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Lower Airway Bacterial Colonization Patterns and Species-Specific Interactions in Chronic Obstructive Pulmonary Disease

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ABSTRACT Little is known about interactions between nontypeable *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* in the lower respiratory tract in chronic obstructive pulmonary disease (COPD) patients. We characterized colonization by these four bacterial species, determined species-specific interactions, and estimated the effects of host factors on bacterial colonization among COPD patients. We conducted a prospective cohort study in veterans with COPD that involved monthly clinical assessment and sputum cultures with an average duration of follow-up of 4.5 years. Cultures were used for bacterial identification. We analyzed bacterial interactions using generalized linear mixed models after controlling for clinical and demographic variables. The outcomes of interest were the relationships between bacteria based on clinical status (stable or exacerbation). One hundred eighty-one participants completed a total of 8,843 clinic visits, 30.8% of which had at least one of the four bacteria isolated. *H. influenzae* was the most common bacterium isolated (14.4%), followed by *P. aeruginosa* (8.1%). In adjusted models, *S. pneumoniae* colonization was positively associated with *H. influenzae* colonization (odds ratio [OR], 2.79; 95% confidence interval [CI], 2.03 to 3.73). We identified negative associations between *P. aeruginosa* and *H. influenzae* (OR, 0.15; 95% CI, 0.10 to 0.22) and *P. aeruginosa* and *M. catarrhalis* (OR, 0.51; 95% CI, 0.35 to 0.75). Associations were similar during stable and exacerbation visits. Recent antimicrobial therapy was associated with a lower prevalence of *S. pneumoniae*, *H. influenzae*, *and M. catarrhalis*, but not *P. aeruginosa*. Our findings support the presence of specific interspecies interactions between common bacteria in the lower respiratory tracts of COPD patients. Further work is necessary to elucidate the mechanisms of these complex interactions that shift bacterial species.

KEYWORDS bacterial colonization, COPD, interactions, exacerbations

Chronic obstructive pulmonary disease (COPD) incurs a substantial public health burden and has high morbidity and mortality in the United States and worldwide (1, 2). The progressive course of COPD is accelerated by acute exacerbations that are associated with more frequent hospitalizations and death (3–5). It is recognized that most exacerbations are caused by bacterial infections; *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* are the four most common pathogens (6). Bacterial colonization and infection by *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* typically occur earlier in the course of COPD, while acquisition of *P. aeruginosa* is more prevalent in advanced disease (6). Overall, acqui-

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sition of a new strain of any of these four pathogens is associated with an exacerbation (7, 8). Chronic colonization of the lower airway by these pathogens in COPD is proinflammatory and is a likely contributor to COPD pathogenesis. Understanding determinants of bacterial acquisition and persistence in the lower airways in COPD is therefore important and as yet poorly understood.

Bacterial colonization patterns in COPD could be influenced by therapeutic interventions, especially antimicrobials, or by host factors, such as age, smoking history, and disease severity (9–12). In addition, interspecies bacterial interactions can influence which species become established and persist in a given environment, altering the composition of a microbial community and potentially influencing disease incidence and severity (12–16). Bacterial species can interact synergistically to promote persistent colonization; alternatively, bacteria can compete for the same environmental niche and reduce colonization. To date, literature on respiratory bacterial interactions is limited to the nasopharynx in young children and has not yet been extended to studies of lower respiratory tract colonization or to adults with COPD.

We characterized bacterial colonization patterns and interactions among the four primary bacterial pathogens in COPD patients during both stable and exacerbation clinical states. The goals of our study were to (i) describe the prevalence of bacterial colonization with *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, and *P. aeruginosa*; (ii) determine species-specific interactions; and (iii) estimate the influence of host factors on colonization with the four selected species. We hypothesized that interspecies interactions are an important independent determinant of bacterial colonization patterns in COPD and that this interaction is different in stable disease versus exacerbation. The interactions described in our study represent bacterial cocolonization; however, we use the term "interactions" here in order to maintain consistency with previously published work (11, 12, 17–19).

MATERIALS AND METHODS

Study design and participants. We analyzed data from the Bacterial Infection in COPD study, a prospective study of individuals with COPD recruited through an outpatient clinic in the Veterans Affairs Medical Center, Buffalo, NY, between April 1994 and June 2014 (7). Participants were evaluated monthly and whenever they had symptoms suggestive of an exacerbation. The Human Studies Subcommittee of the Veterans Affairs New York Healthcare System approved the study protocol, and all participants gave written informed consent.

A total of 183 adults enrolled in the study. We excluded two adults who did not have any follow-up visits. Of the total number of clinic visits ($n = 8{,}953$), 8,843 (99%) visits had sputum culture data available for 7,464 (83%) stable and 1,379 (15%) exacerbation visits. Sputum samples were not available for 110 (1.2%) clinic visits (27 exacerbation and 83 stable visits).

