

## The changing prevalence of lower airway infections in young children with cystic fibrosis

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## AT A GLANCE COMMENTARY

### Scientific Knowledge on the Subject

Historically, *Staphylococcus aureus* pneumonia was the commonest cause of death in children with CF. Following the introduction of antimicrobial treatments, other pathogens emerged as dominant organisms in what appeared to be an age-dependent sequence with *S. aureus* and *Haemophilus influenzae* appearing early in disease followed by *Pseudomonas aeruginosa*. The association of infections with worse disease outcomes has focussed attention on treatment of these organisms to improve survival. However, most studies of infections in young children with CF rely on data obtained from upper-airways samples which may not have accurately reflected lower airways infections. Furthermore, improved care, early diagnosis and aggressive antibiotic treatment may have influenced the pattern of acquisition of lower airway pathogens and its contribution to lung disease.

### What This Study Adds to the Field:

Data from this unique longitudinal observational cohort indicate that infections with *P. aeruginosa* and *Aspergillus* species are prevalent from infancy. The presence of any individual bacterial pathogen does not predict the emergence of future dominant organisms. Reductions in the prevalence of *S. aureus* and *P. aeruginosa*, coinciding with a more aggressive treatment approach, has resulted in *Aspergillus* species becoming the commonest isolated pathogen in early life. Given the changing patterns of infection over time and the continued improvements in morbidity and mortality, the clinical significance and priority for treatment of organisms such as *Aspergillus* requires further investigation to facilitate the development of rational, future antimicrobial interventions to prevent progressive lung disease in early life.

This article has an online data supplement, which is accessible from this issue's table of content online at [www.atsjournals.org](http://www.atsjournals.org)

## ABSTRACT

Rationale: Historical studies suggest that airway infection in cystic fibrosis (CF) initiates with *Staphylococcus aureus* and *Haemophilus influenzae* with later emergence of *Pseudomonas aeruginosa*. *Aspergillus* species are regarded as relatively infrequent, late occurring infections.

Objectives: To assess the prevalence and change in prevalence of early lower airway infections in a modern cohort of children with CF.

Methods: All infants diagnosed with CF after newborn screening, participating in the AREST-CF cohort study between 2000-2018, were included. Participants prospectively underwent bronchoalveolar lavage (BAL) at 3-6 months, 1 year and annually up to 6 years of age. Lower airway infection prevalence was described. Changes in prevalence patterns were assessed longitudinally using generalized estimating equations controlling for age and repeated visits.

Measurements and Main Results: A total of 380 infants underwent 1,759 BALs. The overall prevalence and median age of first acquisition of the most common infections were: *S. aureus* 11%, 2.5yrs, *P. aeruginosa* 8%, 2.4yrs, *Aspergillus* species 11%, 3.2yrs, *H. influenzae* 9%, 3.1yrs. During the study, a significant decrease in prevalence of *P. aeruginosa* ( $p<0.001$ ) and *S. aureus* ( $p<0.001$ ) was observed with significant change towards more aggressive treatment. Prevalence of *Aspergillus* infections did not significantly change ( $p=0.669$ ).

Conclusion: *Aspergillus* species and *P. aeruginosa* are commonly present in the lower airways from infancy. The decrease in prevalence of *P. aeruginosa* and *S. aureus* since 2000, coinciding with more aggressive therapeutic approach, has resulted in *Aspergillus* becoming the most commonly isolated pathogen in young children. Further research is warranted to understand the implication of these findings.

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**Key words:** *Aspergillus*; *Pseudomonas aeruginosa*; Bronchoalveolar lavage;

## INTRODUCTION

Pathophysiological studies of cystic fibrosis (CF) lung infections have historically described a set of bacterial pathogens that are frequently acquired in an age-dependent sequence. Early infections were reported to be caused mainly by *Staphylococcus aureus* and *Haemophilus influenzae* (1, 2). Whereas, *Pseudomonas aeruginosa* was considered to appear later in the course of disease, after airways are “primed” by the earlier infections, to gradually become the predominant pathogen isolated in the respiratory secretions (2-4). More recent studies have shown that infection with *P. aeruginosa* occurs much earlier than believed previously with a median age of first culture isolation ranging between 21 and 30 months (5-7). This early occurrence of *P. aeruginosa* is also reported in current patient registries. Nonetheless, data from these registries still show *S. aureus* and *H. influenzae* to be the predominant organisms cultured in preschool patients with CF (8, 9). Other infections, routinely cultured in adult patients with CF, such as *Aspergillus* species and *Stenotrophomonas maltophilia*, are not commonly reported to occur in preschool children with CF. Importantly, many of these studies reporting the epidemiology of infections in patients with CF, including patient registries, use data from cultures obtained from upper respiratory tract secretions and not the lower airways. Upper respiratory cultures have been shown to lack sensitivity to accurately detect lower respiratory infections and are not routinely used to assess for the presence of fungal infections (5, 10-13). Furthermore, *S. aureus* and *H. influenzae* are organisms that often benignly colonize the upper respiratory tract and thus upper respiratory cultures may over-represent these pathogens (2).

Infection propagating inflammation is considered to be a major cause of the progressive lung disease seen in patients with CF (1, 14-16). Major changes in clinical practice over the past two decades e.g. newborn screening, increasingly aggressive pseudomonal eradication regimens, antibiotic stewardship, improved airway clearance techniques and novel CFTR

modulator therapies (17-19), are all likely to have had an impact on the frequency and nature of pulmonary infections. Correctly identifying changes in the epidemiology of lower respiratory infection early in life in CF is essential for understanding the potential impact of available and emerging therapeutic interventions and of infection control measures in order to prevent progressive lung damage.

The aims of this study were to describe the epidemiology of lower respiratory infection in a modern cohort of prospectively followed and aggressively treated children with CF diagnosed after newborn screening, to assess the associations between different infections and to evaluate the change in infection prevalence during the 18 years of the study.

