some of the key indicators for the interactions.[36,70,71] Specifically, products from the bacteria such as hydrogen peroxide and other secreted products also contribute to the interaction and competition of these species in the respiratory tract.[70] In a study of children with upper respiratory tract infections, *S. aureus* is found to be protective for colonization by *H. influenzae*.[67] Evidence suggests that the role of these polymicrobial interactions tends to be dynamic with synergistic and antagonistic effects. In mouse models, new strains of *S. aureus* were found to inhibit invasion by new S. *aureus* strains in the nasal passage of the host.[37] However in the same study, co-colonization of *S. aureus* and *S. pneumoniae* enabled infection by *H. influenzae* in the nasopharynx.[37]

Although S. *pneumoniae* was not frequently present in this analysis, evidence from *in vitro* assays suggests that the neuraminidase of *S. pneumoniae* is capable of desialylating the lipopolysaccharide of *H. influenzae*, providing a competitive edge in colonization.[68] Co-colonization of *H. influenzae* and *S. pneumoniae* resulted in clearance of *S. pneumoniae* by the complement pathway.[38] While different strains of *H. influenzae* and *S. pneumoniae* are able to co-exist in nasal passages of mouse models[37], *in vitro* studies have demonstrated that production of hydrogen peroxide by *S. pneumoniae* inhibits growth of *H. influenzae*, but results are not reciprocated for *H. influenzae* on *S. pneumoniae* in interspecies competition.

There are many characteristics of *H. influenzae* that allow for frequent colonization, primarily, biofilm production and the ability for *H. influenzae* to evade the host response through intracellular infection of epithelial cells. Both of these characteristics may contribute to persistent infection in the host's lung.[71–73] Additional insight into the field of genomics has also indicated the ability of certain strains of *H. influenzae* to colonize the respiratory tract resulting in simultaneous infections among COPD patients.[74] Bacterial genetic islands involved in metabolic functions are associated with COPD have also been identified.[75] Variation in genomes among different strains of *H. influenzae* isolated from COPD patients likely determine its ability to trigger COPD exacerbations.[75] The difference in strains also produce different inflammatory responses in COPD which could contribute to recurrent exacerbation episodes.[76]

In this study, the covariate representing antimicrobial therapy in the models suggested an association with a decrease in *H. influenzae* colonization. We observed no significant findings in different classes of antibiotics prescribed among the cohort possibly due to a lack of statistical power. Pettigrew et al. demonstrated a difference in the diversity of the nasal microbial community when comparing children with and without otitis media. This difference contributed to the risk of colonization with *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* and was dependent on antibiotic use within the past 6 months which suggests a prolonged effect of antibiotics on the human immune system.[77]

The study's main strength is the description of the frequency of pathogens present in sputum among COPD patients over the course of a year. Due to the prospective, longitudinal design of the parent study, we were also able to evaluate the role of antibiotics in promoting or reducing colonization of specific bacterial pathogens over the course of one year.

Further analysis would prove beneficial in assessing the interaction of medications, host immune response, and susceptibility to bacterial infections in the progression of COPD. The study did not consider interactions of other medications, specifically corticosteroids, potent anti-inflammatory drugs that are often used in controlling of symptoms of COPD and AECOPD. In particular, inhaled steroids compared to oral or IV steroids are not well understood in the long-term management of disease, especially in

AECOPD. A meta-analysis of randomized control trials published in the 1980s and 1990s demonstrated that inhaled corticosteroids improved FEV_1, and thus controlled progression of stable disease. However, it was not shown to be beneficial to the rate of exacerbations.[78] Another limitation of the current analysis was the lack of available data on other variables such as comorbidities affecting use of antibiotics and vulnerability to infections. Finally, this analysis was not able to examine different strains of the five primary pathogens in the interaction of antimicrobial therapy and the role of persistent *H. influenzae* infection.

Antibiotics have the ability to alter the lung microbiome and potentially facilitate the progression of COPD and other respiratory diseases. COPD patients already have impaired airway function that is further exacerbated by the host immune response to respiratory infections. Bacterial infections disrupt the existing microbial communities and increase certain species not commonly found in healthy airways.[44] Management of COPD and prevention of AECOPD would not only reduce health care costs but potentially decrease the amount of injury to the lung tissue that ultimately contributes to the increasing severity of COPD and recurrent COPD exacerbations. Personalized treatment has been suggested for treatment of CF patients with an emphasis on identifying polymicrobial interactions and catering treatment to pathogens that can ultimately improve patient outcomes.[61]

Lastly, this analysis and further analyses of data from the parent study have the potential to increase our understanding of COPD. The use and abuse of antibiotics worldwide may play a role in COPD in the US and elsewhere.[79] In China, *H. influenzae* is commonly found in patients hospitalized with an acute exacerbation of COPD.[64] The policy for prescribing antibiotics in China is less stringent than in the US, which has implications for antimicrobial resistance.[80] In a country where antibiotics are overprescribed and used for minor ailments, antibiotic resistance is not only a concern among COPD patients, but also among patients suffering from other respiratory infections, and may contributed to increased risk of hospitalizations and mortality. Future research focusing on polymicrobial interactions and lung microbiome or the ecology of the respiratory tract, antibiotic resistance, other medications, and the host immune response will provide insight into the prevention and management of COPD.

