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Chronic MRSA and *P. aeruginosa* Co-infection and Rate of Lung Function Decline Among Patients with Cystic Fibrosis

By

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Rollins School of Public Health, Emory University

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Abstract

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By Maret Maliniak

Background: Chronic *Pseudomonas aeruginosa* infection is a well-known risk factor for increased lung function decline among patients with cystic fibrosis (CF). More recently, studies have found that chronic MRSA infection may also contribute to lung function decline. However, the association between chronic MRSA and *P. aeruginosa* co-infection and rate of lung function decline has not been widely studied.

Objective: This study aims to characterize CF patients with chronic MRSA and *P. aeruginosa* co-infection and examine whether these patients have a higher rate of lung function decline, measured by forced expiratory volume in the first second (FEV_1, given as % predicted) per year, compared to patients with other levels of infection.

Population: Cystic fibrosis patients (≥6 years old) attending a CF center in Atlanta, GA and included in the patient registry between 2007 and 2013 comprised the study cohort.

Study Design: Repeated measures mixed effects modeling was used to compare the rates of FEV_1 decline (% predicted/year) between patients chronically co-infected with MRSA and *P. aeruginosa* to patients with chronic *P. aeruginosa* alone, chronic MRSA alone, intermittent MRSA or *P. aeruginosa*, and no colonization of *P. aeruginosa* or MRSA, adjusting for known covariates.

Results: Patients with chronic co-infection were found to have a baseline FEV_1 of 62.9% predicted (standard deviation: 25.8) and a significantly more rapid rate of decline in FEV_1 % predicted compared to patients without MRSA or *P. aeruginosa* adjusting for confounders and using all values of FEV_1 recorded during follow-up (mean rate: -1.06 FEV_1 % predicted per year; 95% CI: -2.08, -0.05). However, the rate of FEV_1 decline was not significantly greater than that of patients with chronic *P. aeruginosa*, chronic MRSA alone, or intermittent infection, suggesting that chronic co-infection may not increase lung function decline beyond that of other levels of infection.

Conclusions: Chronic co-infection of MRSA and *P. aeruginosa* is associated with lower baseline lung function and continued decline in FEV_1 % predicted; however, the rate of decline in FEV_1 % predicted may not be greater than that of other levels of infection. Future studies should investigate this association in the broader CF population.

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Above all, this thesis is dedicated to my sister, Callie, who is and always will be my inspiration in life and for fighting for a cure for cystic fibrosis.

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BACKGROUND/LITERATURE REVIEW

Cystic fibrosis: Background and Epidemiology

Cystic fibrosis (CF) is a life-threatening, autosomal recessive disorder that affects approximately 70,000 people worldwide, with 30,000 residing in the United States (U.S.) (1). In 2011, individuals with cystic fibrosis living in the U.S. had a median life expectancy of 36 years (1). Progressive damage to multiple body systems, primarily the lungs and the digestive system, reduce the life expectancy of CF patients to half that of normal individuals (1). As indicated by its recessive nature, a child must receive a copy of the mutant CF allele by both heterozygote parents to develop cystic fibrosis (2). Heterozygote carriers of one normal CF allele and one mutant CF allele typically are unaware of their carrier status as they are entirely asymptomatic (2). In the U.S., more than 10 million people are carriers with about one in 29 Caucasian Americans having a copy of the CF mutant allele (3).

The history of cystic fibrosis is not well known. A passage from the 1857 "Almanac of Children's Songs and Games from Switzerland" cautioned that the "child will soon die whose forehead tastes salty when kissed" (4). During this time period, newborns with cystic fibrosis did not survive childhood; however, it is thought that heterozygote carriers survived to pass the CF gene because the gene provided some protection against other life-threatening diseases at the time, such as tuberculosis and cholera (5). It was not until 1938 that Dr. Dorothy Anderson would be the first to comprehensively describe CF disease, calling it "cystic fibrosis of the pancreas" because of autopsies of CF children that showed destruction of the pancreas (2). Furthermore, it would not be until 1953 that Dr. Paull DiSant' Agnese demonstrated excess salt in the sweat of children with CF, which is now used as a diagnostic tool called the sweat test (2).

A mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene causes cystic fibrosis (2). The protein product of this gene is an adenosine 3',5'-monophospate (cyclic AMP)-dependent protein kinase (Protein Kinase A)-activated and adenosine triphosphate (ATP)-gated anion channel that controls ion transport in and out of the cell (2). The mutated CFTR gene causes this channel protein to (i) malfunction and (ii) disrupt the transport of ions such as Cl^- (2). As a result, sodium hyperabsorption and chloride hypoabsorption at the apical airway surface lead to dehydration of airway surface fluid (6). This creates a viscous mucus layer in the respiratory tract that cannot be removed by cilia and reduces bacterial clearance (6). As a consequence, the airway is prone to chronic bacterial infections leading to chronic inflammation, oxidative stress, and progressive airway damage (2, 6). In 80% to 95% of people with CF, chronic respiratory infection and associated airway inflammation are the main causes of respiratory failure and death (7).

Despite knowledge of the CF gene structure, the function of the CFTR protein, and the resulting pathology, CF is extremely complex with many aspects of the disease still debated or unknown (2). One major obstacle for curing CF is the existence of more than 1,800 known mutations of the CF gene (8). The most common CFTR mutation is the ΔF508 mutation with approximately 87% of CF individuals in the U.S. having at least one copy of this mutation and 47% having two copies (9). Worldwide, the ΔF508 mutation is found in approximately two-thirds (66%) of all CF chromosomes, making it universally the most common CF gene mutation (8). However, the prevalence of the ΔF508 mutation varies depending on geographic region and ethnic population such that the frequency has been found to be as high as 100% in the Faroe Islands of Denmark and as low as 24.5% in Turkey (8). The United States, Canada, the United Kingdom, Australia, and New Zealand have similar CFTR mutation profiles with a high prevalence of the ΔF508 mutation and a

small number of other mutations (ranging from 5 in New Zealand to 10 in the U.S.), which together account for approximately 80% to 86% of all alleles in CF patients in these countries (8). Spain, Greece, Turkey, and Bulgaria have more mutational heterogeneity with an average of 25 mutations accounting for 84% of CF alleles (8). Bobadilla et al. suggest that this geographic heterogeneity is likely attributed to the Mediterranean region historically being a gateway between Europe and the Middle East (8). The G542X mutation is most common in the Mediterranean region of Europe and the Middle East with a prevalence of 8.0% in Spain compared to 2.4% in the United States (8). Even in countries with a high prevalence of the ΔF508 mutation such as the United States, there exists heterogeneity between racial/ethnic groups such that African-Americans have a lower prevalence of the ΔF508 mutation (48.0%) and a higher prevalence of more rare CF mutations such as the 3120+1G→A mutation (12.2%) compared to Caucasian Americans (8). These differences in the genetic mutation causing cystic fibrosis are associated with differing mechanistic defects in epithelial cells such that some CFTR genotypes have greater CFTR activity than others, resulting in a less severe clinical presentation (10). Although there is some evidence of a genotype-phenotype relationship, considerable variability exists among individuals with the same genotype, and genotype is often not the strongest predictor of lung disease severity (10).

CF disease occurs most often in Caucasians of European descent; however, CF does occur in other ethnic populations and could go undetected or misdiagnosed because of its extreme rarity in other racial/ethnic groups (11). More than 90% of individuals with cystic fibrosis are Caucasian, affecting 1 in 2,500 Caucasians compared to 1 in 4,000 Hispanics, 1 in 15,000 African Americans, and 1 in 100,000 Asian Americans (1). In the U.S., where newborn screening for CF occurs, the median age of diagnosis is 5 months (9). Because of

the relative rarity of cystic fibrosis, many countries, mostly Western and developed countries, have created national registries that attempt to capture the clinical and demographic information of the entire CF population in their country (12). Table 1 shows the geographic distribution of persons with cystic fibrosis internationally according to national and international registry data reports (12).

TABLE 1. The reported number of persons with cystic fibrosis by country from national and international cystic fibrosis registry data reports, 2008-2012 (12).

Country	Number of persons with CF (Year reported)	Estimated coverage (%) of CF population
Australia (13)	3,156 (2012)	95
Belgium** (14)	1,138 (2010)	>90
Brazil (15)	1,798 (2010)	--
Canada (16)	3,913 (2011)	~100
Europe* (17)	18,999 (2008-2009)	--
France** (17)	5,640 (2009)	90
Germany** (17)	5,048 (2009)	90
Ireland** (17)	1,021 (2008)	90
The Netherlands**(17)	1,249 (2009)	97
New Zealand (18)	423 (2012)	--
United Kingdom (19)	10,078 (2012)	~100
United States (9)	27,111 (2011)	~90

*Data from the European Cystic Fibrosis Patient Registry (17)
**Provide data to the European Cystic Fibrosis Patient Registry

In the United States, where the largest CF population in the world resides, the Cystic Fibrosis Foundation (CFF) collects information from approximately 90% of individuals with cystic fibrosis in the U.S. (9). In 2011, information from 27,111 people with CF attending more than 110 CFF-accredited care centers were included in the Cystic Fibrosis Foundation Patient Registry (9). Annually, the Cystic Fibrosis Foundation publishes a patient registry data report, which describes the CF population by examining secular trends in median

survival; survival by birth cohort; the number of adults and children with CF; sociodemographic characteristics (state residence, education, marital status, employment, insurance type); the distribution of CF mutations/genotype; median BMI by age; median FEV_1 by age; medication usage; tobacco usage; common secondary complications (bone disease, CF-related diabetes, depression, arthritis/arthropathy) by age; and other complications such as asthma, gastroesophageal reflux, liver disease, and distal intestinal obstructive syndrome (9).

The Annual Patient Registry Report also includes information on the various bacterial and fungal pathogens common in cystic fibrosis. The CF Care Guidelines for Infection Control recommend that care providers obtain respiratory tract cultures at least quarterly for CF patients with stable pulmonary status and at times of pulmonary exacerbation, often resulting in hospitalization (20). According to the *Patient Registry Annual Report 2011*, approximately 45% of patients followed these guidelines by having 4 or more respiratory cultures (sputum or throat) (9). Although not reported, the proportion of patients with at least one sputum culture per year is likely much higher. The collection of respiratory tract cultures allows for estimation of the prevalence of common respiratory pathogens colonized in the lungs of individuals with CF. These estimated prevalences are reported each year in the annual data report but are limited because (i) not all CF patients in the U.S. attend a CF care center and (ii) patients provide a variable number of sputum cultures each year (9). Additionally, the report only provides the prevalence of patients with at least one positive culture for a respiratory pathogen without disaggregation of patients with intermittent or transient colonization and chronic colonization (9). Furthermore, a single positive culture may or may not indicate infection by that pathogen as colonization does not necessarily equate infection, which results in an immune and inflammatory response in the host (21).