## **METHODS**

### *Study population*

All patients participating in the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) program at Princess Margaret Hospital (Perth, WA, Australia) and Royal Children's Hospital (Melbourne, VIC, Australia) between January 2000 and February 2018, were included in the study. A more detailed version of the methods is provided in the online supplement. The AREST CF program consists of assessments at 3 months of age, 12 months of age and annually until six years of age (20). The assessments include a bronchoscopy with bronchoalveolar lavage (BAL) when clinically stable and a detailed clinical assessment. Since 2005 a chest CT scan was also performed at the time of the bronchoscopy. The program was approved by the ethics committee at each institution and written informed consent was obtained from parents prior to each annual review.



### *Bronchoscopy, BAL Collection and processing*

Following induction of general anaesthesia, a flexible bronchoscopy through a laryngeal mask airway was performed. BAL was collected in the right middle lobe and lingula (or worst affected lobe on chest CT scans) and sent for microbiological processing to test for the presence of bacteria, fungi and viruses and for analysis of markers of inflammation (20-22).

### *Clinical routines during the study period*

Chronic patient management in both centres followed international and national guidelines (17, 23-25). Clinical practice at Princess Margaret Hospital (PMH) and Royal Children's Hospital (RCH) was to attempt *P. aeruginosa* eradication when detected on BAL. At PMH the eradication protocol included two weeks of intravenous antibiotics followed by four weeks of inhaled tobramycin and oral ciprofloxacin (26). At RCH at first the protocol included a three-month treatment with inhaled tobramycin and oral ciprofloxacin for the 1<sup>st</sup> and 3<sup>rd</sup> months and since 2016 a two-month treatment with inhaled tobramycin.

At PMH, routine therapy for respiratory exacerbations included intravenous, inhaled or oral antibiotics targeting *P. aeruginosa*. Routine therapy for respiratory exacerbations at RCH was driven by previous cultures (including oropharyngeal swabs), when these were unavailable or negative, gentamicin or later ceftriaxone and/or flucloxacillin were usually used as first line empirical therapy. Importantly, children with CF followed at both centres were routinely prescribed antistaphylococcal prophylaxis (Amoxicillin-clavulanic acid) from diagnosis until 2 years of age (27).

### *Statistical analysis*

Data were summarized by standard descriptive statistics analysing the point prevalence of the most common pathogens cultured from the BAL samples at each age. Differences between groups were analysed using Kruskal-Wallis and Mann Whitney U tests as applicable.

The change in infection prevalence over the study period was investigated using generalized estimating equation (GEE) models clustering for repeated visits in the same patients and adjusting for the patients' age at BAL. Statistical significance for a linear change in prevalence over time was calculated from these models and the model output was used to plot the predicted prevalence for each pathogen over time using a polynomial fit. Mixed effects models were used to evaluate the change in treatment routines and outcomes during the study period and to evaluate if the probability of specific airway pathogens after 1 year follow-up is associated with the presence of any pathogen at baseline. All analyses were performed using Stata version 15 (StataCorp, TX, USA).

## RESULTS

Three hundred and eighty patients were included in the study. These patients underwent 1759 annual assessments in which BAL samples were obtained and available for microbiological and cytokine analysis. Patients' characteristics are presented in Table 1.

### Infection prevalence

The most commonly cultured pathogens from the BAL samples of patients included in this study were *Aspergillus* species (11%), *S. aureus* (11%), non-typeable *H. influenzae* (9%) and *P. aeruginosa* (8%). Other less commonly encountered infections in BAL samples were *Streptococcus pneumoniae* (3%), *Stenotrophomonas maltophilia* (3%), *M. catarrhalis* (1.6%), methicillin-resistant *Staphylococcus aureus* (MRSA) (0.7%), *Burkholderia* species (0.3%), *Achromobacter xylosoxidans* (0.2%) and nontuberculous mycobacteria (0.1%). Importantly, 55% of BAL samples were negative for an infection.

The median ages of first acquisition of the most commonly encountered infections were, for *Aspergillus* species 3.2yrs, for *P. aeruginosa* 2.4yrs, for *S. aureus* 2.5yrs and for *H. influenzae* 3.1yrs. The median age of first infection was not significantly different between *P. aeruginosa* and *S. aureus*, and both were significantly lower than the median age of first infection with *Aspergillus* species and *H. influenzae* ( $p < 0.05$ ) (see table 2).

There were no significant differences in the overall prevalence of infections with *P. aeruginosa* or *Aspergillus* species between medical centres (RCH and PMH), but infections with *S. aureus* and *H. influenzae* were significantly more prevalent in patients from RCH than PMH (15% vs 8%,  $p = 0.004$  and 15% vs 6%,  $p < 0.001$ , respectively) as has been previously reported (28). The point prevalence and cumulative incidence of infections according to age at BAL are presented in Figure 1. By 6 years of age 89% of patients experienced at least one lower respiratory tract infection as detected on BAL, 32% of patients experienced at least one lower airway infection with *P. aeruginosa* and 40% of patients experienced at least one infection with *Aspergillus* species, see table 2 for additional information on infection prevalence and incidence.

### **Evaluating associations between infections**

The multivariate mixed effect model evaluating risk for a future infection showed that the risk for a lower respiratory tract infection with *P. aeruginosa*, *Aspergillus* species, or *S. Aureus* was significantly increased with more than double the odds if the same pathogen was detected in the BAL in the previous year. None of the other pathogens seemed to increase the risk of later acquisition of *P. aeruginosa*. The risk for a future infection with *S. aureus* was also increased in children infected with *H. influenzae* (OR 2.2, 95% CI (1.2 – 4.0)) at baseline, but decreased for those infected with *P. aeruginosa* (OR 0.3, 95% CI 0.1-0.9) (Table 3).

## Co-infection

Out of 790 BAL cultures in which a pathogen was identified, in 24% (192) more than one pathogen was cultured (“co-infection”). A coinfection was most common with BALs positive for both *S. aureus* and *H. influenzae*, and least common for BALs positive for infections with *Aspergillus* species (table 2). Figure E1 in the online supplement presents the change of co-infections with age.