Data collection. Participants completed an in-depth interview conducted by trained personnel at enrollment, which collected information on demographics, baseline respiratory health history, occupational exposures, smoking history, medical history, current medications, and baseline pulmonary function tests. Subsequently, at each monthly visit, sputum and serum samples were collected, and the participants were also questioned about their chronic respiratory symptoms (dyspnea, cough, sputum production, viscosity, and purulence), and responses were graded 1 (at the usual level), 2 (somewhat worse than usual), or 3 (much worse than usual). Clinical assessment of the cause was prompted by minor worsening of two or more symptoms or major worsening of one or more symptoms. If the patient had a fever (a temperature of ≥38.3°C), appeared ill, or had signs of consolidation on lung examination, a chest film was obtained to rule out pneumonia. If other causes of symptom worsening, such as pneumonia, upper respiratory tract infection, and congestive heart failure, were ruled out clinically, the participant was considered to have a COPD exacerbation at that visit. The determination of whether the patient had stable disease or an exacerbation was made by one of two physicians (T.F.M. or S.S.) prior to receipt of sputum culture results.

Sputum samples. Sputum samples were spontaneously expectorated morning samples homogenized by incubation at 37°C for 15 min with an equal volume of 0.1% dithiothreitol. All the individuals enrolled in the study were patients with chronic bronchitis who received extensive, repeated instruction in providing adequate sputum samples. The samples were assessed macroscopically for adequacy prior to processing (20). Serial dilutions of homogenized sputum in phosphate-buffered saline were placed on blood, chocolate, and MacConkey agar plates. Bacteria were identified using standard techniques. A P6-specific monoclonal antibody was used to distinguish *H. influenzae* from *Haemophilus haemolyticus*. Sputum supernatants and pellets were stored at −70°C. Study personnel processing the samples were blinded to the participant's clinical status.

The dynamics of bacterial colonization of the respiratory tract in adults with COPD include patterns that involve the isolation of identical strains from sputum with intervening negative cultures. This

TABLE 1 Characteristics of participants enrolled in the COPD study, Buffalo, NY, April 1994 to June 2014

Characteristic[a]	No. (%) or mean ± SD (n = 181)
Age (yr)	67 ± 9.2
Race	
White	159 (88)
Black	22 (12)
Smoking status	
Former smoker	121 (67)
Current smoker	60 (33)
Pack-yr of smoking	79 ± 36
FEV_1 (liters)	1.64 ± 0.68
FEV_1 (% predicted)	49 ± 18
Charlson comorbidity index	4.2 ± 1.5
Duration in study (yr)	4.5 ± 4.0
Median (IQR)	3.5 (1.5, 5.8)
Antibiotic use between clinic visits	
None	7,078 (80)
Any	1,765 (20)

[a]IQR, interquartile range.

phenomenon is known as "gaps" and is defined as a period of negative sputum cultures preceded and followed by clinic visits at which apparently identical strains were isolated. Previous findings have shown that persistent colonization occurs between these gaps and that sputum cultures underestimate the frequency of colonization of the respiratory tract in COPD (8, 20). Therefore, we presumed that colonization was present between clinic visits where identical strains were isolated.

Statistical analyses. The rates of lower airway colonization by the four potential bacterial pathogens at monthly visits over the course of the study were characterized using chi-square or Fisher's exact tests, as appropriate. The main outcomes of interest were the relationships between bacteria overall and based on clinical status (stable and exacerbation).

Colonization by *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, and *P. aeruginosa* was examined using a generalized linear mixed model with random intercept to account for repeat visits. We used the logit link in the model, along with an unstructured covariance structure (SAS version 9.4; SAS Institute, Inc., Cary, NC). Since each participant contributed multiple sputum samples, we used a repeated-measures design to take into account variability of multiple samples from each participant. *P. aeruginosa* colonization was examined as a separate covariate in each model to reduce multiple comparisons between organisms. Further, in chronic airway diseases, including cystic fibrosis (CF) and bronchiectasis, *P. aeruginosa* is the predominant organism in the lower respiratory tract. We hypothesized that *P. aeruginosa* colonization was associated with a decrease in colonization by the other three organisms, which was another *a priori* reason for it to be treated as a separate covariate.

Each model included the presence or absence of other bacterial pathogens and additional host factors—age, race, Charlson comorbidity index (21), duration in primary study, pack-years of smoking, smoking status (current versus former), forced expiratory volume in 1 s (FEV_1) (percent predicted), and recent antimicrobial therapy (between the last and current visits)–since these factors have been shown to influence colonization patterns (9–12). For each model, we estimated odds ratios (ORs) for the response pathogen given the presence of each predictor pathogen alone and then jointly, including the additional host factors. To examine the effects of covariates on each of the pathogens of interest based on clinical status, we first modeled colonization of each pathogen individually before separately examining whether bacterial interactions differed according to stable and exacerbation visits.