CF Respiratory Pathogens

From as early as infancy, individuals with cystic fibrosis colonize organisms such as *Staphylococcus aureus, Haemophilus influenzae,* and *Pseudomonas aeruginosa* (9). Among the 27,111 individuals included in the 2011 CFF Patient Registry, the most prevalent pathogen found in 67.9% of CF airways was *Staphylococcus aureus* (methicillin-sensitive and methicillin-resistant), followed by *Pseudomonas aeruginosa* which was found in 50.6% of CF airways (Table 2) (9).

TABLE 2. Estimated percent of patients with select respiratory pathogens by age group. Adapted from the 2011 Cystic Fibrosis Foundation *Patient Registry Annual Report 2011*(9).*

	Age group (years)								
	0-1	**2-5**	**6-10**	**11-17**	**18-24**	**25-34**	**35-44**	**45+**	**Total**
S. aureus	58%	66%	78%	79%	70%	60%	50%	45%	68%
P. aeruginosa	23%	24%	31%	48%	65%	76%	75%	72%	51%
MRSA	10%	17%	27%	30%	31%	26%	20%	20%	26%
H. influenzae	25%	32%	28%	17%	12%	9%	6%	6%	17%
S. maltophilia	15%	10%	16%	20%	17%	11%	11%	12%	14%
B. cepacia complex	<1%	<1%	1-2%	3%	5%	5%	5%	5%	3%

Abbreviations: *B. cepacia* complex, *Burkholderia cepacia complex; H. influenza*, *Haemophilus influenza*; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*; *S. aureus*, *Staphylococcus aureus*; *S. maltophilia, Stenotrophomonas maltophilia*
*All estimates are approximated

As seen in 2011 and previous years, the prevalence of these two pathogens is age-dependent, such that the prevalence of *S. aureus* peaks during childhood and adolescence then decreases during adulthood while the prevalence of *P. aeruginosa* is lower during childhood and adolescence and peaks during adulthood (9, 22). More than half of infants (<2 years) born with CF colonize *S. aureus* while less than one-quarter of CF infants colonize *P. aeruginosa* (9). Between two and 17 years of age, the majority of individuals continue to colonize *S. aureus* while the percent of individuals colonizing *P. aeruginosa* increases almost

linearly with age beginning around 2 to 5 years old until the prevalence peaks at approximately 76% among individuals 25-34 years of age (9). In the oldest age group reported (45 years and older), the majority of patients colonize *P. aeruginosa* while less than half colonize *S. aureus* (9).

The estimated prevalence of *S. aureus* among CF patients includes all patients with a positive respiratory culture for methicillin-sensitive *S. aureus* (MSSA) and/or methicillin-resistant *S. aureus* (MRSA). Until 1996, the CF Foundation did not distinguish between MSSA and MRSA; however, because of the increasing prevalence of MRSA among individuals with CF in the U.S. and worldwide, the CF Foundation began reporting the prevalence of MRSA separately (9, 23). Unlike the prevalence of *P. aeruginosa,* which has decreased between 2001 (58.7%) and 2011 (50.6%), the prevalence of MRSA detected in CF lungs has increased substantially from 7.3% in 2001 to 17.2% in 2005 to 25.9% in 2011 for an increase of nearly 20% in 10 years according to patient registry annual data reports (9, 22). Additionally, a study examining the change in respiratory microbiology in cystic fibrosis patients from 1995 to 2005 found a statistically significant 28.7% increase in the prevalence of MRSA among adolescents 11-17 years old, which was the largest percent change for all age groups during the ten-year time period (23). In 2011, MRSA was detected in all age groups from infancy to patients above 45 years of age but had a peak prevalence of about 30-31% among CF individuals aged 11-24 years (9).

Although less common than *S. aureus* and *P. aeruginosa*, other respiratory pathogens found in CF airways include *Haemophilus influenzae, Stenotrophomonas maltophilia,* and *Burkholderia cepacia* complex (9). *Haemophilus influenzae* was detected in 16.5% of all CF patients in 2011 and followed a similar trend as *S. aureus* with a higher proportion of CF individuals colonizing *H. influenzae* during childhood compared to adulthood (9). *S. maltophilia* was

colonized in 14.0% of all CF patients in 2011 and had a peak prevalence of 20.0% among the 11 to 17 year age group (9). For all other age groups, the proportion colonizing this pathogen was around 15% with little variation (9). *B. cepacia* complex was found in only 2.6% of CF airways in 2011 but is considered a significant CF pathogen as it has been found to decrease lung function and to largely exclude an individual with CF from being accepted for a lung transplant (9, 24). *B. cepacia* complex is found in less than 1% to 3% of CF children (<18 years) and approximately 5% of CF adults (>18 years) (9).

Researchers have linked the changing prevalences of these respiratory pathogens with age to the progression of lung damage that occurs as individuals with CF get older (7). For example, it is thought that early childhood infection with *S. aureus* (primarily MSSA), *H. influenzae,* and other pathogens may damage the epithelial cell surfaces and lead to increased attachment and later displacement by *P. aeruginosa* (7). Previously, providers prescribed anti-staphylococcal medication to treat early infection with *S. aureus*; however, several studies, notably a randomized control trial and a Cochrane systematic review, have demonstrated that anti-staphylococcal treatment actually increases the rate of *P. aeruginosa* acquisition particularly among young CF patients (25-27). This led the US CF Pulmonary guidelines to recommend avoiding treatment of MSSA with anti-staphylococcal antibiotics in order to prevent acquisition of *P. aeruginosa* (28). This "trade-off" between remaining MSSA-positive to prevent *P. aeruginosa* infection is well justified as there is greater empirical evidence of *P. aeruginosa* infection causing lung damage and clinical deterioration than that of MSSA infection (29).

Pseudomonas aeruginosa

Respiratory pathogens greatly impact the morbidity and mortality of cystic fibrosis patients, and *Pseudomonas aeruginosa* is considered the most significant of these respiratory

pathogens (5, 30). *P. aeruginosa* is a common bacterium found in soil, water, skin flora, and most man-made environments including hospitals (30). Although individuals without cystic fibrosis are not harmed by *P. aeruginosa*, people who are immunocompromised such as those with cystic fibrosis, diabetes, AIDS, or cancer can be harmfully infected by *P. aeruginosa* in the pulmonary tract, urinary tract, and the blood (30). In CF patients, *P. aeruginosa* can be acquired through these natural reservoirs or by other CF patients, which is the primary reason for strict infection control guidelines in CF care centers and the discouragement of physical contact between CF patients (30). It is thought that the microbial pathogenesis of *P. aeruginosa* begins with the colonization of the oropharynx and follows with the lower respiratory tract where infection may result (30). Evidence suggests that during the early stages of infection, *P. aeruginosa* strains are non-mucoid, transient, and susceptible to antibiotics; however, after a period of time one or two mucoid strains establish themselves and chronic infection develops, beginning a continuous cycle of inflammatory responses and progressive lung tissue damage (30). Researchers have not discovered the microbiological mechanisms that lead to the establishment of particular mucoid strains but have found mucoid *P. aeruginosa* to be associated with the overproduction of an exopolysaccharide that provides protection from antibiotics (30, 31).

In CF, about half of patients colonize *P. aeruginosa* by the time they are 11 years old (9). Furthermore, research suggests that upon colonization, most patients continue to either intermittently or chronically colonize *P. aeruginosa* (32). Because sputum samples are most commonly used to detect the presence of respiratory pathogens such as *P. aeruginosa*, it can be difficult to distinguish infection from colonization (32). To provide insight into these differences, Lee et al. formulated the Leed's criteria for defining stages of *P. aeruginosa*

colonization and infection, which were validated by comparison of sputum results to antibody levels (32). The Leed's criteria for defining *P. aeruginosa* are as follows (32):

- **Chronic infection**: When more than 50% of months, when samples had been taken, were *P. aeruginosa* culture positive.
- **Intermittent infection**: When 50% or less of months, when samples had been taken, were *P. aeruginosa* culture positive.
- **Free of infection:** No growth of *P. aeruginosa* during the previous twelve months, having previously been *P. aeruginosa* culture positive.
- **Never:** *P. aeruginosa* never cultured from sputum or cough swab.

In 2006, the Leed's criteria were further evaluated and shown to agree with both clinical status and levels of *P. aeruginosa* antibodies (21). The authors also found that at least 4 airway cultures were required in different months spread over the year to accurately classify *P. aeruginosa* infection stage according to the Leed's criteria (21). However, Lee et al. recommended that at least 6 sputum samples or eight samples for cough swab or nasopharyngeal aspirate in separate months in a year were needed to accurately classify *P. aeruginosa* infection status (32). Consequently, investigators seeking to conduct studies on the clinical effect of *P. aeruginosa* among CF patients should consider (i) the method of culture collection and (ii) the frequency of culture collection while following the Leed's criteria for classifying an individual's infection status.

Extensive research has been dedicated to the effect of *P. aeruginosa* infection on the morbidity and mortality of CF patients. In a 2011 review of CF clinical microbiology, Hauser et al. cited a multitude of studies that have found a significant association between *P. aeruginosa* infection and (i) increased mortality, (ii) poorer lung function, (iii) worse chest radiologic imaging scores, (iv) slower patient growth, (v) increased frequency of daily cough,

and (vi) increased hospitalization associated with pulmonary exacerbation (30). Dating back to 1976, Wilmott et al. found that only 53% of CF children with chronic *P. aeruginosa* infection survived to 16 years of age compared to 84% of CF children who remained *P. aeruginosa*-negative (33). In a 2002 study of more than 3,000 CF children, Emerson et al. found that early acquisition of *P. aeruginosa* among patients one to five years old had a 2.6 times higher risk of mortality after eight years of follow-up compared to their *P. aeruginosa*-negative counterparts (34). This study also found a significant difference in the percent predicted forced expiratory volume in the first second (FEV_1 % predicted) between patients positive and negative for *P. aeruginosa* (34). FEV_1 % predicted is the most common clinical measurement of pulmonary function used by the CF Foundation, CF researchers, and CF providers (9). The CF Foundation defines different levels of disease severity according to a patient's FEV_1 % predicted as (9):

- Normal: ≥90% predicted
- Mild lung disease: 70 to 89% predicted
- Moderate lung disease: 40 to 69% predicted
- Severe lung disease: <40% predicted

In the study by Emerson et al., patients positive for *P. aeruginosa* had a mean FEV_1 of 74.6% predicted while patients negative for *P. aeruginosa* had a mean FEV_1 of 88.2% predicted, suggesting more progressed disease among young patients with *P. aeruginosa* compared to young patients without *P. aeruginosa* (34).