## Change in infection prevalence, 2000 - 2018

During the study period, there were significant decreases in the prevalence of lower airway infections with *P. aeruginosa* ( $p < 0.001$ ), *S. aureus* ( $p < 0.001$ ) and *H. influenzae* ( $p = 0.023$ ), as detected by BAL. *P. aeruginosa* infection prevalence gradually decreased from 12.3% to 9.8% to 5.9% and *S. aureus* infection prevalence gradually decreased from 15.7% to 13.0% to 9.1% during the years 2000-2006, 2006-2012 and 2012-2018 respectively, (Table 4). The relative magnitude of the decrease in prevalence was similar in each medical centre and the rate of decline was gradual over the study period (Figure 2). The prevalence of the other encountered lower airway pathogens (*Aspergillus* species, *S. maltophilia*, *S. pneumoniae* and *M. catarrhalis*) did not significantly change during the study period (Figure 2, and Table 4). The decrease in prevalence of *P. aeruginosa*, *S. aureus* and *H. influenzae* has resulted in *Aspergillus* species emerging as the most commonly isolated lower airway pathogen cultured in recent years.

## Change in clinical routines and outcomes, 2000 - 2018

Change in clinical routines and outcomes are presented in Tables 5 and E1. Overall, during the study period there was a significant increase in the use of chronic therapies for routine treatment of CF lung disease (azithromycin, hypertonic saline and dornase alpha) as well as increase use of antipseudomonal oral and inhaled antibiotics (ciprofloxacin and inhaled

tobramycin) (Tables 5 and E1). Intravenous antibiotic treatment (both outpatients and inpatient) which included treatment for eradication of newly acquired *P. aeruginosa* infections did not significantly change during the study period which is suggestive for a more aggressive approach targeting *P. aeruginosa* despite increasing proportions of negative cultures.

When assessing changes in outcome measures during the study period. Body mass index (BMI), forced expiratory volume in one second (FEV1) at 6 years of age and number of admissions for respiratory exacerbations did not significantly change. However, lower respiratory inflammation, including neutrophil elastase levels and neutrophil percentage in BAL samples significantly decreased during the study period (Table 5).

## DISCUSSION

In this study to better understand the epidemiology of lower respiratory infections in young children with CF, we demonstrate that lower airway infections with *P. aeruginosa*, *Aspergillus* species, *S. aureus* and *H. influenzae* are all prevalent from early infancy. We observed that the prevalence of *P. aeruginosa*, *S. aureus* and *H. influenzae* significantly decreased in the last two decades and that these changes coincided with the general increased use of oral and inhaled antipseudomonal antibiotics and inhaled mucolytic therapies. An important observation resulting from the decrease in prevalence was that *Aspergillus* species are now the most prevalent lower respiratory pathogens identified in our cohort of children. The emergence of *Aspergillus* species as the most dominant pathogens suggests that further studies should be undertaken to determine the clinical significance of this observation and the contribution of *Aspergillus* infections to early progressive lung disease.

Infections with *P. aeruginosa* begin in early childhood, and *P. aeruginosa* gradually becomes the major respiratory pathogen in patients with CF (5-7). A commonly proposed concept is that infections with *P. aeruginosa* follow earlier infections with other pathogens which “prime” the airway for colonization (2, 3). In our cohort of young children, although *P. aeruginosa* is one of the earliest encountered lower respiratory infections we show that the risk for lower airway infections with *P. aeruginosa* is not significantly increased by prior infection with *S. aureus* or *H. influenzae*.

Another major finding in our study is the significant decrease in the prevalence of lower airway *P. aeruginosa*, *S. aureus* and *H. influenzae* infections during the study period. Due to the observational nature of the study, causality cannot be assessed for this observation, but a similar decrease in incidence and prevalence of *P. aeruginosa* infections in older patients with CF, and recently also in infants diagnosed after newborn screening, has previously been reported (29-31). Our study focuses on a young cohort of children, mostly naïve to previous infections and represents most of the children in the two centres with comprehensive follow up from diagnosis following newborn screening and direct evaluation of lower airway pathogens (13). The decrease in prevalence of common bacterial pathogens was gradual and centre independent. During the study period, patient management significantly changed towards a more aggressive treatment approach with increased use of antipseudomonal antibiotics and utilization of chronic therapies. This is consistent with international guidelines (17, 27, 32) and is also evident in data from the Cystic Fibrosis Foundation patient registry (8). Other factors such as improved infection control, airway clearance techniques and antibiotic stewardship were not evaluated in this study but may have also contributed to the change in infection prevalence. These factors clearly represent a general improvement in care, in line with current international guidelines, that are likely to have contributed to the decrease in prevalence of bacterial pathogens in ours and other cohorts (29-31, 33)

*S. aureus* is often considered to be the first pathogen cultured from respiratory secretions in young patients with CF (1-3) with an overall reported prevalence of 30% to 70% (2, 8, 9). In our cohort, the median age of first infection with *S. aureus* was not significantly different from that of *P. aeruginosa* and the overall prevalence of *S. aureus* lower airway infection was only 11%. We speculate that this low prevalence in *S. aureus* infection and increased age of first acquisition in our cohort is due to the routine prescription of antibiotic prophylaxis against *S. aureus* used in both participating centres. Furthermore, lower airway sampling utilised in our study which avoids upper airway contamination seen with oropharyngeal swabs and sputum cultures may also have contributed to the lower prevalence of *S. aureus* and *H. influenzae* compared with studies that rely on oro-pharyngeal cultures (10). The antistaphylococcal prophylactic routine as well as the gradual increased use of azithromycin during the study period, may also explain the gradual decline in the prevalence of *S. aureus* and *H. influenzae* infections contrary to recent reports (8). Importantly, previous studies assessing antistaphylococcal prophylaxis raised concerns of increased incidence of overall *P. aeruginosa* infections and risk for first acquisition of *P. aeruginosa* infections in the patients receiving therapy (34, 35). however, as mentioned above, we have observed a significant decrease in *P. aeruginosa* infections during our study period despite the routine use of *S. aureus* prophylactic therapy possibly in view of an aggressive *P. aeruginosa* eradication and treatment approach.

*Aspergillus species*, and mainly *Aspergillus fumigatus*, are prevalent fungal pathogens isolated from the CF airway (36, 37). In patients with CF, they are usually regarded as a late occurring pathogen, possibly after frequent exposure to broad-spectrum antibiotic therapy (3, 38, 39). Surprisingly therefore, our study demonstrates that *Aspergillus* species are the commonest organism cultured, usually as an isolated pathogen, from the lower respiratory airways of young patients with CF. Other, recent studies, that have evaluated lower airway

samples in pediatric patients with CF, also reported a higher prevalence of *Aspergillus* infections than expected (40, 41) strongly suggesting that our observations are generalizable in the context of modern approaches to CF therapy. These studies evaluated lower airways whereas most centres routinely use oropharyngeal samples for pathogen surveillance in preschool children with CF. Oropharyngeal samples are not routinely processed for fungal pathogens and lack sensitivity for their detection (40, 41).