RESULTS

The characteristics of the study participants are presented in Table 1. The mean age of participants at study entry was 67 ± 9.2 years, and most were white (88%). The mean baseline Charlson comorbidity index was 4.2 ± 1.5, and the mean FEV_1 percent predicted was 49% ± 18%. Participants were followed up for a mean of 4.5 years (median, 3.5 years). Antibiotic use between clinic visits occurred for 1,765 visits (20%), yielding 1,949 total antibiotic courses. The most common antibiotics were azithromycin (20.6%), trimethoprim-sulfamethoxazole (10.9%), and amoxicillin-clavulanic acid (10.5%) (Table 2).

Bacterial colonization during stable and exacerbation visits. The distribution and colonization patterns of the four potential bacterial pathogens during stable and

TABLE 2 Antibiotic utilization between clinic visits among participants enrolled in the COPD study, Buffalo, NY, April 1994 to June 2014

Antibiotic group	Antibiotic	No. (%) on antibiotic (n = 1,949)[a]
Penicillins	Amoxicillin	171 (8.8)
	Amoxicillin/clavulanic acid	205 (10.5)
	Other	46 (2.4)
Cephalosporins	Cephalexin	50 (2.6)
	Ceftriaxone	46 (2.4)
	Other	66 (3.4)
Macrolides	Azithromycin	401 (20.6)
	Clarithromycin	21 (1.1)
Fluoroquinolones	Ciprofloxacin	118 (6.1)
	Levofloxacin	164 (8.4)
	Moxifloxacin	151 (7.7)
	Other	119 (6.1)
Folate antagonists	Trimethoprim/sulfamethoxazole	213 (10.9)
Lincosamide	Clindamycin	52 (2.7)
Tetracycline	Doxycycline	39 (2.0)

[a]Antibiotic records were unavailable for 41 subject visits. The following antibiotics used in <20 visits are not included in the table: erythromycin (14 uses), vancomycin (12 uses), metronidazole (10 uses), tetracycline (8 uses), and gentamicin (2 uses).

exacerbation visits are presented by duration in the study (Table 3). Overall, at least one species was isolated from 30.8% (2,722 of 8,843) of the total visits. Of the 8,843 visits, *H. influenzae* was the most common bacterial species identified, in 14.4% (n = 1,277) of visits, *M. catarrhalis* was present in 6.9% (n = 606), and *S. pneumoniae* was present in 5.7% (n = 500). *P. aeruginosa* was present in 8.1% (n = 716) of visits. Polymicrobial colonization, defined as two or more of the four pathogens detected at a visit, was observed in 5% of visits.

When the bacteria were examined individually, the rate of *H. influenzae* colonization when no other cocolonizing bacteria were present was higher at exacerbation than at stable visits (13.3% versus 10.4%; P = 0.003). Similarly, *M. catarrhalis* colonization was significantly more frequent at exacerbation than at stable visits (9.1% versus 3.4%; P = 0.0001).

S. pneumoniae colonization alone at stable visits, when no other pathogens were present, was 2.8% (211/7,464) compared to 2.6% (36/1,379) during exacerbation visits (P = 0.72). Colonization by *P. aeruginosa* alone was slightly higher at exacerbation visits than at stable visits, though this difference did not reach statistical significance (7.5% versus 6.4%; P = 0.12). Visits characterized by colonization by two or more pathogens were significantly more common at exacerbation than at stable visits (7.3% versus 4.6%; P = 0.0001).

Interspecies interactions between *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, and *P. aeruginosa*. The outcomes of colonization with *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* are presented in separate models in Table 4. In the model examining *H. influenzae* colonization as the outcome, *H. influenzae* was positively associated with *S. pneumoniae* colonization (OR, 2.79; 95% confidence interval [CI], 2.09 to 3.73), but not *M. catarrhalis* (OR, 1.01; 95% CI, 0.78 to 1.32). *S. pneumoniae* and *M. catarrhalis* cocolonization was positively associated with *H. influenzae* colonization (OR, 2.82; 95% CI, 1.91 to 4.17). Colonization by *P. aeruginosa* resulted in 85% reduction in the odds of *H. influenzae* colonization (OR, 0.15; 95% CI, 0.10 to 0.22).

When stratified based on clinical status (stable versus exacerbation), similar relationships between *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were apparent during stable visits (Table 5). However, colonization by *S. pneumoniae* or the combination of *S. pneumoniae* and *M. catarrhalis* was not associated with *H. influenzae* colonization during exacerbation visits. Colonization by *P. aeruginosa* was negatively associated with *H. influenzae* colonization during both stable and exacerbation visits.