Tables

Table 1: Demographic characteristics of study participants

Baseline characteristics of Patients followed for one year. N=130 patients, N=1,281 visits

Characteristic	**N (%)**	**Mean (SD)**	**Range**				
			Min	**P_{25}**	**P_{50}**	**P_{75}**	**Max**
Sex							
Male	125 (96.9)						
Female	4 (3.1)						
Race							
White	116 (89.9)						
Non-White	13 (10.1)						
Smoking Status							
Former	85 (65.9)						
Current	44(34.1)						
Age (years)		66.7 (9.6)	45	61	68	74	85
Years with disease at enrollment		10.2 (11.3)	0	3	6	13	54
Pack-per-years of smoking		79.7 (36.3)	10	50	75	105	176
FEV1-Liters		1.63 (0.7)	0.47	1.23	1.47	1.93	4.07
FEV1 % Predicted		48.4 (18.5)	13	36	48	58	99
Airway Obstruction							
Severe: FEV_1 <50% of predicted	68(52.3)						
Moderate: $FEV_1$50-69% of predicted	49(37.7)						
Mild: FEV_1 ≥70% of predicted	13(10.0)						

Table 2: Clinic visits with sputum samples within 1 year

N=130 patients, N=1,281 visits

Visits	**N (%)**	**Mean (SD)***	**Range***				
			Min	P_{25}	P_{50}	P_{75}	Max
Total	1,281	9.8 (1.5)	3	9	10	11	12
Scheduled	1,210 (94.5)	9.8 (1.4)	4	9	10	11	12
Unscheduled [¥]	71 (5.5)	9.4 (3.0)	3	8	11	11	12

*Mean number and distribution of visits for any patient for 1 year. P_{25}, P_{50}, P_{75} are interquartile ranges.

[¥] Unscheduled visits are an indicator for exacerbation episodes. The mean and range represents the distribution of additional unscheduled visits in the entire study population that contribute to the total number of visits.

Table 3: Bacteria in sputum samples

(N=130 patients)[a]

Bacteria	**N (%)**
Any Pathogen	58(44.6)
H. influenzae	26 (20.0)
S. aureus	19 (14.6)
M. catarrhalis	7 (5.4)
P. aeruginosa	4 (3.1)
S. pnuemoniae	2 (1.5)

[a] Distribution over all subjects of bacteria in any of each subject's samples.

Table 4: Distribution of antibiotic use during the year of observation

(N=130 patients)

Antibiotic	**N (%)[a,b]**
Total in 1 year	
0	61 (46.9)
1	52 (40.0)
2-3	17 (13.1)
Class	
Penicillin	22 (16.9)
Cephalosporin	10 (7.7)
Macrolide	16 (12.3)
Fluoroquinolone	18 (13.8)
Sulfonamide-base	19 (14.6)
Other	5 (3.8)

[a] Percentages may not sum to 100% due to rounding

[b] Distribution over all subjects of antibiotic class use in any of each subject's samples.

Table 5: Predicted outcome of colonization of H. influenzae in the presence of S. aureus, S. pneumoniae, M. catarrhalis, and P. aeruginosa, and antibiotics in sputum from COPD patients †

N= 130 subjects, N=1,281 samples

Parameters	**OR (95% CI)***	**p-value**
S. aureus		0.080
Absent (reference)	1.0	
Present	0.49 (0.22-1.09)	
S. pneumoniae		0.510
Absent (reference)	1.0	
Present	1.27 (0.62-2.62)	
M. catarrhalis		0.951
Absent (reference)	1.0	
Present	0.97 (0.36-2.62)	
P. aeruginosa		0.126
Absent (reference)	1.0	
Present	0.39 (0.12-1.3)	
Antimicrobial drug therapy in the past 21 days		0.077
No (reference)	1.0	
Yes	0.55 (0.28-1.07)	

*OR, odds ratio, CI, confidence interval.

†Repeated measures logistic regression using GEE and autoregressive correlation method. The model included variables defining the presence or absence of the above bacteria and antibiotics taken within the past 21 days from the time of sputa collection.

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