Researchers have also found that early treatment of *P. aeruginosa* infection can lead to successful eradication and better outcomes in CF patients. In a longitudinal study conducted at a CF center in Florence, Italy, 58 young CF patients who colonized *P. aeruginosa* for the first time were immediately treated with a combination of inhaled colistin and oral

ciprofloxacin and 47 (81%) patients achieved successful eradication (35). Notably, the average lung function decline was -4.69% FEV_1 predicted per year (standard deviation: 2.95) among those who did not achieve successful eradication compared to -1.63% FEV_1 predicted per year (standard deviation: 1.60) among those that did achieve successful eradication ($p<0.05$) (35). This study provided evidence that early eradication of *P. aeruginosa* can prevent subsequent clinical decline. This study, among others, also established that rate of lung function decline can be a better indicator of the progression of lung disease in cystic fibrosis compared to single measures of FEV_1 which have significant variability (36).

Despite available therapies and eradication approaches, recent studies of risk factors for rate of decline in FEV_1 show *P. aeruginosa* infection remains a significant, independent risk factor of decline among children, adolescents, and young adults with CF (36-38). Evidence for the older adult population is not large, particularly because only in the past few decades have CF patients begun to survive into their 30s and 40s. In one study of CF adults, the association of *P. aeruginosa* and rate of lung function decline was limited to mucoid and multi-drug resistant types of *P. aeruginosa* (37). The authors found that for young adult patients (18-24 years) positive for multi-drug resistant *P. aeruginosa*, the rate of lung function decline was -2.49% FEV_1 predicted per year compared to -1.88% FEV_1 predicted per year for patients without multi-drug resistant *P. aeruginosa* ($p=0.004$) (37). However, in the >25 year age group, multiple antibiotic-resistant *Pseudomonas aeruginosa* was not significantly associated with lung function decline; this was also found in a larger study of multi-drug resistant *P. aeruginosa* among the general CF population (37, 39).

Although there is some emerging mixed evidence of the effect of differing types of *P. aeruginosa*, particularly that of antibiotic resistant strains among varying age groups, *P.*

aeruginosa remains the most significant pathogen contributing to morbidity and mortality in cystic fibrosis.

Methicillin-resistant *Staphylococcus aureus* (MRSA)

In the past decade, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased internationally and alarmingly so in the United States. In 2011, approximately 25.9% of CF patients in the U.S. CF Foundation patient registry had a positive respiratory culture for MRSA, increasing from 7.3% in 2001 (9). Internationally, the prevalence of MRSA in CF patients is much lower but has been recorded as high as 13% in Ireland in 2007 (29).

Similarly to *P. aeruginosa*, *Staphylococcus aureus* is a pervasive bacterium that is not always pathogenic (29). In CF patients, it is one of the earliest bacteria found in the lungs, and although some early studies supported anti-staphylococcal treatment, later studies showed that eradication of methicillin-sensitive *S. aureus* (MSSA) increased acquisition of *P. aeruginosa,* which is considered more harmful than MSSA (27). There are similar concerns for treatment of MRSA with some believing that treatment may lead to the emergence of further resistance (29).

The microbiologic difference between methicillin-sensitive and methicillin-resistant *S. aureus* is the acquisition of the *mec*A gene, which makes it resistant to methicillin and other β-lactam antibiotics (29). The first outbreaks of MRSA were in hospitals in the 1960s; however, since the 1990s a community-associated (CA)-MRSA has emerged which tends to be different genetically than hospital-associated (HA)-MRSA and be more resistant to antibiotics (29). The prevalence of CA-MRSA and HA-MRSA is unknown in the general CF population (29). One prospective study of 82 patients with MRSA at a pediatric and adult CF clinic found that 72% of patients had a type of MRSA associated with healthcare

associated infections while 28% had a CA-MRSA type of which 17% were characterized as USA300 strains (40).

The evidence for the clinical impact of MRSA among CF patients has been conflicting. As of 2013, there have been two national studies and several smaller, mostly CF center studies, examining the association between MRSA and lung function decline, primarily measured as FEV_1 % predicted per year (40-47). Three of these studies found statistically significant, independent associations between MRSA and FEV_1 % predicted decline per year, controlling for other important factors shown to contribute to rate of lung function decline such as *P. aeruginosa* infection, *Burkholderia cepacia* complex infection, CF-related diabetes, pancreatic insufficiency, sex, baseline FEV_1 % predicted, and age (41, 44, 47). A 10-year national Cystic Fibrosis Foundation registry study of 17,357 CF patients aged 8 to 45 years found that chronic MRSA, defined as three or more positive MRSA cultures during follow-up, independently contributed to a 43% faster rate of decline among patients 8 to 21 years of age infected with MRSA (-2.06% FEV_1 predicted/ year) compared to CF patients of the same age group without MRSA (-1.44% FEV_1 predicted/ year) (difference: -0.62% FEV_1 predicted/year; 95% confidence interval: -0.70, -0.54) (41). However, there was not a significant difference in the rate of decline in FEV_1 % predicted among CF patients 22 to 45 years of age (41). One potential explanation for the null association found in adults is a phenomenon called the "basement effect" which occurs when CF lung disease is advanced, baseline FEV_1 is low, and any further inflammation introduced by MRSA does not have a detectable clinical effect (41). Another recent study conducted at a CF clinic in Brussels found that chronic MRSA infection, defined as three or more MRSA cultures during at least six months follow-up, was associated with a faster rate of lung function decline compared to a control group without MRSA (difference: -0.80% FEV_1 predicted/year; $p=0.026$) (47).

Although the investigators did not examine co-infection of chronic MRSA and chronic *P. aeruginosa*, they did observe a higher proportion of chronic *P. aeruginosa* colonization among patients with chronic MRSA compared to the control group, though the difference was not statistically significant (47).

Other studies have found non-significant differences in the rate of lung function decline between patients with and without MRSA. A 5-year national study conducted by the Epidemiologic Study of Cystic Fibrosis (ESCF) among 5,090 patients found that, although CF patients with incident MRSA had a faster rate of decline both before and after MRSA acquisition compared to patients who remained MRSA negative, the rate of FEV_1 decline did not significantly change after MRSA detection compared to before detection ($p=0.145$) (42). The authors determined that MRSA may not independently increase the rate of lung function decline but serve as an indicator of more advanced disease (42). As in other studies, the authors did not examine interaction between MRSA and *P. aeruginosa* infection. Finally, smaller studies in local CF centers have found a more rapid rate of decline in FEV_1 among MRSA-positive patients but without significant differences when compared to MRSA-negative patients (40, 45, 46).

Although the question of whether MRSA independently contributes to a faster rate of clinical decline in CF patients is still somewhat debatable, it has garnered attention in the CF community as an important pathogen, particularly because of the rapidly growing prevalence among CF patients.

Co-infection of MRSA and *Pseudomonas aeruginosa*

The association between MRSA and *P. aeruginosa* co-infection and rate of lung function decline has not been widely studied. Most studies have not examined interaction between these two pathogens but rather controlled for the presence of the other (40-42, 47).

A recent study conducted at two CF centers in France found a 0.1% higher rate of FEV_1 predicted decline per year among patients with combined MRSA and *P. aeruginosa* colonization compared to patients with neither *S. aureus* (MRSA or MSSA) nor *P. aeruginosa* infection (p=0.03) (45). Rate of FEV_1 decline was not significantly different for those with MRSA alone (p=0.08) or *Pseudomonas aeruginosa* alone (p=0.11) compared to patients without *S. aureus* or *P. aeruginosa* (45). Although the authors noted that 77% of patients with MRSA had persistent MRSA (detected in more than 1 year) and that they did not experience a higher rate of decline compared to patients without persistent MRSA (p=0.68), the authors did not appear to examine differences between chronic and intermittent colonization of MRSA and/or *P. aeruginosa* in their longitudinal analysis (45). Few studies conducted in the U.S., where the prevalence of MRSA is highest in the world among CF patients, have examined the impact of co-infection of MRSA and *P. aeruginosa* infection, chronic or not, on the rate of lung function decline. Researchers have investigated co-infection of *P. aeruginosa* and *S. aureus* but with no distinction between MRSA and MSSA. One study conducted at a U.S. adult CF center found that concurrent infection with both *P. aeruginosa* and *S. aureus* was more prevalent among patients with rapidly declining lung function (-8.1% FEV_1 predicted/year; standard deviation: 4.0) compared to patients moderately declining in lung function decline (–1.1% FEV_1 predicted/year; standard deviation: 1.2) (p=0.01) (38). In another small study of CF children less than 3 years old, patients who initially colonized *P. aeruginosa* and *S. aureus* had a 10-year survival estimate of 57%, which was significantly lower than those with normal respiratory flora, *S. aureus* alone, or *P. aeruginosa* alone whose 10-year survival ranged from 92% to 100% (48).

Previous in vitro studies support the biological plausibility of an interaction between MRSA and *P. aeruginosa*. Two studies using cystic fibrosis sputum found that *P. aeruginosa*

simultaneously suppressed the growth of *S. aureus* while increasing the prevalence of more virulent small colony variant *S. aureus* that were aminoglycoside-resistant (22, 49). However, the investigators did not examine MRSA specifically. A more recent study examining the interaction between USA300 MRSA, a community-acquired MRSA (CA-MRSA) strain, and *P. aeruginosa* in cutaneous wounds found that the presence of *P. aeruginosa* resulted in induced expression of USA300 MRSA virulence factors, Panton-Valentine leukocidin (PVL) and α-hemolysin, which increased the severity of the wound (50). Without a similar study, researchers cannot determine whether the same interaction would occur in the cystic fibrosis lung and if it would deleteriously impact lung function. There is some evidence that it would. In a small study of 33 cystic fibrosis patients, PVL+ MRSA was associated with an increased rate of decline in FEV_1 (51). These studies suggest that a biological interaction between these two pathogens is possible and could result in a more severe expression of *Staphylococcus aureus* and subsequent clinical deterioration. However, further research with the specific objective of assessing the biological interaction of MRSA and *P. aeruginosa* in the CF lung is needed.

There is a paucity of literature examining chronic co-infection of MRSA and *P. aeruginosa* in cystic fibrosis patients, particularly in the United States where the prevalence of MRSA in the CF population is the highest worldwide. This study aims to investigate the clinical impact of chronic MRSA and *P. aeruginosa* co-infection by (i) characterizing patients chronically co-infected with MRSA and *P. aeruginosa* and (ii) examining the association between chronic co-infection and rate of lung function decline compared to other levels of infection among patients with cystic fibrosis.