Based on our longitudinal observations, the dominance of *Aspergillus* species may be explained by the declining prevalence of other common bacterial pathogens and increased use of antibiotics. However, with the relatively common isolation of *Aspergillus* species at 3 months of age and overall stable prevalence during the 18 years of the study despite the increase use of antibiotics, it is possible that *Aspergillus* infection might be inherent to the pathophysiology of early CF lung disease as previously suggested (2, 42, 43). Since we have previously reported that *Aspergillus* species are associated with lower airway neutrophilic inflammation in young children (44), the role of these organisms in the progression of early lung disease requires further investigation. Currently, no guidelines recommend routine treatment of *Aspergillus* infection and therapy of *Aspergillus* infections is aimed largely at patients meeting criteria for a diagnosis of allergic bronchopulmonary aspergillosis.

However, several studies suggest that respiratory infections with *Aspergillus* are associated with worse clinical outcomes (36, 41, 44-47) and that treatment might be beneficial (48). Thus, there is a need to improve fungal surveillance and culturing detection techniques in young children to better understand the prevalence and clinical significance of early *Aspergillus* infections in order to develop appropriate interventions.

The study design which includes annual lower respiratory BAL cultures may have underestimated the prevalence of transient infections. However, the large number of patients included and the longitudinal design are likely to have provided robust estimates of



prevalence of persistent infection, particularly since the trends were similar in two geographically separated centres. Patients participating in the study were routinely prescribed anti-staphylococcal prophylaxis in the first 2 years of life. Hence, our results may not reflect infection rates in populations with infrequent use of antistaphylococcal antibiotics.

The main strengths of the study are the large number of patients included from two geographically distinct CF centres over nearly two decades, high participation rates, standardised sampling of lower respiratory secretions and the longitudinal design. This has enabled us to accurately describe the prevalence of *lower* respiratory pathogens in young patients with CF that is essential in order to best understand the inflammatory responses to lung infections in early life (1, 13, 15, 16).

Whilst our observations are the result of a unique surveillance approach, the conclusions are likely to be generalizable. The decline in prevalence of common pathogens that we have observed in young children is also reported for older patients from international data registries. Importantly, our data explain the emergence of *Aspergillus* species as common pathogens. Furthermore, the changing patterns of infection have been associated with increased antibiotic use consistent with international recommendations and as reported in national patient registries.

The clinical significance of the dominance of *Aspergillus species*, requires further investigation in order to develop rational, antimicrobial interventions aimed at preventing progressive lung disease in young children with CF.

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**TABLES**

Table 1. Study population characteristics at the annual reviews

Characteristic	
Study participants	380
Gender (Male, %)	185 (49)
Pancreatic sufficiency, n (%)	57 (15)
Homozygous Delta F508, n (%)	184 (48)
Meconium ileus, n (%)	64 (17)
Patients per centre, PMH/RCH	214/166
Total N annual visits (with BAL)	1759
N visits at assessments age 3 months	268
N visits at assessments age 12 months	293
N visits at assessments age 2 years	289
N visits at assessments age 3 years	268
N visits at assessments age 4 years	237
N visits at assessments age 5 years	217
N visits at assessments age 6 years	187
N visits per patient, median (range)	5 (1-7)
Age at visit in years, median (range)	2.9 (0.2 – 6.7)

PMH – Princess Margaret Hospital; RCH – Royal Children’s Hospital; BAL – bronchoalveolar lavage

**Table 2.** Specific prevalence characteristics of the most commonly cultured pathogens

	<i>P. aeruginosa</i>	<i>Aspergillus</i> sp.	<i>H. influenzae</i>	<i>S. aureus</i>
Total number of positive BAL's	143/1759 (8%)	192/1759 (11%)	163/1759 (9%)	199/1759 (11%)
Co-infections <i>P. aeruginosa</i>	-	23/192 (12%)	23/163 (14%)	27/199 (14%)
Co-infections <i>Aspergillus</i> sp.	23/143 (16%)	-	21/163 (13%)	24/199 (12%)
Co-infection <i>H. influenzae</i>	23/143 (16%)	21/192 (11%)	-	44/199 (22%)
Co-infection <i>S. aureus</i>	27/143 (19%)	24/192 (13%)	44/163 (27%)	-
Co-infection any pathogen	60/143 (42%)	65/192 (34%)	86/163 (53%)	93/199 (47%)
Median age first acquisition, yrs (interquartile range) *	2.4 (1.1-3.9)	3.2 (2.0-4.9)	3.1 (2.1-4.3)	2.5 (1.1-4.0)
Prevalence at age 6 yrs	20/187 (11%)	33/187 (18%)	26/187 (14%)	27/187 (14%)
Cumulative incidence by age 6 yr	86/266 (32%)	106/266 (40%)	100/266 (38%)	107/266 (40%)

Co-infection is defined positive if both pathogens were cultured in samples taken from the same BAL procedure.. See figure E1 in the online supplement for analysis of co-infections specified by age. Cumulative incidence applies to 266 children who reached the age of 6 or more at time of analysis. In the calculation of cumulative incidence missing BALs were considered negative. \*Significance Kruskal-Wallis for difference in distribution in age of acquisition of first infection between any of the pathogens:  $p < 0.001$ , significance Mann-Whitney U test *P. aeruginosa* vs. *Aspergillus*:  $p=0.007$ , *P. aeruginosa* vs. *H. Influenzae*:  $p=0.012$ , *P. aeruginosa* vs. *S. aureus*:  $p=0.675$ , *Aspergillus* vs. *H. Influenzae*:  $p=0.524$ , *Aspergillus* vs. *S. aureus*:  $p=0.001$ , *H. Influenzae* vs. *S. aureus*:  $p=0.006$ . BAL – bronchoalveolar lavage; yrs – years.