TABLE 3 Distribution of 4 major respiratory pathogens collected from adults with COPD, Buffalo Veterans Affairs Medical Center, Buffalo, NY, USA, 1994 to 2014

Parameter	Value [no. (%)] for participants in study for:										
	Total	<1 yr		1–3 yr		>3–5 yr		>5–10 yr		>10 yr	
		Stable	Exac[b]	Stable	Exac	Stable	Exac	Stable	Exac	Stable	Exac
Participants	181	32 (18)		50 (28)		34 (19)		49 (27)		16 (9)	
Visits[a]	8,843	155	37	819	184	1,252	220	3,171	540	2,150	425
Bacteria present											
0 pathogens	6,231 (70)	130 (84)	33 (89)	673 (82)	121 (66)	884 (71)	117 (53)	2,236 (71)	308 (57)	1,490 (69)	239 (56)
1 pathogen											
S. pneumoniae	247 (2.8)	4 (2.6)	2 (5.4)	27 (3.3)	11 (6.0)	7 (0.56)	3 (1.4)	55 (1.7)	20 (3.7)	101 (4.7)	17 (4.0)
M. catarrhalis	380 (4.3)	3 (1.9)	1 (2.7)	26 (3.2)	16 (8.7)	36 (2.9)	20 (0.1)	76 (2.4)	35 (6.5)	114 (5.3)	53 (13)
P. aeruginosa	579 (6.5)	5 (3.2)	1 (2.7)	10 (1.2)	2 (1.1)	98 (7.8)	26 (12)	246 (7.8)	40 (7.4)	116 (5.4)	35 (8.2)
H. influenzae	957 (11)	7 (4.5)	0	52 (6.4)	22 (12)	185 (15)	32 (15)	373 (12)	78 (14)	157 (7.3)	51 (12)
2 pathogens											
S. pneumoniae, H. influenzae	128 (1.4)	0	0	1 (0.12)	2 (1.1)	10 (0.8)	0	50 (1.6)	9 (1.7)	49 (2.3)	7 (1.7)
S. pneumoniae, M. catarrhalis	37 (0.4)	0	0	17 (2.1)	3 (1.6)	5 (0.4)	1 (0.45)	4 (0.13)	1 (0.19)	5 (0.23)	1 (0.24)
S. pneumoniae, P. aeruginosa	51 (0.6)	0	0	1 (0.12)	0	0	0	8 (0.25)	3 (0.56)	35 (1.6)	4 (0.94)
H. influenzae, M. catarrhalis	115 (1.3)	0	0	2 (0.24)	1 (0.54)	14 (1.1)	7 (3.2)	54 (1.7)	20 (3.7)	13 (0.60)	4 (0.94)
H. influenzae, P. aeruginosa	41 (0.5)	0	0	1 (0.12)	0	1 (0.08)	2 (0.91)	26 (0.82)	7 (1.3)	3 (0.14)	1 (0.24)
M. catarrhalis, P. aeruginosa	39 (0.4)	0	0	2 (0.24)	0	4 (0.32)	4 (1.8)	8 (0.25)	8 (1.5)	8 (0.37)	5 (1.2)
3 pathogens											
S. pneumoniae, H. influenzae, M. catarrhalis	32 (0.4)	0	0	2 (0.24)	0	2 (0.16)	1 (0.45)	6 (0.19)	0	19 (0.88)	2 (0.47)
S. pneumoniae, H. influenzae, P. aeruginosa	3 (0.03)	0	0	0	0	0	0	1 (0.03)	1 (0.19)	1 (0.05)	0
S. pneumoniae, M. catarrhalis, P. aeruginosa	2 (0.02)	0	0	0	0	0	0	1 (0.03)	0	0	1 (0.24)
H. influenzae, M. catarrhalis, P. aeruginosa	1 (0.01)	0	0	0	0	0	0	0	1 (0.19)	0	0

[a]Bacterial cultures were missing for 110 subject visits.
[b]Exac, exacerbation.

TABLE 4 Determinants and interactions of colonization with the four major bacterial pathogens in COPD patients, Buffalo, NY, USA, April 1994 to June 2014