ABSTRACT

Background: Chronic *Pseudomonas aeruginosa* infection is a well-known risk factor for increased lung function decline among patients with cystic fibrosis (CF). More recently, studies have found that chronic MRSA infection may also contribute to lung function decline. However, the association between chronic MRSA and *P. aeruginosa* co-infection and rate of lung function decline has not been widely studied.

Objective: This study aims to characterize CF patients with chronic MRSA and *P. aeruginosa* co-infection and examine whether these patients have a higher rate of lung function decline, measured by forced expiratory volume in the first second (FEV_1, given as % predicted) per year, compared to patients with other levels of infection.

Population: Cystic fibrosis patients (≥6 years old) attending a CF center in Atlanta, GA and included in the patient registry between 2007 and 2013 comprised the study cohort.

Study Design: Repeated measures mixed effects modeling was used to compare the rates of FEV_1 decline (% predicted/year) between patients chronically co-infected with MRSA and *P. aeruginosa* to patients with chronic *P. aeruginosa* alone, chronic MRSA alone, intermittent MRSA or *P. aeruginosa*, and no colonization of *P. aeruginosa* or MRSA, adjusting for known covariates.

Results: Patients with chronic co-infection were found to have a baseline FEV_1 of 62.9% predicted (standard deviation: 25.8) and a significantly more rapid rate of decline in FEV_1 % predicted compared to patients without MRSA or *P. aeruginosa* adjusting for confounders and using all values of FEV_1 recorded during follow-up (mean rate: -1.06 FEV_1 % predicted per year; 95% CI: -2.08, -0.05). However, the rate of FEV_1 decline was not significantly greater than that of patients with chronic *P. aeruginosa*, chronic MRSA alone, or intermittent infection, suggesting that chronic co-infection may not increase lung function decline beyond that of other levels of infection.

Conclusions: Chronic co-infection of MRSA and *P. aeruginosa* is associated with lower baseline lung function and continued decline in FEV_1 % predicted; however, the rate of decline in FEV_1 % predicted may not be greater than that of other common levels of infection. Future studies should investigate this association in the broader CF population.

INTRODUCTION

Cystic fibrosis (CF) is a life-threatening, autosomal recessive disorder that affects approximately 30,000 people in the United States (U.S.) and 70,000 worldwide (1). Due to a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, an imbalance in the sodium and chloride ions creates a viscous mucus layer in the respiratory tract that impedes bacterial clearance (2). As a consequence, the airway is prone to chronic bacterial infections that contribute to both morbidity and mortality among individuals with cystic fibrosis (2, 6).

Pseudomonas aeruginosa has long been appreciated as the most pathogenic microbe in cystic fibrosis and infects about half of patients by the time they are 11 years old and three-quarters by adulthood (9). Furthermore, upon colonization, most patients continue to either intermittently or chronically colonize *P. aeruginosa* (32). Chronic *P. aeruginosa* has been associated with increased mortality, poorer lung function, worse chest radiologic imaging scores, slower patient growth, increased frequency of daily cough, and increased hospitalization (21, 30, 32).

In a single decade, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) more than tripled from 7.3% in 2001 to approximately 25.9% in 2011 among CF patients in the Cystic Fibrosis Foundation (CFF) patient registry (9). In response, there is a growing body of literature investigating the clinical impact of MRSA in CF. A national study of the CFF patient registry found that patients with MRSA had a median survival time 6.2 years shorter than patients without MRSA (52). However, studies examining MRSA's longitudinal impact on decline in forced expiratory volume in the first second (FEV_1) have been less clear. Three recent studies have found chronic MRSA infection to significantly increase the rate of FEV_1 decline (41, 44, 47). Other studies, including a national study conducted by the

Epidemiologic Study of Cystic Fibrosis (ECSF), have found non-significant differences in FEV_1 decline between patients with and without MRSA, suggesting that MRSA infection is simply an indicator of more advanced disease (40, 42, 45).

The association between chronic MRSA and *P. aeruginosa* co-infection and rate of lung function decline among CF patients has not been widely studied despite some previous evidence of an association. A two-year study conducted in France found a significantly higher rate of lung function decline among patients with combined MRSA and *P. aeruginosa* colonization compared to patients without *S. aureus* or *P. aeruginosa* colonization (45). A study conducted at an adult U.S. CF center found *P. aeruginosa* and *S. aureus* co-infection was significantly more prevalent among patients with rapidly declining lung function than patients with moderate lung function decline but made no distinction between methicillin-sensitive and methicillin-resistant *S. aureus* (38). Co-infection has also been associated with a higher mortality. In a small study of CF children less than 3 years old, patients who initially colonized *P. aeruginosa* and *S. aureus* had a 10-year survival estimate of 57%, which was significantly lower than those with normal respiratory flora, *S. aureus* alone, or *P. aeruginosa* alone whose 10-year survival ranged from 92% to 100% (48).

There is a paucity of literature examining the association of chronic co-infection between MRSA and *P. aeruginosa* and rate of FEV_1 decline, particularly in the United States where the prevalence of MRSA in the CF population is the highest in the world. This study aims to investigate the clinical impact of chronic MRSA and *P. aeruginosa* co-infection by (i) characterizing patients chronically co-infected with MRSA and *P. aeruginosa* and (ii) examining the association between chronic co-infection and rate of lung function decline compared to other levels of infection among patients with cystic fibrosis.

METHODS

This was a retrospective cohort study utilizing data from the patient registry of three large CF centers in Atlanta, Georgia affiliated with Emory University and Children's Healthcare of Atlanta, Inc., between January 1, 2007 and December 31, 2013. All patients had previously consented to be included in the patient registry (Port-CF). A total of 782 CF patients were seen during this time. The study was approved by the Emory University Institutional Review Board (IRB) [IRB00070522].

Patients

Patients were included in the analysis if they were ≥6 years of age, followed for at least 2 years in the patient registry, and met the minimum criteria for number of respiratory tract cultures and spirometry measurements. Patients <6 years of age were excluded to avoid unreliable spirometry data. A one-year lead-in period was established for each patient in order to determine MRSA and *P. aeruginosa* infection status and baseline covariates. To adequately assess chronicity of MRSA and *P. aeruginosa*, patients were included if they had at least 4 respiratory tract culture results recorded during the one-year lead-in period as has been done previously (53). Spirometry must have been performed at least once during the lead-in period to assess baseline FEV_1 and three times during follow-up to estimate rate of decline in FEV_1.

Clinical Data

Patient clinical and demographic data were extracted from Port-CF. Clinical data are recorded in Port-CF at each clinic visit, which are typically quarterly and during times of disease exacerbation when hospitalized or treated with intravenous (IV) antibiotics at the patient's home. Demographic data include sex, race/ethnicity, age at each encounter, and

CF center. Clinical data include patient's genotype, spirometry measurements, and secondary complications. Patient genotype was categorized as homozygous ΔF508 mutation, heterozygous ΔF508 mutation, other, and unknown. Baseline FEV_1 % predicted was defined as the patient's first FEV_1 % predicted recorded during the lead-in period and was categorized as <40% predicted, 40- <70% predicted, 70- <80% predicted, and ≥100% predicted. Pancreatic insufficiency was a dichotomous variable defined by pancreatic enzyme usage. CF-related diabetes (CFRD) status was defined as CFRD with or without fasting hyperglycemia and was classified as yes/no. Additional secondary complications were categorized in the same way (yes/no) and defined by documented presence during the lead-in period. Body mass index (BMI) was calculated as a patient's body mass (kg) divided by the square of his/her height (m^2). Values of FEV_1 % predicted, BMI, and other covariates that were considered invalid were set to missing before analysis.

The primary outcome of interest was mean rate of FEV_1 % predicted decline, which is the most common clinical measure of lung function decline in scientific studies of cystic fibrosis (54). Values of FEV_1 % predicted were calculated using the equations of Hankinson et al. and Wang et al. according to patient's sex, age, and race (55, 56). All values of FEV_1 % predicted were used in the initial analysis. This was a similar approach as VandenBranden et al., Ren et al., and Konstan et al (57-59). Results of the initial analysis were then compared to results using only FEV_1 measurements taken during times of clinical stability to check for bias from the inclusion of values taken during times of pulmonary exacerbation. FEV_1 % predicted values recorded during hospitalization and home IV treatments were set to missing before secondary analysis.

Infection Groups

MRSA and *P. aeruginosa* status were determined from respiratory tract cultures obtained from sputum (71%), induced sputum (0.2%), throat/nasal culture (28%), or bronchoscopy (0.9%) during the one-year lead-in period.

During the one-year lead-in period, *P. aeruginosa* infection status was classified as never, intermittent, or chronic based on Leed's criteria, a previously validated definition for chronic *P. aeruginosa* (21, 32, 53). As defined in previous studies, MRSA was considered chronic if detected in more than three respiratory cultures during the lead-in period (40, 41, 47). Chronic co-infection was defined as >50% of patient respiratory cultures positive for *P. aeruginosa* and ≥3 MRSA positive cultures during the lead-in period. Chronic *P. aeruginosa* alone was defined as >50% of cultures positive for *P. aeruginosa* and <3 MRSA positive cultures. Chronic MRSA alone was defined as ≤50% of cultures positive for *Pseudomonas aeruginosa* and ≥3 MRSA positive cultures. Intermittent *P. aeruginosa*/MRSA infection was defined as ≤50% of cultures positive for *P. aeruginosa* and <3 MRSA-positive cultures but colonizing *P. aeruginosa* and/or MRSA at least once during the lead-in period. The reference group comprised patients who did not colonize *P. aeruginosa* or MRSA during the lead-in period.

Statistical Analysis

Descriptive statistics were calculated to summarize patient characteristics. Continuous variables were compared using analysis of variance or Kruskal-Wallis test as appropriate. Categorical variables were compared using chi-square tests.

Repeated measures mixed-effects modeling with a random slope and intercept to account for natural heterogeneity among patients was used to determine the effect of chronic MRSA and *P. aeruginosa* co-infection on FEV_1 % predicted per year, adjusting for

known covariates determined a priori through a review of the literature. Covariates were assessed during the lead-in period and included baseline age, sex, race/ethnicity, baseline FEV_1 % predicted, BMI, positive respiratory tract cultures for other bacteria (MSSA, *Burkholderia cepacia* complex, *Haemophilus influenzae,* and *Aspergillus fumigatus*), pancreatic insufficiency, CF-related diabetes, and asthma. To assess the association between infection status and rate of decline in FEV_1 % predicted, models included an interaction term of infection status with time. Time was measured as age in years. The exposure variable and all potential covariates were included in the initial model. Sex, age at baseline, presence of *Burkholderia cepacia* complex, and CF-related diabetes were included in the final model regardless of statistical significance. Other potential confounders were assessed through backwards elimination by removing the least significant variables ($p > 0.05$) one by one and comparing each subsequent model to the initial model to ensure control of confounding. Because of collinearity and poor model fit, baseline values for BMI and pancreatic insufficiency, were not used; rather BMI was treated as a time-varying variable and study data from all years were used to assess pancreatic enzyme use.