**Table 3.** Association of pathogen at baseline with pathogens at 1-year follow-up

	<i>P. aeruginosa</i> after 1-year follow-up	<i>Aspergillus sp.</i> after 1-year follow-up	<i>H. influenzae</i> after 1-year follow-up	<i>S. aureus</i> after 1-year follow-up
Pathogen at baseline:	OR (CI) p-value	OR (CI) p-value	OR (CI) p-value	OR (CI) p-value
<i>P. aeruginosa</i>	2.4 (1.1 – 5.1) p=0.024	1.3 (0.7 – 2.5) p=0.357	0.5 (0.2 – 1.2) p=0.146	0.3 (0.1 – 0.9) p=0.023
<i>Aspergillus sp.</i>	1.7 (0.9 – 3.0) p=0.084	2.4 (1.2 – 4.5) P=0.008	0.9 (0.4 – 1.7) p=0.721	0.7 (0.3 – 1.5) p=0.353
<i>H. influenzae</i>	1.3 (0.7 – 2.6) p=0.381	1.1 (0.6 – 2.1) p=0.713	1.4 (0.7 – 2.8) p=0.373	2.2 (1.2 – 4.0) p=0.010
<i>S. aureus</i>	1.3 (0.7 – 2.3) p=0.451	0.9 (0.5 – 1.7) p=0.874	1.3 (0.2 – 1.2) p=0.146	2.4 (1.3 – 4.6) p=0.008

Results from 4 separate multivariable mixed effects models in which multiple 1-year follow-up periods per patient were included, the correlation within the same patient was accounted for by the within-person random effect covariance matrix. All respiratory pathogens at baseline included as possible predictors to assess their independent predictive ability, models were corrected for age and pancreatic sufficiency.



**Table 4.** Infection prevalence, according to the year of study and patients' age at time of bronchoscopy (repeat visits included).

	2000-2006				2006-2012				2012- 2018			
	0-2yr n=92	3-4yr n=57	5-6yr n=22	All n=171	0-2yr n=272	3-4yr n=239	5-6yr n=211	All n=722	0-2yr n=304	3-4yr n=236	5-6yr n=326	All n=866
P. aeruginosa % (n)	9.8% (9)	14.0% (8)	18.2 % (4)	<b>12.3%</b> <b>(21)</b>	7.8% (21)	10.9% (26)	11.4% (24)	<b>9.8%</b> <b>(71)</b>	4.3% (13)	5.9% (14)	7.4% (24)	<b>5.9%</b> <b>(51)</b>
Aspergillus sp. % (n)	4.3% (4)	12.3% (7)	13.6 % (3)	<b>8.2%</b> <b>(14)</b>	6.6% (18)	14.2% (34)	13.7% (29)	<b>11.2%</b> <b>(81)</b>	3.6% (11)	11.0% (26)	18.4% (60)	<b>11.2%</b> <b>(97)</b>
S. aureus % (n)	13.0 % (12)	19.3% (11)	18.2 % (4)	<b>15.7%</b> <b>(27)</b>	8.1% (22)	15.0% (36)	17.1% (36)	<b>13.0%</b> <b>(94)</b>	4.3% (13)	10.6% (25)	12.3% (40)	<b>9.1%</b> <b>(78)</b>
H. influenzae % (n)	6.5% (6)	7.0% (4)	0% (0)	<b>5.8%</b> <b>(10)</b>	5.1% (14)	16.3% (39)	13.2% (28)	<b>11.2%</b> <b>(81)</b>	1.3% (4)	9.7% (23)	13.8% (45)	<b>8.3%</b> <b>(72)</b>

n - number of annual assessments with bronchoalveolar lavage.

**Table 5.** Evaluation of the effect of calendar year on changes in treatment routines and outcomes during the study period (2000 to 2018).

Management	0-2yr	3-4yr	5-6yr	All
Routine medication use in the year prior to the annual assessment	(n=366 assessments in 208 patients)	(n=397 assessments in 238 patients)	(n=501 assessments in 239 patients)	(n=1264 assessments in 285 patients)
Odds Ratio - per 1 year increase in calendar year (95% confidence interval), p value				
Azithromycin use	<b>1.7 (1.1-2.9), 0.028</b>	<b>1.5 (1.1-2.0), 0.015</b>	<b>1.3 (1.1-1.6), 0.013</b>	<b>1.3 (1.1-1.5), &lt;0.001</b>
Hypertonic saline use	NA	1.3 (1.0-1.7), 0.098	1.2 (1.0-1.4), 0.089	<b>1.2 (1.1-1.4), 0.010</b>
Dornase alpha use	<b>2.1 (1.1-4.1), 0.028</b>	<b>1.8 (1.3-2.4), 0.001</b>	<b>2.1 (1.6-2.8), &lt;0.001</b>	<b>1.7 (1.5-2.0), &lt;0.001</b>
Ivacaftor use	NA	NA	NA	NA
Ivacaftor-lumacaftor use	NA	NA	NA	NA
Oral Augmentin use	1.1 (1.0-1.3), 0.081	1.0 (0.9-1.1), 0.851	0.9 (0.8-1.0), 0.051	1.0 (0.9-1.1), 0.825
Inhaled tobramycin use	<b>1.3 (1.1-1.5), 0.008</b>	<b>1.2 (1.1-1.3), 0.008</b>	<b>1.1 (1.0-1.2), 0.021</b>	<b>1.1 (1.1-1.2), &lt;0.001</b>
Ciprofloxacin use	NA	1.1 (0.9-1.3), 0.311	<b>1.1 (0.9-1.3), 0.069</b>	<b>1.1 (1.0-1.2), 0.026</b>
Use of intravenous antibiotics	<b>1.1 (1.0-1.2), 0.046</b>	0.9 (0.8-1.0), 0.297	1.0 (0.9-1.2), 0.965	1.0 (0.9-1.1), 0.648
<hr/>				
Outcome	0-2yr	3-4yr	5-6yr	All
Inflammation	(n=555 measurements in 284 patients)	(n=483 measurements in 271 patients)	(n=518 measurements in 244 patients)	(n=1556 measurements in 320 patients)
Difference - per one year increase in calendar year (95% confidence interval), p value				
Interleukin 8 levels (log scale)	0.03 (-0.01-0.06), 0.064	-0.01 (-0.04-0.04), 0.945	0.01 (-0.03-0.05), 0.656	0.01 (-0.02-0.03), 0.581
Neutrophil elastase (log scale)	<b>-0.05 (-0.07--0.03), &lt;0.001</b>	<b>-0.06 (-0.1--0.02), 0.004</b>	<b>-0.06 (-0.1--0.01), 0.020</b>	<b>-0.05 (-0.1--0.03), &lt;0.001</b>
% neutrophil on BAL cell count	<b>-1.7 (-2.1--1.3), &lt;0.001</b>	<b>-1.6 (-2.2--1.0), &lt;0.001</b>	<b>-0.9 (-1.6--0.23), 0.009</b>	<b>-1.5 (-1.9--1.1), &lt;0.001</b>
Admission for respiratory exacerbations	0.06 (-0.12-0.23), 0.521 (n=281 assessments in 161 patients)	-0.13 (-0.71-0.44), 0.647 (n=302 assessments in 198 patients)	0.10 (-0.08-0.28), 0.287 (n=247 assessments in 185 patients)	-0.01 (-0.13-0.13), 0.965 (n=833 assessments in 271 patients)
FEV1 at 6 years of age (z-score)	-	-	-	-0.05 (-0.13-0.04), 0.273 (n=132 patients)
BMI at 6 year of age	-	-	-	0.02 (-0.08-0.12), 0.657 (n=152 patients)