Characteristic	No. (%) of samples (n = 8,843)	Adjusted OR (95% CI)[a]		
		H. influenzae	*M. catarrhalis*	*S. pneumoniae*
Bacteria present				
S. pneumoniae, *M. catarrhalis*				
Neither	7,808 (88)	1.0 (reference)		
S. pneumoniae only	429 (4.9)	**2.79 (2.09–3.73)**		
M. catarrhalis only	535 (6.0)	1.01 (0.78–1.32)		
Both	71 (0.8)	**2.82 (1.91–4.17)**		
H. influenzae, *S. pneumoniae*				
Neither	7,229 (82)		1.0 (reference)	
H. influenzae only	1,114 (13)		1.11 (0.86–1.44)	
S. pneumoniae only	337 (3.8)		0.86 (0.61–1.20)	
Both	163 (1.8)		0.95 (0.63–1.44)	
H. influenzae, *M. catarrhalis*				
Neither	7,108 (80)			1.0 (reference)
H. influenzae only	1,129 (13)			**2.81 (2.10–3.77)**
M. catarrhalis only	458 (5.2)			0.82 (0.57–1.18)
Both	148 (1.7)			**2.30 (1.44–3.67)**
P. aeruginosa				
Absent	8,124 (92)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Present	719 (8)	**0.15 (0.10–0.22)**	**0.51 (0.35–0.75)**	1.34 (0.89–2.02)
Age (yr) (10-yr increase)		0.79 (0.43–1.46)	1.19 (0.77–1.83)	1.49 (0.75–2.96)
Antibiotic therapy between visits				
No		1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes		**0.81 (0.67–0.98)**	**0.62 (0.48–0.79)**	**0.62 (0.45–0.84)**
Race				
White		1.0 (reference)	1.0 (reference)	1.0 (reference)
Nonwhite		1.14 (0.37–3.55)	1.01 (0.45–2.27)	0.24 (0.05–1.14)
Charlson comorbidity index (1-point increase)		1.02 (0.73–1.44)	1.06 (0.83–1.35)	0.75 (0.49–1.13)
Duration in study (1-yr increase)		1.04 (0.95–1.14)	1.05 (0.99–1.12)	1.03 (0.94–1.13)
Smoking status				
Former smoker		1.0 (reference)	1.0 (reference)	1.0 (reference)
Current smoker		0.82 (0.35–1.93)	0.81 (0.45–2.27)	1.54 (0.62–3.84)
Pack-yrs of smoking (10-pack increase)		1.10 (0.99–1.23)	1.06 (0.98–1.14)	0.98 (0.87–1.11)
FEV_1 % predicted (10% increase)		1.02 (0.82–1.25)	0.96 (0.82–1.11)	0.94 (0.74–1.20)

[a]Significant ORs and 95% CIs are shown in boldface. Each model included variables representing presence of absence of other bacteria, as well as all other variables used.

Neither *S. pneumoniae* nor *H. influenzae* was significantly associated with *M. catarrhalis* colonization individually or when they cocolonized; this did not differ for stable and exacerbation visits. Colonization by *P. aeruginosa* was associated with a 49% reduction in the odds of *M. catarrhalis* colonization (OR, 0.51; 95% CI, 0.35 to 0.75). The relationship between *M. catarrhalis* and *P. aeruginosa* persisted during stable visits only.

An increase in *S. pneumoniae* colonization was seen with *H. influenzae* (OR, 2.81; 95% CI, 2.10 to 3.77) and when *H. influenzae* and *M. catarrhalis* were both present (OR, 2.30; 95% CI, 1.44 to 3.67). These relationships were statistically significant only during stable visits (Table 5). *P. aeruginosa* colonization was positively associated with *S. pneumoniae* (OR, 1.67; 95% CI, 1.04 to 2.68) during stable visits but not at exacerbation visits (OR, 0.78; 95% CI, 0.34 to 1.81).

Effects of host factors on bacterial colonization. Table 3 shows that antibiotic therapy between visits was negatively associated with colonization by *H. influenzae* (OR, 0.81; 95% CI, 0.67 to 0.98), *M. catarrhalis* (OR, 0.62; 95% CI, 0.48 to 0.79), and *S. pneumoniae* (OR, 0.62; 95% CI, 0.45 to 0.84). Similar relationships were found when the data were stratified by clinical status (Table 4). Colonization by *P. aeruginosa* was not significantly associated with antibiotic utilization.

TABLE 5 Outcomes of colonization with *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* in COPD patients during stable and acute exacerbation visits