Additional sensitivity analyses were performed. These included removing patients >45 years of age as they represent a milder phenotype in cystic fibrosis and assessing the effect of mucoid-type *P. aeruginosa* infection. Finally, patients who cultured both MRSA and *P. aeruginosa,* regardless of chronicity, were compared to those who never cultured both pathogens to examine whether any co-infection was associated with an increased rate of FEV_1 % predicted decline.

All statistical analyses were conducted in SAS Version 9.3 (Carey, NC). The SAS procedure PROC MIXED was used for mixed-effects modeling accounting for repeated measures. Statistical significance was accepted at $p < 0.05$.

RESULTS

A total of 354 patients met the inclusion criteria for analysis (Figure 1). Forty (11.3%) patients had chronic MRSA and *P. aeruginosa* co-infection, 105 (29.7%) had chronic *P. aeruginosa* alone, 43 (12.2%) had chronic MRSA alone, 71 (20.1%) had intermittent MRSA/ *P. aeruginosa*, and 95 (26.8%) did not have MRSA or *P. aeruginosa* during the lead-in period. Patients with chronic co-infection colonized an average of 5.4 (standard deviation: 1.9) *P. aeruginosa*-positive cultures and 5.1 (standard deviation: 1.5) MRSA-positive cultures during the lead-in period (Figure 2A). During subsequent follow-up, patients with chronic co-infection continued to colonize both *P. aeruginosa* and MRSA with an average of approximately three positive cultures of each pathogen per year (Figure 2B). Patients who did not culture *P. aeruginosa* or MRSA during the lead-in period often cultured at least one of these pathogens during subsequent follow-up. Twenty-one (5.9%) patients remained free of both *P. aeruginosa* and MRSA during the entire study period.

Baseline characteristics by infection group are presented in Table 1. Infection groups were significantly different in baseline age, FEV_1 % predicted, BMI, MSSA status, *H. influenzae* status, mucoid *P. aeruginosa* status, *Aspergillus* status, and number of FEV_1 measurements recorded during patient hospitalization ($p<0.05$). Patients with chronic co-infection were most similar in age to patients with *P. aeruginosa* alone. Sixty percent of chronically co-infected patients were children (<18 years) compared to 46.7% of chronic *P. aeruginosa* alone patients, 93.0% of chronic MRSA alone patients, 84.5% of intermittent

patients, and 86.3% of patients with no *P. aeruginosa* or MRSA. Patients with chronic co-infection had the lowest baseline FEV_1 % predicted (mean: 62.9%; standard deviation: 25.8) while patients with no MRSA or *P. aeruginosa* had the highest baseline FEV_1 % predicted (mean: 86.3%; standard deviation: 20.7). Thirty-eight (95%) patients with chronic co-infection and all 105 (100%) patients with chronic *P. aeruginosa* alone cultured mucoid *P. aeruginosa* at least once during the lead-in period. Patients with chronic co-infection had the highest mean number of FEV_1 measurements recorded when in the hospital.

Patients were followed for an average of 4.6 years (standard deviation: 1.5; range: 1.0-6.0 years) after the lead-in period. There were no differences in the average follow-up time between infection groups ($p=0.44$). The average number of respiratory cultures and FEV_1 measurements documented during the lead-in period were 5.1 cultures (standard deviation: 1.3) and 7.4 FEV_1 measurements (standard deviation: 3.7). Patients with chronic co-infection had the highest number of respiratory cultures and FEV_1 measurements taken during the lead-in period. Differences between infection groups for number of respiratory cultures and FEV_1 measurements were significant ($p <0.0001$).

Mean values of FEV_1 % predicted at baseline, end of follow-up, and change from baseline to end of follow-up by infection status are presented in Table 2. Patients with chronic co-infection, chronic *P. aeruginosa* alone, and chronic MRSA alone had similar mean changes in FEV_1 % predicted between baseline and end of follow-up. The greatest change in FEV_1 % predicted occurred among patients with chronic MRSA alone (mean difference: -11.6; standard deviation: 15.3). Patients with chronic co-infection had the highest variability in mean change of FEV_1 % predicted (mean: -10.5; standard deviation: 23.8).

The unadjusted mean rate of decline in FEV_1 % predicted for all study patients was -1.62 FEV_1 % predicted per year (95% CI: -1.95, -1.29). The adjusted mean rates of decline in FEV_1 % predicted by infection group are presented in Table 2 and Figure 3. Using all measurements of FEV_1 % predicted, patients with chronic co-infection and chronic *P. aeruginosa* alone had significantly more rapid rates of decline compared to patients without MRSA or *P. aeruginosa,* adjusting for covariates. The adjusted mean rate of decline in FEV_1 % predicted was -1.06 FEV_1 % predicted per year (95% CI: -2.08, -0.05) for patients with chronic co-infection and -0.96 FEV_1 % predicted per year (95% CI: -1.74, -0.18) for patients with chronic *P. aeruginosa* alone. Patients with chronic co-infection had a 11.0%, 62.2%, and 94.0% more rapid rate of decline in FEV_1 % predicted than patients with chronic *P. aeruginosa* alone, chronic MRSA alone, and intermittent infection, respectively; however, the differences in rates between patients with chronic co-infection and patients from each of these groups were not significant (p=0.83; p=0.50; p=0.34, respectively). Mean rates of decline for patients with chronic MRSA and intermittent MRSA/ *P. aeruginosa* were not significantly different than patients without MRSA or *P. aeruginosa*. Only chronic *P. aeruginosa* remained a significant predictor of subsequent FEV_1 decline when analysis was limited to values of FEV_1 taken during times of clinical stability.

As a sensitivity analysis, patients >45 years of age (n=6) were removed from analysis as these patients may represent a milder phenotype in cystic fibrosis. Results were nearly identical to the initial analysis (see Appendix Table 1). To examine whether any co-infection regardless of chronicity had an impact on rate of lung function decline, an analysis comparing patients who colonized both MRSA and *P. aeruginosa* at least once during the lead-in period (n=85) to patients who did not colonize both pathogens (n=269) was done. Adjusting for the same covariates as in the previous analysis, any co-infection was not found

to significantly increase rate of decline in FEV_1 % predicted (mean rate: -0.29; 95% CI: -0.97, 0.39) (see Appendix Table 2). Because 95-100% of patients with either chronic co-infection or chronic *P. aeruginosa* alone had colonized mucoid-type *P. aeruginosa* during the lead-in period, no additional analyses examining the effect of mucoid-type *P. aeruginosa* were conducted.

DISCUSSION

In this retrospective cohort study of adult and pediatric CF patients attending three CF centers in Atlanta, Georgia, chronic co-infection of MRSA and *P. aeruginosa* was found to significantly increase the rate of decline in FEV_1 % predicted compared to patients without MRSA or *P. aeruginosa,* adjusting for covariates and using all measures of FEV_1 (mean rate: -1.09 FEV_1 % predicted per year; 95% CI: -2.11, -0.07). However, there was not a significant difference between the rates of decline in FEV_1 % predicted for patients with chronic co-infection when compared to patients with chronic *P. aeruginosa* alone, chronic MRSA alone, or intermittent infection of these two pathogens. These results suggest that patients with chronic co-infection may have a more rapid rate of decline than patients without MRSA or *P. aeruginosa* but does not lead to significant increases in rate of decline beyond that of chronic *P. aeruginosa* alone, chronic MRSA alone, or even intermittent infection, which are more common levels of infection among CF patients. Furthermore, when limited to FEV_1 measurements taken during times of clinical stability, the effect of chronic co-infection on rate of FEV_1 decline became non-significant, indicating potential bias by FEV_1 values taken during times of pulmonary exacerbation that could have been only temporary and reversible. However, lack of significance may also be a result of the small number of chronically co-infected patients and high variability within this group.

Furthermore, patients with chronic co-infection appeared to have deteriorated lung function at baseline (mean FEV_1: 62.9%), which could underestimate the true effect of chronic co-infection because patients with lower FEV_1 at baseline have been observed to have a slower rate of FEV_1 decline (59).

In addition to assessing the impact of chronic co-infection on lung function decline, this study provided patient clinical and demographic characteristics associated with five different levels of infection status, which are not readily available in national Cystic Fibrosis Foundation patient registry reports. Notably, patients by level of infection were significantly different with regard to baseline age, FEV_1 % predicted, BMI, and presence of other microorganisms including MSSA, *H. influenzae*, mucoid *P. aeruginosa*, and *Aspergillus* status. Patients with chronic co-infection had the lowest average FEV_1 % predicted at baseline, which was nearly 10 percentage points less than the baseline average for patients with chronic *P. aeruginosa* alone, suggesting that deterioration in lung function had already occurred before the start of the study. The mean age of patients with chronic co-infection was 19.2 years (range: 6.0-49.7 years), which was similar to patients with chronic *P. aeruginosa* alone but nearly 8 years older than the mean age of patients with chronic MRSA alone, intermittent infection, and no MRSA/ *P. aeruginosa* infection. Because this study did not examine incident chronic co-infection, it was not possible to determine age of onset associated with chronic co-infection or whether patients acquired MRSA or *P. aeruginosa* infection first or simultaneously, though the latter is unlikely. When examining change in infection status among patients with at least 4 respiratory cultures during follow-up, patients with chronic *P. aeruginosa* alone during the lead-in period were more likely to develop chronic co-infection during follow-up compared to patients with chronic MRSA alone during the lead-in period (19/103=18.4% vs. 3/43=7.0%, respectively). This suggests that patients may

have chronic *P. aeruginosa* alone before developing chronic co-infection. However, with the growing prevalence of MRSA in the CF population and the young age associated with chronic MRSA alone in this study, patients may acquire chronic MRSA at a younger age and then acquire chronic *P. aeruginosa* later in adolescence or young adulthood. To observe this transition would require a longer follow-up time than in the present study. Future research should explore whether previous infection status affects incidence of chronic co-infection and subsequent lung function decline.

The prevalence of chronic co-infection in this study was 11.3% while any co-infection was found in 24.0% of patients, which is higher than in a previous study in France that found 10.8% of pediatric and adult patients were infected with both MRSA and *P. aeruginosa* regardless of chronicity (45). The two-year cohort study found that patients with co-infection had a significantly more rapid rate of decline in FEV_1 % predicted compared to patients without *S. aureus* or *P. aeruginosa*; all other levels of infection including *P. aeruginosa* alone and MRSA alone were found to be non-significant (45). Because the authors did not consider chronicity of infection and had a different reference group, it is difficult to compare their results to the results found in this study. For example, the findings of this study suggest the effect of co-infection is limited to that of chronic co-infection as any co-infection was not associated with a more rapid rate of lung function decline (mean rate: -0.29; 95% CI: -0.96, 0.39). Furthermore, chronic *P. aeruginosa* alone was significantly associated with lung function decline in this study. This difference is most likely due to the chronicity and mucoid nature of *P. aeruginosa* in this study, which have previously been found to significantly increase rate of lung function in CF patients (60).