Results from mixed effects logistic regression models for assessment of patient management and mixed effects linear regression models for assessment of patients' outcome. analyses are clustered for repeated measures in the same patient and corrected for pancreatic insufficiency, age, gender and homozygosity for ΔF508. NA –number of patients too small to perform analyses (use of ciprofloxacin under 2 years, n=5; use of hypertonic saline under 2 years, n=2; use of ivacaftor under 6 years, n=2; use of ivacaftor-lumacaftor under 6 years n=0). BAL – bronchoalveolar lavage; FEV1 – forced expiratory volume in one second; BMI – body mass index.

## Figure Legends

**Figure 1. A.** point prevalence and **B.** Cumulative incidence, of the 7 most commonly cultured lower respiratory tract infections by patients' age at the time of the bronchoscopy.

**Figure 2.** Change in lower respiratory infection prevalence during the study period (2000 to 2018) for the 7 most commonly cultured lower respiratory tract infections, adjusted for age and repeated visits using the output of a GEE model plotted with date of study visit as a fractional polynomial (twoway fptest procedure in Stata). Coefficient of the GEE models for a linear trend over time: *P. aeruginosa*, -0.09, 95% CI -0.14--0.04 (p<0.001); *S. aureus*, -0.09, 95% CI -0.13--0.05 (p<0.001); *Aspergillus* species, -0.01, 95% CI -0.05-0.03 (p=0.669); *H. influenzae*, -0.04, 95% CI -0.08--0.01 (p=0.023); *S. maltophilia* -0.03, 95% CI -0.14-0.02 (p=0.529); *S. pneumoniae*, 0.01, 95% CI -0.06-0.07 (p=0.874); *M. catarrhalis*, 0.03, 95% CI -0.08-0.14 (p=0.571).