Characteristic	No. (%) of samples		Adjusted OR (95% CI)[a]					
			H. influenzae		*M. catarrhalis*		*S. pneumoniae*	
	Stable (n = 7,464)	Exac[b] (n = 1,379)	Stable	Exac	Stable	Exac	Stable	Exac
Bacteria present								
S. pneumoniae, *M. catarrhalis*								
Neither	6,693 (90)	1,115 (81)	1.0 (reference)	1.0 (reference)				
S. pneumoniae only	350 (4.7)	79 (5.7)	**3.75 (2.69–5.24)**	1.21 (0.64–2.28)				
M. catarrhalis only	360 (4.8)	175 (13)	1.06 (0.76–1.46)	0.90 (0.57–1.43)				
Both	61 (0.8)	10 (0.7)	**3.96 (2.50–6.28)**	1.09 (0.49–2.42)				
H. influenzae, *S. pneumoniae*								
Neither	6,165 (83)	1,064 (77)			1.0 (reference)	1.0 (reference)		
H. influenzae only	888 (12)	226 (16)			1.15 (0.84–1.58)	1.11 (0.73–1.69)		
S. pneumoniae only	270 (3.6)	67 (4.9)			0.83 (0.56–1.24)	0.67 (0.33–1.38)		
Both	141 (1.9)	22 (1.6)			0.96 (0.60–1.53)	0.74 (0.33–1.69)		
H. influenzae, *M. catarrhalis*								
Neither	6,126 (82)	982 (71)					1.0 (reference)	1.0 (reference)
H. influenzae only	917 (12)	212 (15)					**3.86 (2.76–5.39)**	1.18 (0.64–2.19)
M. catarrhalis only	309 (4.1)	149 (11)					0.80 (0.53–1.22)	0.60 (0.28–1.28)
Both	112 (1.5)	36 (2.6)					**3.09 (1.81–5.28)**	0.71 (0.26–1.89)
P. aeruginosa								
Absent	6,886 (92)	1,238 (90)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Present	578 (7.7)	141 (10)	**0.13 (0.08–0.22)**	**0.26 (0.12–0.56)**	**0.37 (0.22–0.62)**	0.92 (0.53–1.61)	**1.67 (1.04–2.68)**	0.78 (0.34–1.81)
Age (yr) (10-yr increase)			0.74 (0.38–1.41)	0.83 (0.47–1.44)	1.38 (0.78–2.42)	0.97 (0.67–1.39)	1.62 (0.79–3.32)	0.87 (0.43–1.79)
Antibiotic therapy between visits								
No			1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Yes			**0.77 (0.62–0.97)**	0.73 (0.50–1.05)	**0.69 (0.50–0.93)**	**0.44 (0.29–0.66)**	**0.59 (0.41–0.85)**	0.70 (0.40–1.22)
Race								
White			1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Nonwhite			1.39 (0.41–4.68)	0.77 (0.28–2.10)	0.89 (0.30–2.64)	1.04 (0.55–1.94)	0.17 (0.03–1.00)	0.14 (0.02–1.28)
Charlson comorbidity index (1-point increase)			1.01 (0.70–1.45)	1.08 (0.79–1.49)	1.01 (0.73–1.39)	1.08 (0.86–1.35)	0.71 (0.45–1.11)	1.08 (0.69–1.68)
Duration in study (1-yr increase)			1.02 (0.93–1.12)	1.01 (0.94–1.09)	1.03 (0.95–1.12)	**1.05 (1.00–1.10)**	1.04 (0.94–1.15)	1.02 (0.92–1.11)
Smoking status								
Former smoker			1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Current smoker			0.78 (0.31–1.94)	0.92 (0.43–1.94)	1.15 (0.53–2.49)	**0.40 (0.24–0.67)**	1.75 (0.68–4.55)	0.79 (0.31–2.04)
Pack-yrs of smoking (10-pack increase)			**1.12 (1.00–1.26)**	1.02 (0.93–1.12)	1.05 (0.95–1.17)	1.03 (0.98–1.09)	0.98 (0.86–1.12)	0.98 (0.88–1.11)
FEV_1 % predicted (10% increase)			1.03 (0.83–1.29)	1.04 (0.86–1.25)	0.96 (0.78–1.17)	0.97 (0.85–1.10)	0.94 (0.73–1.20)	0.98 (0.75–1.28)

[a]Significant ORs and 95% CIs are shown in boldface. Each model included variables representing presence or absence of other bacteria, as well as all other variables used.
[b]Exac, exacerbation.

Smoking was positively associated with *H. influenzae* colonization during stable visits (each 10-pack-year increase, OR, 1.12; 95% CI, 1.00 to 1.26) and negatively associated with *M. catarrhalis* colonization during exacerbation visits (current smoker, OR, 0.40; 95% CI, 0.24 to 0.67). Additional host factors, including age, race, Charlson comorbidity index, and FEV_1 percent, were not significantly associated with bacterial colonization.

DISCUSSION

We examined lower respiratory tract colonization with S. *pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *P. aeruginosa* in a COPD cohort, and our models indicate significant interspecies interactions. During stable COPD, *S. pneumoniae* and *H. influenzae* appear to have a positive relationship, while colonization with *M. catarrhalis* does not appear to interact with these two pathogens. *P. aeruginosa* has a negative impact on *H. influenzae* and *M. catarrhalis* colonization, but not on *S. pneumoniae*, where actual enhancement of colonization was seen. In exacerbations, the relationship between *S. pneumoniae* and *H. influenzae* was no longer seen; however, the negative interaction of *P. aeruginosa* with *H. influenzae* and *M. catarrhalis* persisted. Reduction of interspecies interactions at exacerbation could represent the dominance of a newly acquired bacterial strain and the associated host immune-inflammatory response. Not surprisingly, recent antibiotic therapy was negatively associated with colonization by *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* but not colonization by *P. aeruginosa*.