The impact of chronic MRSA alone is not clear. Although patients with chronic MRSA alone had an 11.6 % (standard deviation: 15.3) decrease in mean FEV_1 % predicted from 83.0% predicted at baseline to 71.4% predicted at the end of follow-up, the mean rate of decline (mean: -0.64; 95% CI: -1.66, 0.39) was not significantly different from that of patients without MRSA or *P. aeruginosa.* These results are in contrast to that of Dasenbrook et al. who found that chronic MRSA still significantly increased the rate of lung function decline by approximately 0.5 FEV_1 % predicted per year when limited to patients 8-21 years of age without *P. aeruginosa* and milder disease (41).

The present study's findings support those of other studies that have found an association between co-infection and deteriorated lung function. One study among patients at an adult U.S. CF center found that concurrent infection with both *P. aeruginosa* and *S. aureus* was more prevalent among patients with a -8.1 FEV_1 % predicted per year (standard deviation: 4.0) rate of decline compared to patients with a –1.1% FEV_1 % predicted per year (standard deviation: 1.2) rate of decline ($p=0.01$) (38). However, no distinction was made between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus.* Additionally, a recent study among CF children found co-infection to be a significant predictor of initial low FEV_1 and that children in the low FEV_1 group had more positive cultures for MRSA and *P. aeruginosa* both before and after initial spirometry (61).

This study was limited by the use of retrospective registry data. Another limitation was the establishment of infection status based on a single year of observation, which did not allow for determination of incident infection or infection status to vary with time. Model fit is generally better when time-dependent variables are utilized that account for changes over time rather than time-independent variables which were utilized in this study

with the exception of body mass index. The use of a one-year lead-in period did strengthen interpretation of the impact of infection status on subsequent lung function decline by ensuring that infection preceded lung function decline. This study was further limited by the potential for misclassification of infection status during the lead-in period that may have changed during follow-up. To examine potential misclassification, the number of cultures positive for MRSA and *P. aeruginosa* during the lead-in period and subsequent follow-up were calculated (Figure 2A and B). This calculation revealed clear differences between infection groups during both time periods that support the baseline classification scheme.

The relatively small sample size was another limitation of this study. Restricting analysis to patients with at least four respiratory cultures and one spirometry measurement during the patient's first year of observation led to the exclusion of 148 patients. An additional 156 patients were excluded for having fewer than three FEV_1 measurements spanning at least one year of follow-up. An analysis of excluded patients ≥6 years of age (n=304) showed that excluded patients were significantly older (mean age=21.6 years; standard deviation: 13.3; p-value: <0.0001), had a lower baseline FEV_1 % predicted (mean=74.3; standard deviation: 28.3; p-value=0.004), were more likely to be pancreatic sufficient (p<0.0001) and have CF-related diabetes (p=0.03) than included patients (see Appendix Table 3). The unadjusted mean rate of decline in FEV_1 among excluded patients was -1.50 % predicted per year (95% CI: -1.72, -1.29), which was similar to that of included patients. Infection status for excluded patients was determined using all patient respiratory cultures; by this method, included and excluded patients did not differ by infection status (p=0.54). Because chronicity of infection was central to the research question of interest, the requirement of at least four respiratory cultures during the lead-in period was deemed necessary for classification. However, future studies should consider whether fewer

respiratory cultures could be used to accurately classify infection status and prevent large exclusion of patients.

Because this analysis was conducted among patients attending three CF clinics in Atlanta, Georgia, the results of this study may not generally apply to all cystic fibrosis patients. Further research in other CF care centers should be conducted to determine whether this association persists in the broader CF population.

In conclusion, although chronic co-infection status was associated with a significantly more rapid decline in FEV_1 % predicted compared to patients without MRSA or *P. aeruginosa*, results suggest that a synergistic relationship between MRSA and *P. aeruginosa* may not exist given that the rate of decline in co-infected patients was not significantly greater than that of patients with chronic *P. aeruginosa* alone, chronic MRSA alone, or intermittent MRSA/ *P. aeruginosa* infection. Additionally, the association between chronic co-infection and lung function decline lacked significance when FEV_1 measurements were limited to those taken during times of clinical stability. Nevertheless, patients with chronic co-infection had the lowest FEV_1 % predicted at baseline and continued to lose lung function during follow-up, warranting further research into the impact of chronic co-infection especially in light of the growing prevalence of MRSA and co-infection in this population.

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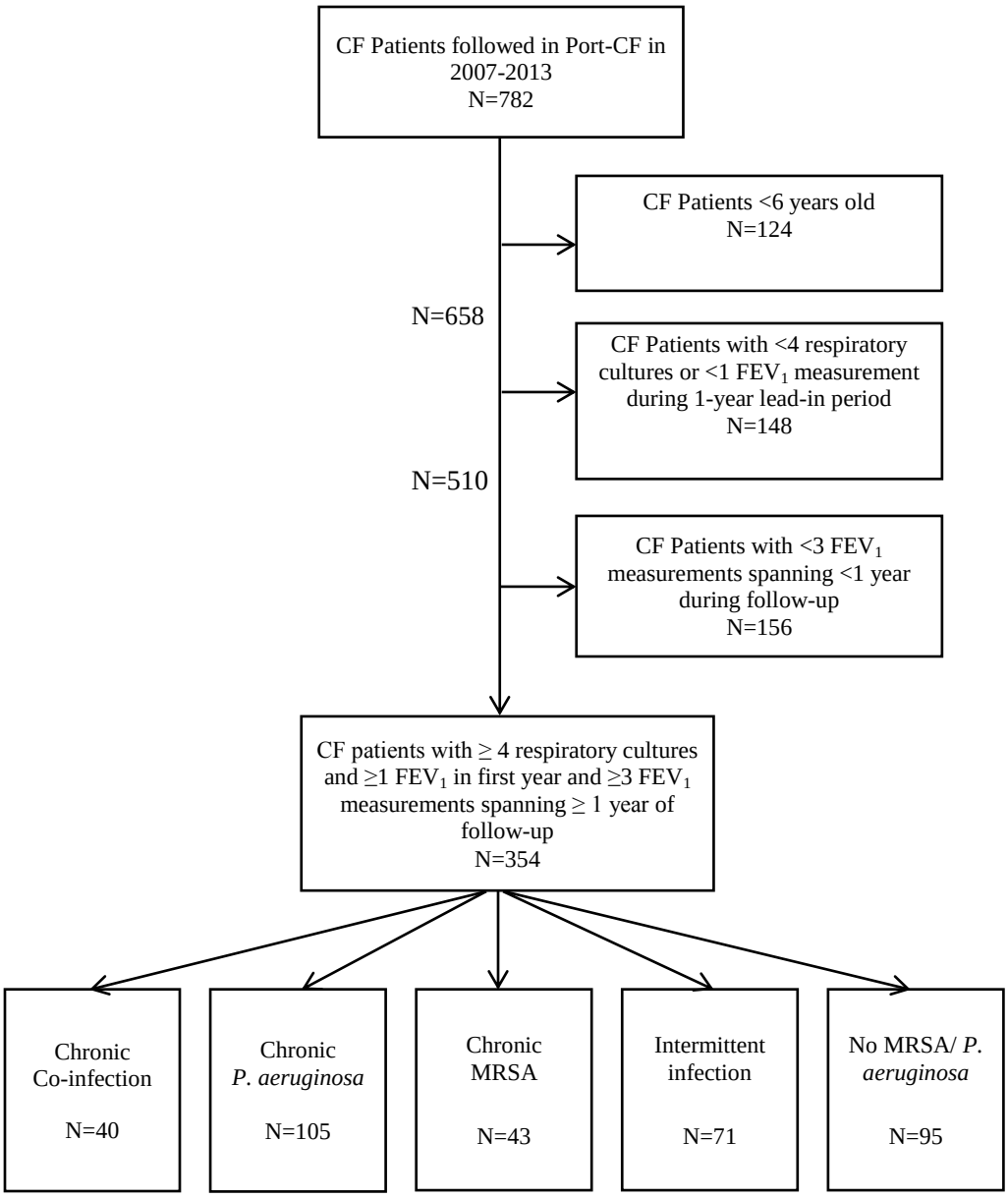
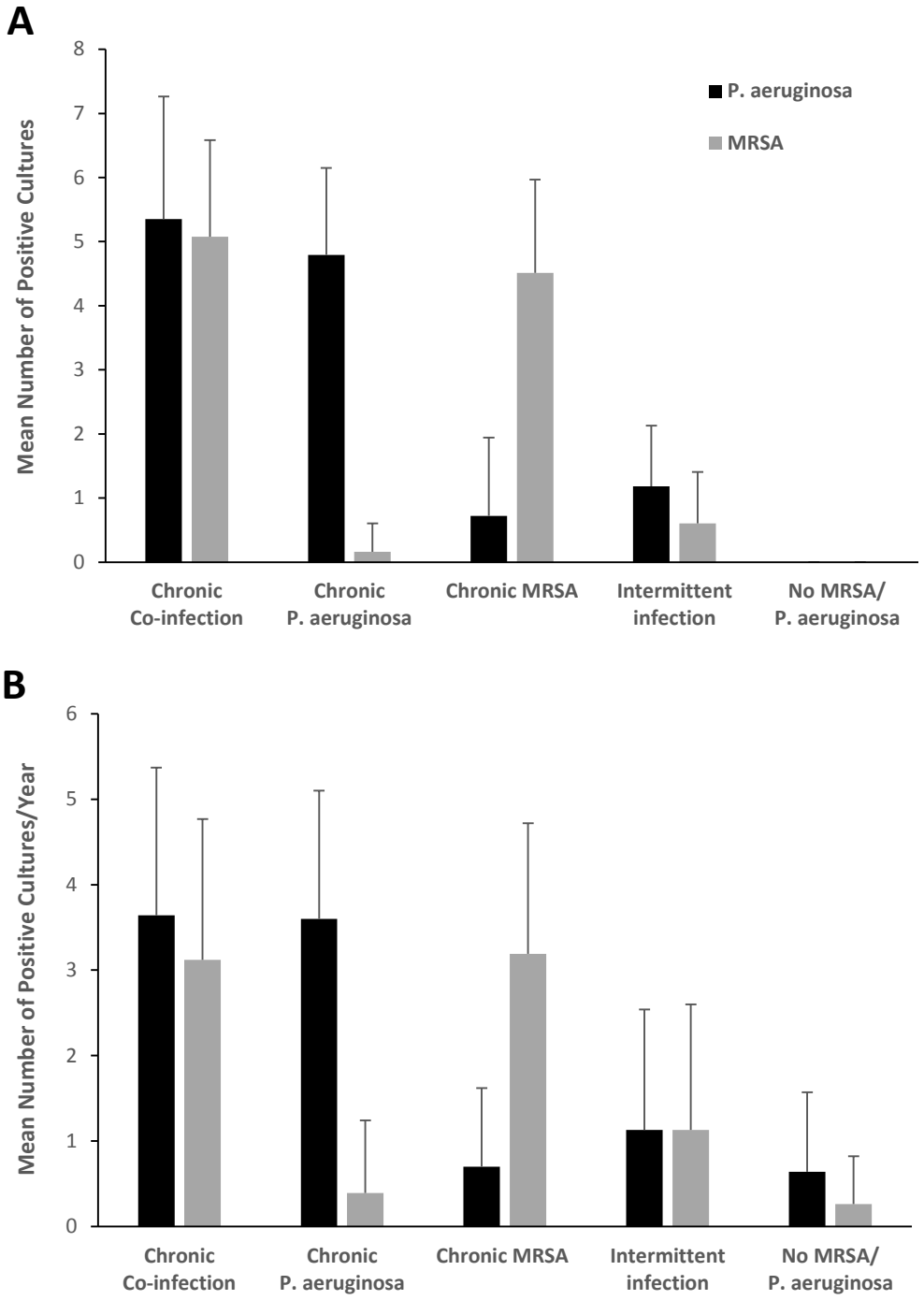
FIGURE 1. Diagram illustrating patient selection into study cohort, 2007-2013.