Figure 1

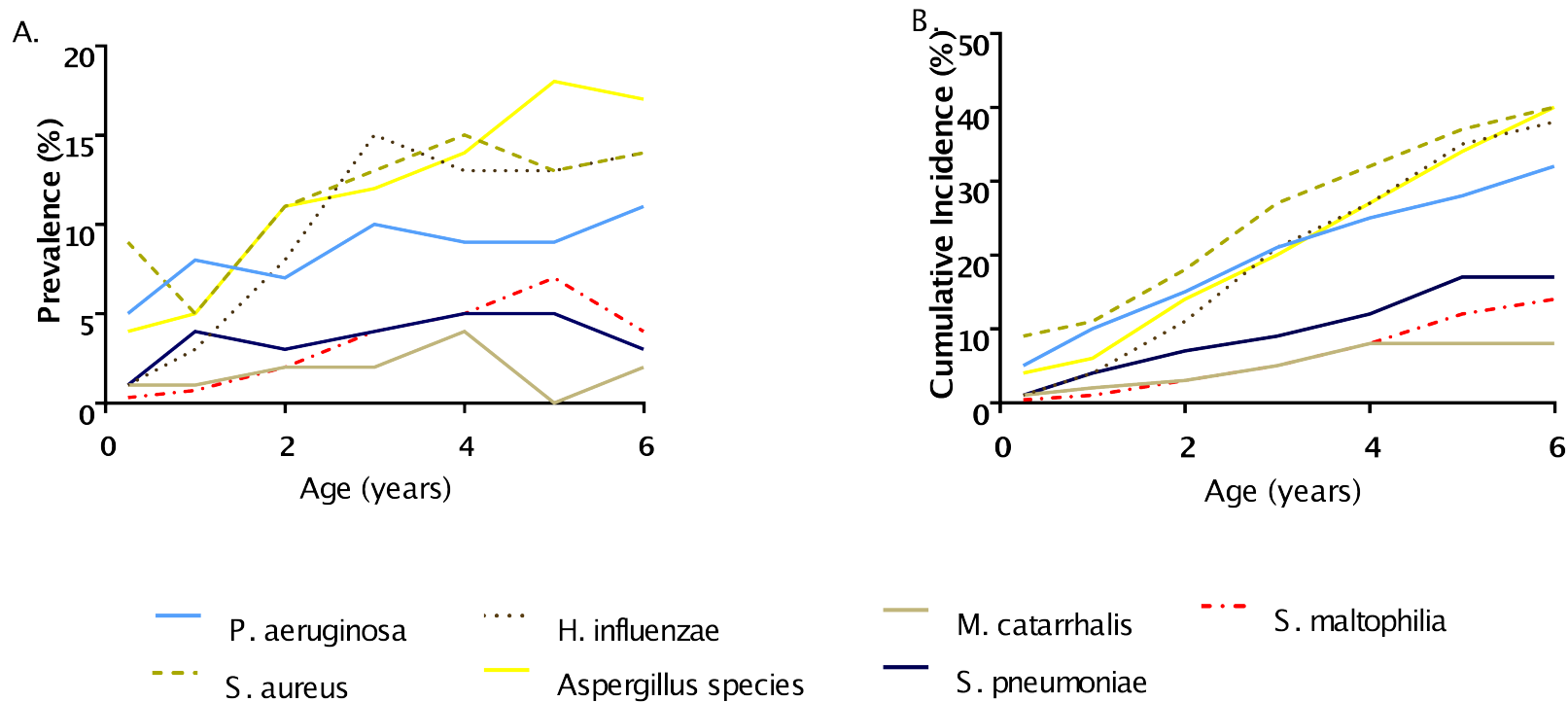
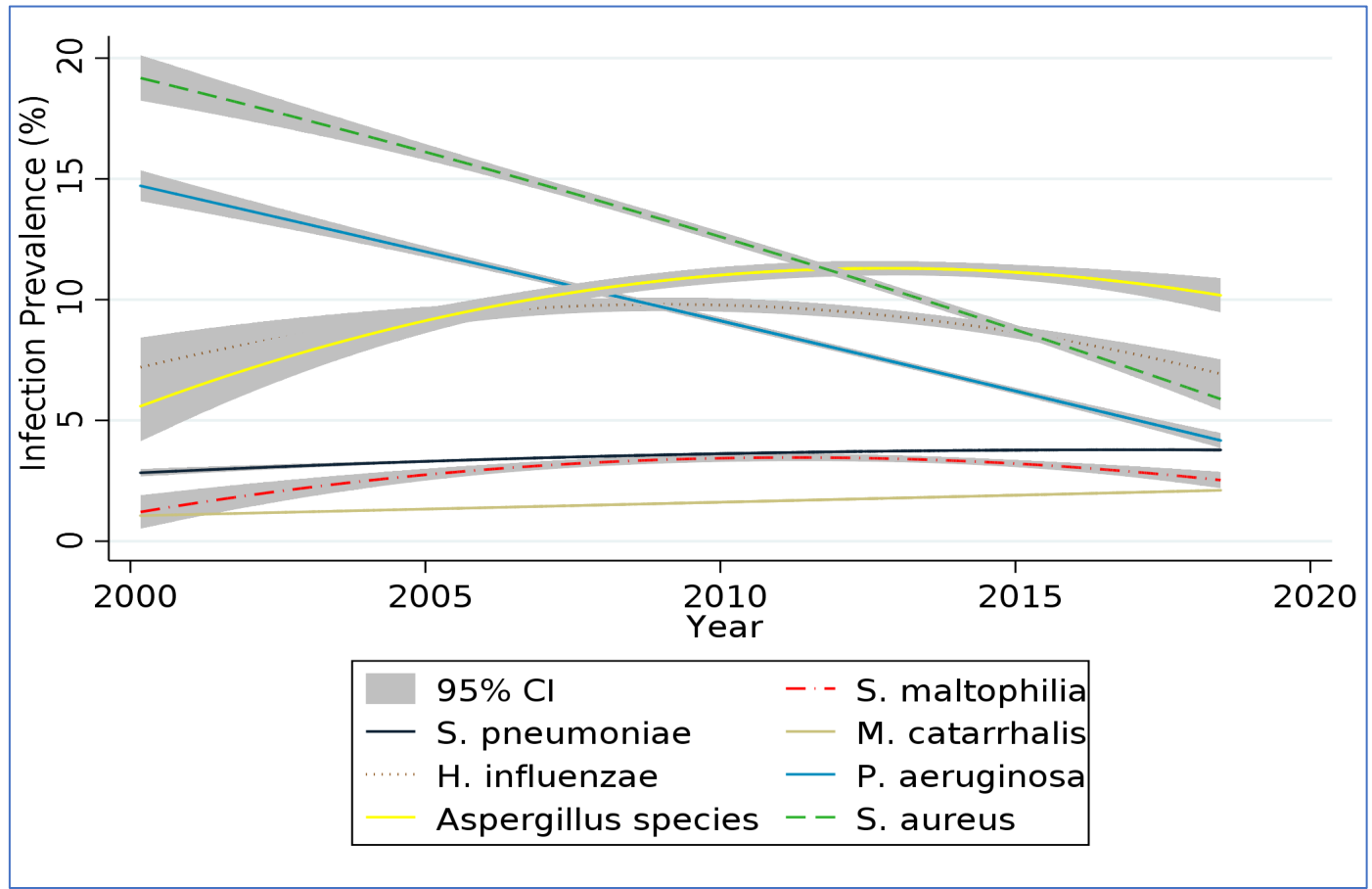


Figure 2



**The changing prevalence of lower airway infections in young children with cystic  
fibrosis**

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Ranganathan, Stephen M Stick, Daan Caudri on behalf of AREST CF

**Online Data Supplement**

## **METHODS**

### *Study population*

All patients participating in the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) programme at Princess Margaret Hospital (Perth, WA, Australia) and Royal Children's Hospital (Melbourne, VIC, Australia) between January 2000 and February 2018, were included in the study. The AREST CF program is a prospective cohort study recruiting patients with CF, all but 3 children included were diagnosed following NBS. The program consists of annual assessments at 3 months of age, 12 months of age and annually until six years of age, as previously described (1). The annual assessments include a bronchoscopy with bronchoalveolar lavage (BAL) performed under general anaesthesia when clinically stable and a detailed clinical assessment including a medication questionnaire. A detailed clinical assessment was also performed at each routine clinical visit. Since 2005 a volumetric controlled chest CT scan was also performed at the time of the bronchoscopy. The program was approved by the ethics committee at each institution and written informed consent was obtained from parents prior to each annual review.

### *Bronchoscopy, BAL Collection*

Following induction of general anaesthesia and chest CT scanning, a flexible bronchoscopy through a laryngeal mask airway was performed. Suction of pulmonary secretions was delayed until the tip of the bronchoscope was below the level of the carina to avoid upper airway contamination. BAL was collected in the right middle lobe and lingula (or worst affected lobe as seen on chest CT scans).