Ours is the first clinical study of bacterial interactions in COPD within a prospective study design. The literature regarding interactions between these organisms is limited to studies of nasopharyngeal colonization in children. Madhi et al. (14) investigated nasopharyngeal interactions between *S. pneumoniae* and *H. influenzae* in healthy children in South Africa. They reported a synergistic association in which children colonized by *S. pneumoniae* were more likely to be colonized by *H. influenzae* than children not colonized by *S. pneumoniae* (73.9% versus 29.3%; $P < 0.0001$). Jacoby et al. (13) investigated nasopharyngeal microbial interactions among children in Western Australia and found synergistic associations between *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. Chien et al. investigated nasopharyngeal bacterial interactions in healthy young Peruvian children and reported a positive association between *S. pneumoniae* and *H. influenzae* by both culture and quantitative PCR (qPCR) (17). Our results are similar, having identified positive interactions between *S. pneumoniae* and *H. influenzae*. Conversely, Pettigrew et al. investigated nasopharyngeal bacterial interactions in children with upper respiratory tract infections; the authors found competitive interactions between *S. pneumoniae* and *H. influenzae*, as well as *H. influenzae* and *M. catarrhalis* (12). Interestingly, the competitive balance between *S. pneumoniae* and *H. influenzae* was altered by the addition of *M. catarrhalis*. Cocolonization with *H. influenzae* and *M. catarrhalis* was associated with a positive interaction with *S. pneumoniae*. Similar negative interactions were found by Xu et al. in their analysis of nasopharyngeal colonization in children at the onset of acute otitis media (11). Our results extend the positive associations between *S. pneumoniae* and *H. influenzae* seen in most studies of the upper airway to the lower airway in COPD.

The presence of *P. aeruginosa* in COPD is associated with an accelerated decline in lung function, more frequent exacerbations, and a greater requirement for antibiotic therapy (8, 22–25). Our results provide support for the hypothesis that negative interactions exist between *P. aeruginosa*, *H. influenzae*, and *M. catarrhalis* in COPD. Colonization with *P. aeruginosa* is better characterized in other chronic lung diseases, including CF and bronchiectasis. In CF, the most commonly isolated bacterium in young patients is *Staphylococcus aureus*. In older adults with CF, however, *P. aeruginosa* is the predominant organism, eventually representing over 80% of bacteria in the lung (26, 27). Multiple mechanisms have been proposed to explain the competitive interactions associated with *P. aeruginosa* in CF. Pernet et al. showed that colonizing *P. aeruginosa* triggers host cells to produce type IIA secreted phospholipase A2, a host enzyme with bactericidal activity capable of inhibiting *S. aureus* (28). Filkins et al. found that *P.*

aeruginosa drives *S. aureus* from aerobic respiration to fermentative metabolism, which reduces *S. aureus* viability and eventually results in the predominance of *P. aeruginosa* in the community (29). In bronchiectasis, competitive interactions associated with *P. aeruginosa* are further supported by Rogers et al., who demonstrated interspecies competition between *H. influenzae* and *P. aeruginosa* (30). When *H. influenzae* or *P. aeruginosa* was present in the opposite dominant group, the two species were found to be in very low abundance, i.e., patients in the *P. aeruginosa*-dominant group competitively excluded *H. influenzae* (*P. aeruginosa* mean abundance ± standard deviation [SD], 87.3% ± 13.4%; *H. influenzae*, 0.56% ± 0.77%). These findings are consistent with strong competitive effects between *P. aeruginosa* and *H. influenzae*, moving toward competitive exclusion. Given the poor prognosis associated with *P. aeruginosa* colonization in COPD and other lung diseases, it is important to further understand the mechanistic and clinical implications of these interactions. Interventions that rationally alter the lung microbiota could be envisaged, or novel drug targets could be selected to enable or inhibit these interactions, which may competitively suppress *P. aeruginosa* colonization and subsequent infection.

The mechanisms of bacterial interaction between *S. pneumoniae* and *H. influenzae* have previously been explored. Weimer et al. showed that β-lactamase-producing nontypeable *H. influenzae* was able to protect biofilm-resident *S. pneumoniae* from amoxicillin treatment in the chinchilla middle ear (31). Interestingly, the study also showed that a β-lactamase-deficient *H. influenzae* strain was able to protect biofilm *S. pneumoniae* from amoxicillin, indicating that β-lactamase plays a role in interspecies synergy but that other factors may also lead to bacterial protection. A study by Cope et al. demonstrated synergy *in vitro* between *H. influenzae* and *S. pneumoniae*, with both species reaching higher cell densities in coculture than in monoculture (32). In the coculture biofilm setting, *H. influenzae* contact with *S. pneumoniae* resulted in upregulation of *pilA* expression, which is known to encode the major subunit of Tfp, by *H. influenzae* (33). Conversely, some studies have demonstrated competitive interactions between *S. pneumoniae* and *H. influenzae*. *In vitro* experiments have shown that *S. pneumoniae* and *H. influenzae* coculture leads to a rapid decrease in *H. influenzae* through the action of hydrogen peroxide (34). However, pneumococcal strains modified not to produce hydrogen peroxide lost their ability to kill *H. influenzae*. An *in vivo* mouse model showed that when *H. influenzae* colonized with *S. pneumoniae* in the nasopharynx, *S. pneumoniae* was rapidly cleared (35). The competitive interactions between *H. influenzae* and *S. pneumoniae* were dependent on cellular components of *H. influenzae* activating host complement and neutrophils. Multiple disruptions in innate defense mechanisms are prevalent in COPD, which may explain why pathogen persistence is seen in spite of abundant inflammation in the lower airways in the disease. We believe this could also explain the synergism between the two pathogens that dominates in this study.