Abbreviations: CF, cystic fibrosis; FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*;

FIGURE 2. Average number of respiratory cultures positive for *P. aeruginosa* and MRSA by baseline infection status during the lead-in period (Panel A) and follow-up per year (Panel B), 2007-2013.



Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*

TABLE 1. Baseline patient characteristics by infection status, 2007-2013

	Infection Status					
	Chronic Co-infection	**Chronic *P. aeruginosa***	**Chronic MRSA**	**Intermittent infection**	**No MRSA/ *P. aeruginosa***	***P*-value***
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
All patients	40 (100)	105 (100)	43 (100)	71 (100)	95 (100)	
Age, year						
Mean (SD)	19.2 (9.1)	21.0 (11.5)	12.8 (8.1)	12.9 (7.8)	11.0 (7.1)	<0.0001
FEV_1 % predicted						
Mean (SD)	62.9 (25.8)	71.9 (25.5)	83.0 (21.1)	86.3 (20.7)	92.4 (21.2)	<0.0001
FEV_1 % predicted group						<0.0001
<40	8 (20.0)	15 (14.3)	2 (4.7)	1 (1.4)	2 (2.1)	
40- <70	16 (40.0)	33 (31.4)	10 (23.3)	18 (25.4)	9 (9.5)	
70- <100	12 (30.0)	42 (40.0)	18 (41.9)	31 (43.7)	48 (50.5)	
≥100	4 (10.0)	15 (14.3)	13 (30.2)	21 (29.6)	36 (37.9)	
Sex						0.32
Male	17 (42.5)	49 (46.7)	25 (58.1)	38 (53.5)	55 (57.9)	
Female	23 (57.5)	56 (53.3)	18 (41.9)	33 (46.5)	40 (42.1)	
Race/ethnicity						0.42
White	35 (87.5)	99 (94.3)	35 (81.4)	67 (94.4)	85 (89.5)	
African-American	3 (7.5)	4 (3.8)	5 (11.6)	3 (4.2)	7 (7.4)	
Other	2 (5.0)	2 (1.9)	3 (7.0)	1 (1.4)	3 (3.2)	
Genotype						0.60
Homozygous delf508	21 (52.5)	57 (54.3)	19 (44.2)	36 (50.7)	49 (51.6)	
Heterozygous delf508	16 (40.0)	40 (38.1)	14 (32.6)	26 (36.6)	37 (38.9)	
Other	3 (7.5)	7 (6.7)	9 (20.9)	8 (11.3)	9 (9.5)	
Unknown	0 (0)	1 (1.0)	1 (2.3)	1 (1.4)	0 (0)	
BMI, kg/m^2						0.002
Mean (SD)	19.4 (4.4)	19.3 (3.0)	18.6 (4.8)	17.8 (3.1)	17.6 (3.1)	
Pancreatic insufficient¶	39 (97.5)	97 (92.4)	40 (93.0)	67 (94.4)	82 (86.3)	0.82

Asthma	3 (7.5)	12 (11.4)	7 (16.3)	12 (16.9)	16 (16.8)	0.51
CF-related diabetes	5 (12.5)	11 (10.5)	1 (2.3)	7 (9.9)	3 (3.2)	0.11
Other microorganisms						
MSSA‡	12 (30.0)	48 (45.7)	16 (37.2)	56 (78.9)	83 (87.4)	<0.0001
B. cepacia complex‡	1 (2.5)	4 (3.8)	1 (2.3)	4 (5.6)	5 (5.3)	0.86
H. influenzae‡	1 (2.5)	1 (1.0)	3 (7.0)	7 (9.9)	9 (9.5)	0.046
Mucoid *P. aeruginosa*‡	38 (95.0)	105 (100)	16 (37.2)	54 (76.1)	0 (0)	<0.0001
Aspergillus‡	4 (10.0)	27 (25.7)	12 (27.9)	18 (25.4)	9 (9.5)	0.006
Measures of FEV_1 taken during patient hospitalization						<0.0001
Mean (SD)	3.5 (4.0)	1.6 (2.2)	1.5 (2.4)	0.9 (1.5)	0.3 (0.9)	

Abbreviations: *B. cepacia, Burkholderia cepacia*; BMI, body mass index; CF, cystic fibrosis; *H. influenzae, Haemophilus influenzae;* FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa;* MSSA, methicillin-sensitive *Staphylococcus aureus;* SD, standard deviation

¶Missing=25

‡Cultured positive for pathogen ≥1 time during baseline period

**P*-value comparing infection groups using Kruskal-Wallis non-parametric test, analysis of variance, and chi-square test as appropriate

TABLE 2. FEV_1 % predicted at baseline, end of follow-up, and difference between first and last FEV_1 % predicted by infection group, 2007-2013

		Baseline FEV_1% predicted		**FEV_1% predicted at end of follow-up**		**Difference**	
Infection status	N	Mean	SD	Mean	SD	Mean	SD
Chronic co-infection	40	62.9	25.8	52.5	26.8	-10.5	23.8
Chronic *P. aeruginosa* alone	105	71.9	25.5	61.2	26.6	-10.7	17.6
Chronic MRSA alone	43	83.0	21.1	71.4	25.6	-11.6	15.3
Intermittent infection	71	86.3	20.6	83.8	24.9	-2.5	18.3
No MRSA/ *P. aeruginosa*	95	92.4	21.2	90.7	22.7	-1.7	14.0

Abbreviations: FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*; SD, standard deviation

TABLE 3. Adjusted mean rate of decline in FEV_1 % predicted by infection group using all FEV_1 values and clinically stable FEV_1 values, 2007-2013*

	All FEV_1 Values		**Clinically Stable FEV_1 Values¶**	
Infection Status	Regression Coefficient‡	95% CI	Regression Coefficient‡	95% CI
Chronic Co-infection	-1.06	-2.08, -0.05	-0.85	-1.84, 0.14
Chronic *P. aeruginosa* alone	-0.96	-1.74, -0.18	-0.89	-1.65, -0.14
Chronic MRSA alone	-0.66	-1.69, 0.37	-0.65	-1.65, 0.35
Intermittent infection	-0.55	-1.42, 0.33	-0.55	-1.40, 0.30
No MRSA/ *P. aeruginosa*	Ref		Ref	

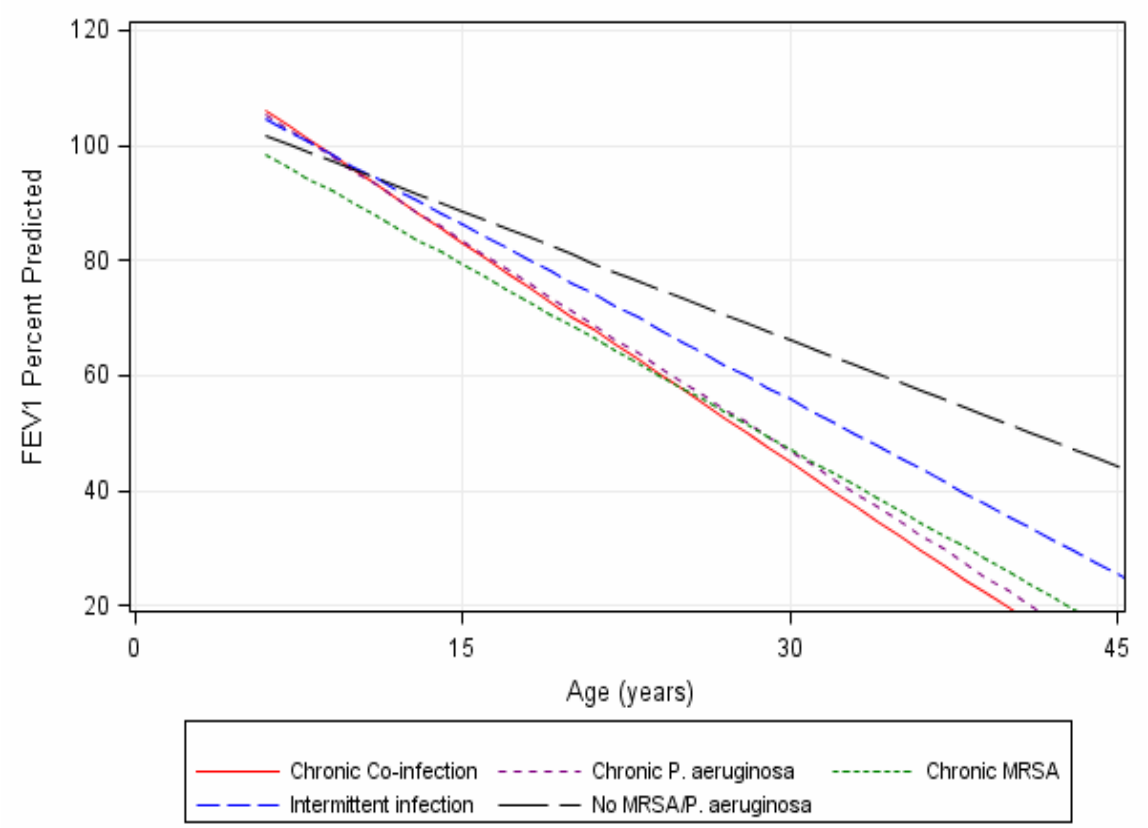
Abbreviations: CI, confidence interval; FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*

*Missing=2 due to missing values for pancreatic enzyme usage

¶2,189 (16.9%) FEV_1 values taken during hospitilization or home intravenous treatment removed from analysis

‡Model adjusted for age, baseline FEV_1 (categorized as <40% predicted, 40-<70 % predicted, 70-<100 % predicted, and >100% predicted), baseline age (categorized as <18 years and ≥18 years), sex, BMI, CF-related diabetes, presence of *Burkholderia cepacia* complex, and pancreatic enzyme use

FIGURE 3. Adjusted mean rate of decline in FEV_1 % predicted by infection status, 2007-2013*



Abbreviations: FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P.aeruginosa, Pseudomonas aeruginosa*
*Analysis described in Table 3 using all FEV_1 values was used for figure

PUBLIC HEALTH IMPLICATIONS AND POSSIBLE FUTURE DIRECTIONS

This study provides evidence that chronic co-infection of MRSA and *Pseudomonas aeruginosa* is associated with deteriorated lung function and loss of lung function over a period of one to six years among patients with cystic fibrosis. Patients with co-infection had the lowest FEV_1 % predicted at baseline and continued to lose lung function throughout the follow-up period and at a more rapid rate than patients without MRSA or *P. aeruginosa* when considering all values of FEV_1. Based on previous literature, this was the first study to examine the association between chronic co-infection and rate of lung function decline among CF patients attending a U.S. CF care center, contributing to the importance of these findings. Further research should be conducted at other CF care centers and in the broader CF population to determine whether the results of this study persist.

The public health implications of this study include the recommendation of prevention and possible treatment of chronic MRSA and *P. aeruginosa* infection among cystic fibrosis patients. The Cystic Fibrosis Foundation stresses the importance of prevention, primarily through proper hand cleaning and avoiding close contact with other cystic fibrosis patients (62). Because of the threat of cross-infection of pathogens such as MRSA and *P. aeruginosa* between patients, the Cystic Fibrosis Foundation recently updated their infection control policies and imposed new restrictions for patients when visiting their CF clinic, hospitalized, or attending CF Foundation events (62). Possible treatments of co-infection in cystic fibrosis patients have not been evaluated. There are existing guidelines for the effective treatment of chronic *P. aeruginosa* infection (63). However, guidelines do not exist for chronic MRSA infection, primarily because MRSA has only recently emerged as an important pathogen in cystic fibrosis and its effect on lung function decline has not been clear. While some studies conclude that MRSA does independently contribute to increased

lung function decline (41), other studies have suggested it is simply a marker of more severe disease (40, 42). Patients with chronic MRSA alone in this study had a lower average FEV_1 % predicted at baseline and at the end of follow-up than patients without MRSA or *P. aeruginosa*; however, patients with chronic MRSA alone did not have a significantly more rapid rate of decline in FEV_1 % predicted compared to patients without MRSA or *P. aeruginosa*. These results are similar to other previous studies that show mixed evidence surrounding the clinical impact of chronic MRSA infection. Regardless, researchers should first determine whether antibiotic treatment is appropriate and effective for patients with MRSA infection. A further need exists for randomized clinical trials and prospective studies to determine the most effective antibiotics for treatment of chronic MRSA, with and without co-infection with *P. aeruginosa* (29).

This study sheds new light on how different levels of infection with MRSA and *P. aeruginosa* are associated with rate of lung function decline, revealing that chronic co-infection with MRSA or *P. aeruginosa* is associated with lower lung function and continued loss of lung function over a period of one to six years. Researchers must further determine if this association exists in the broader CF population. Future studies examining incident co-infection and its association with lung function decline are also needed to better determine whether co-infection independently contributes to increased lung function decline. Finally, continued efforts aimed at prevention and treatment of chronic MRSA infection, with and without *P. aeruginosa* co-infection, are of great importance for the health of the CF community.

APPENDIX

TABLE 1. Adjusted mean rate of decline in FEV_1 % predicted by infection group using all FEV_1 values and clinically stable FEV_1 values for patients ≤45 years of age at baseline (N=347), 2007-2013*

	All FEV_1 Values		**Clinically Stable FEV_1 Values¶**	
Infection Status	Regression Coefficient‡	95% CI	Regression Coefficient‡	95% CI
Chronic Co-infection	-1.11	-2.14, -0.07	-0.88	-1.89, 0.13
Chronic *P. aeruginosa* alone	-0.99	-1.79, -0.20	-0.92	-1.69, -0.15
Chronic MRSA alone	-0.69	-1.74, 0.36	-0.68	-1.70, 0.34
Intermittent infection	-0.54	-1.43, 0.34	-0.54	-1.40, 0.31
No MRSA/ *P. aeruginosa*	Ref		Ref	

Abbreviations: CI, confidence interval; FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*

*Missing=2 due to missing values for pancreatic enzyme usage

¶FEV_1 values taken during hospitilization or home intravenous treatment were removed from analysis

‡Model adjusted for age, baseline FEV_1 (categorized as <40% predicted, 40-<70 % predicted, 70-<100 % predicted, and >100% predicted), baseline age (categorized as <18 years and ≥18 years), sex, BMI, CF-related diabetes, presence of *Burkholderia cepacia* complex, and pancreatic enzyme use

TABLE 2. Adjusted mean rate of decline in FEV_1 % predicted by infection group using all FEV_1 values and clinically stable FEV_1 values, 2007-2013*

	All FEV_1 Values		**Clinically Stable FEV_1 Values¶**	
Infection Status	Regression Coefficient‡	95% CI	Regression Coefficient‡	95% CI
Any co-infection§ (n=85)	-0.29	-0.97, 0.39	-0.24	-0.90, 0.42
No co-infection (n=269)	Ref		Ref	

Abbreviations: CI, confidence interval; FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa*

*Missing=2 due to missing values for pancreatic enzyme usage

¶2,189 (16.9%) FEV_1 values taken during hospitilization or home intravenous treatment removed from analysis

‡Model adjusted for age, baseline FEV_1 (categorized as <40% predicted, 40-<70 % predicted, 70-<100 % predicted, and >100% predicted), baseline age (categorized as <18 years and ≥18 years), sex, BMI, CF-related diabetes, presence of *Burkholderia cepacia* complex, and pancreatic enzyme use

§Any co-infection was defined as at least one positive culture for both MRSA and *P. aeruginosa* during the lead-in period

TABLE 3. Distribution of patient characteristics by included and excluded patients, 2007-2013

	Included Patients	Excluded Patients	
	N (%)	N (%)	*P*-value
All patients	354 (100)	304 (100)	
Baseline age, year			<0.0001
Mean (SD)	15.5 (10.0)	21.6 (13.3)	
Baseline FEV1% predicted			0.004
Mean (SD)	80.6 (25.0)	74.3 (28.3)	
Infection Status			0.54
Chronic Co-infection	40 (11.3)	29 (9.5)	
Chronic P. aeruginosa	105 (29.7)	109 (35.9)	
Chronic MRSA	43 (12.1)	34 (11.2)	
Intermittent MRSA/P. aeruginosa	71 (20.1)	59 (19.4)	
No MRSA/P. aeruginosa	95 (26.8)	73 (24.0)	
FEV_1 % predicted group			0.01
<40	28 (7.9)	41 (13.5)	
40- <70	86 (24.3)	55 (18.1)	
70- <100	151 (42.7)	102 (33.6)	
>=100	89 (25.1)	51 (16.8)	
Missing	0 (0)	55 (18.1)	
Sex			0.80
Male	184 (52.0)	161 (53)	
Female	170 (48.0)	143 (47)	
Race/ethnicity			0.28
White	321 (90.7)	266 (87.5)	
African-American	22 (6.2)	29 (9.5)	
Other	11 (3.1)	9 (3)	
Genotype			<0.0001
Homozygous delf508	182 (51.4)	132 (43.4)	
Heterozygous delf508	133 (37.6)	118 (38.8)	
Other	36 (10.2)	28 (9.2)	
Unknown	3 (0.8)	26 (8.6)	
Pancreatic insufficient	325 (91.8)	251 (82.6)	<0.0001
Missing	25 (7.1)	19 (6.3)	
Asthma	50 (14.1)	53 (17.4)	0.24
CF-related diabetes	27 (7.6)	61 (20.1)	<0.0001
Other microorganisms			
MSSA‡	215 (60.7)	184 (60.5)	0.96

B. cepacia complex‡	15 (4.2)	23 (7.6)	0.07
H. influenzae‡	21 (5.9)	21 (6.9)	0.61
Mucoid *P. aeruginosa*‡	213 (60.2)	194 (63.8)	0.34
Number of FEV_1 % predicted measurements¶			
Mean (SD)	36.6 (17.8)	13.2 (11.8)	<0.0001

Abbreviations: *B. cepacia, Burkholderia cepacia*; BMI, body mass index; CF, cystic fibrosis; *H. influenzae, Haemophilus influenzae;* FEV_1, forced expiratory volume in the first second; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa, Pseudomonas aeruginosa;* MSSA, methicillin-sensitive *Staphylococcus aureus;* SD, standard deviation

‡Cultured positive for pathogen ≥1 time during baseline period

**P*-value comparing included and excluded patients using t-test and chi-square test as appropriate

¶All FEV_1 measurements regardless if taken during a time of clinical stability

IRB Approval Document

EMORY UNIVERSITY

Institutional Review Board

TO: Maret Maliniak
Principal Investigator
Public Health

DATE: November 24, 2013

RE: **Expedited Approval**
IRB00070522
MRSA and Pseudomonas aeruginosa Coinfection on Rate of Pulmonary Function Decline in Cystic Fibrosis

Thank you for submitting a new application for this protocol. This research is eligible for expedited review under 45 CFR.46.110 and/or 21 CFR 56.110 because it poses minimal risk and fits the regulatory category F(5) as set forth in the Federal Register. The Emory IRB reviewed it by expedited process on 11/24/2013 and granted approval effective from **11/24/2013** through **11/23/2014**. Thereafter, continuation of human subjects research activities requires the submission of a renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this approval:

- A complete waiver of HIPAA authorization has been granted for the purposes of identifying cases and conducting the study
- A waiver of all elements of informed consent has been granted

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, and study design), you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you

Sam Roberts, BA CIP
Senior Research Protocol Analyst
This letter has been digitally signed