Recent antibiotic exposure was associated with a lower prevalence of colonization with *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. Chronic microbial colonization contributes to chronic inflammation and progressive loss of lung function (7, 9, 36, 37). The potential role of long-term antibiotic use in COPD has previously been investigated, primarily with respect to long-term macrolide therapy (38). Albert et al. (39) investigated the use of 12 months of daily azithromycin in COPD patients at increased risk of exacerbations and reported a reduction in the frequency of acute exacerbations among patients who received azithromycin. Interestingly, a recent study by Pettigrew et al. showed that macrolides were ineffective at eradicating *H. influenzae* and that fluoroquinolones were a more effective antibiotic in eradicating *H. influenzae* in individuals with COPD (40). For *P. aeruginosa*, we did not observe any association between antibiotic therapy and colonization. A previous analysis of the same cohort reported that antibiotics did not fully account for *P. aeruginosa* clearance, with only 32% of *P. aeruginosa* cases cleared in the presence of any antibiotic therapy (8). Our results reinforce the finding that antibiotics may play only a minor role in *P. aeruginosa* clearance and that factors other than antibiotics may facilitate *P. aeruginosa* clearance

from the respiratory tract in COPD patients. Further studies on the effect of bacterium-host-antimicrobial interactions in the lower respiratory tracts of COPD patients are necessary to better understand the observed shifts in bacterial species. Our findings could guide therapeutic choices in stable COPD and at exacerbation. We confirm that recent antibiotic therapy can render sputum culture results unreliable. Most exacerbations are caused by a dominant pathogen, likely driven by new strain acquisition, supporting targeted antibiotic therapy based on sputum cultures. Propensity for a chronic infected phenotype could be driven by bacterial synergism in stable COPD. Though *Pseudomonas* emergence reduces other Gram-negative bacteria, *S. pneumoniae* is not diminished. Further, current smokers are more likely to be infected with *H. influenzae* than with *M. catarrhalis*.

This study had several strengths, including the prospective study design, the large number of repeated samples, and careful documentation and diagnosis of COPD exacerbations. We examined lower respiratory tract bacterial carriage during exacerbations, a time when patients are at risk of further complications. A limitation of our study is that we did not evaluate bacterium-virus interactions, as viruses were not examined within the primary study. Viruses are known to cause COPD exacerbations and have been detected in up to 15% of sputum samples during stable COPD (6). The mechanisms by which viruses influence bacterial colonization are diverse, and it is possible that these interactions may have influenced those observed (41). Further, the lung microbiota includes multiple bacterial taxa and bacterial families, and the interrelationship between these bacteria most likely influences which species are dominant within the lower airways of COPD patients (42–44). Colonization patterns may also be interrelated with the host immune response and the presence of exacerbation symptoms. A longitudinal study using culture-independent techniques is necessary to further evaluate these bacterial interactions in the lung microbiome, as well as the influence of host factors on microbial composition. The purpose of this study was to evaluate relationships between bacteria over time, not to explore the mechanisms by which the bacteria interact. Further work is necessary to better understand the mechanisms of interaction and clinical significance between bacteria in the lower respiratory tract in COPD patients. The COPD patients in our sample were older and predominantly male, with moderate to severe COPD at baseline. Further study of mild disease in young patients with female gender representation is required to determine if the identified bacterial relationships are applicable across the spectrum of COPD. Finally, based on previous findings, we presumed that colonization was present between clinical visits when an identical strain was isolated (8, 20). These bacteria are present far more often than is revealed by sputum cultures, and by not including gaps, we would underestimate the true frequency of bacterial colonization of the lower respiratory tract in adults with COPD.

In summary, our work presents novel insight into interspecies interactions, including both positive and negative, between common pathogenic bacteria in patients with COPD. A greater understanding of how bacteria interact with each other in the lower respiratory tract may have important implications for the treatment of COPD. Vaccines may also play a role in the microbial ecology of the lower respiratory tract, and whether this is beneficial or detrimental to interspecies interaction is unknown. These interactions may offer novel therapeutic opportunities to alter the characteristics of the airway microbiota. Optimal treatment of COPD may be enhanced through personalized strategies for strain-specific therapy by screening microbial communities and analyzing the probability of strain replacement and subsequently reducing bacterial infection.

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