### *BAL Processing*

BAL samples were sent for analysis of markers of inflammation (free neutrophil elastase activity, cell count with differential and interleukin-8 concentration) and standard

microbiological processing to test for the presence of bacteria, fungi and viruses as previously described (1-3). Briefly, for microbiological processing, non-homogenised nor diluted BAL samples were used. A drop (10 – 50ul) of BAL fluid was inoculated onto agar plates and spread for single colonies. BAL samples were cultured in the following manner: Blood agar (Oxoid, Thermo Fisher, Melbourne, Australia), Cysteine lactose electrolyte deficient agar (Oxoid, Thermo Fisher, Melbourne Australia) and blood agar with ticarcillin (for resistant *P. aeruginosa* identification) were incubated aerobically at 35°C for 48hrs in a CO<sub>2</sub> incubator; For the detection of *Burkholderia cepacia*, at first a PC plate (selective plate for *B.cepacia*) incubated at 35°C for 48hrs in a CO<sub>2</sub> incubator, then at room temperature for another 24hrs was used, or a *Burkholderia cepacia* selective agar (Becton Dickinson, Sydney, Australia) incubated aerobically for 48 hours at 35°C in 5% CO<sub>2</sub> was used; Mannitol salt agar (Oxoid, Thermo Fisher, Melbourne, Australia) for the detection *Staphylococcus aureus* was incubated at 35°C for 48hrs in an aerobic atmosphere. For isolation of *Haemophilus influenzae*, Fildes agar (PathWest Microbiology, Perth, Australia) incubated at 35°C for 48hrs in an anaerobic environment or Chocolate agar (Oxoid, Thermo Fisher, Melbourne, Australia) were used; Colistin Nalidixic acid agar (Oxoid, Thermo Fisher, Melbourne, Australia) was used for the detection of *Streptococcus pneumoniae*; Sabourauds' agar with chloramphenicol (Sigma, Aldrich, Sydney, Australia) was used for detection of yeast/fungi and incubated at 35°C for 48hrs in a CO<sub>2</sub> incubator, then at 28°C for another 14 days;

Bacteria were identified by colony morphology, gram stain and biochemical tests including, Catalase (Hydrogen Peroxide 3%) (Oxoid, Thermo Fisher, Melbourne, Australia) Oxidase (Oxoid Thermo Fisher, Melbourne, Australia), Phadebact™ (MKL Diagnostics, Sollentuna, Stockholm) Staphylococcal latex test, API™ (BioMerieux, Marcy-l'Étoile, France) and automated identification by Vitek™ (BioMerieux, Marcy-l'Étoile, France). Culturing media and detection assays did not significantly change during the study period.



### *Clinical routines during the study period*

Management of patients with cystic fibrosis has changed considerably worldwide during the study period in all aspects of patients' care (infection control, nutrition, airway clearance, aggressive management of bacterial infections and CFTR modulator therapy). These changes have also affected the clinical practice in both participating centres. Overall, both centres participating in the study followed international and national guidelines for chronic management of patients with CF (4-7). Data regarding medications use for patients participating in this study were recorded in each annual assessment and also additionally at each routine clinical visit. Data regarding infection control and manual airway clearance techniques used were not collected in this study. Data on number of admissions and IV antibiotic courses has been collected from the Australian CF Data Registry.

Several clinical routines at participating centres should be specifically noted. Clinical practice at Princess Margaret Hospital (PMH) and Royal Children's Hospital (RCH) was to attempt eradication of *P. aeruginosa* when detected on BAL. At PMH the standard eradication protocol includes two weeks of intravenous antibiotics followed by four weeks of inhaled tobramycin and oral ciprofloxacin, with an eradication rate of 77%, as previously shown (8). At RCH at first the protocol included a three-month treatment with inhaled tobramycin and oral ciprofloxacin for the 1<sup>st</sup> and 3<sup>rd</sup> months and since 2016 a two-month treatment with inhaled tobramycin.

At PMH routine therapy for respiratory exacerbations, even without evidence for a lower airway *P. aeruginosa* infection, often included intravenous, inhaled or oral antibiotics targeting *P. aeruginosa*, with treatment choice depending on the severity of the exacerbation. Routine therapy for respiratory exacerbations at RCH was driven by previous cultures, when these were unavailable or negative, gentamicin or later ceftriaxone and/or flucloxacillin were

usually used as first line empirical therapy. Children with CF followed at both centres were routinely prescribed antistaphylococcal prophylaxis (Amoxicillin-clavulanic acid) from diagnosis until 2 years of age.

### *Statistical analysis*

Data were summarized by standard descriptive statistics analysing the point prevalence of the most common pathogens cultured from the BAL samples at each age. Differences between groups were analysed using Kruskal-Wallis and Mann Whitney U tests as applicable.

The change in infection prevalence over the study period was investigated using generalized estimating equation (GEE) models clustering for repeated visits in the same patients and adjusting for the patients' age at BAL. Statistical significance for a linear change in prevalence over time was calculated from these models and the model output was used to plot the predicted prevalence for each pathogen over time using a polynomial fit. Mixed effects models adjusted for repeated visits in the same patients and corrected for pancreatic insufficiency, age, gender and homozygosity for  $\Delta F508$  were used to evaluate the change in treatment routines and outcomes during the study period. For change in treatment routines, use of medications as reported by patients was analysed both as dichotomous (medication use in the year prior to the annual assessment) and continuous (number of times the patient reported the use of the medication in the year prior to the annual assessment) variables. In the manuscript, analyses of dichotomous outcome was presented unless analyses of outcomes as continuous variables showed additional or different information.

To evaluate if the probability of specific airway pathogens after 1 year follow-up is associated with the presence of any pathogen at baseline, a prediction model was built using mixed effects logistic regression models correcting for age and pancreatic sufficiency. In these models, each available follow-up period with paired data on the BAL at baseline and 1

year later was considered in the analysis. All analyses were performed using Stata version 15 (StataCorp, TX, USA).

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**Table E1.** Use of pulmonary therapies in the AREST-CF cohort during the study period, 2000-2018.

Year of annual assessment	2000	2004	2008	2012	2016	2017
Medication <sup>#</sup>						
Azithromycin use	-	0%	5.7%	10.4%	16.1%	29.6%
Hypertonic saline use	-	0%	4.8%	2.6%	6.3%	9.9%
Dornase alpha use	-	0%	2.9%	10.4%	25.9%	35.8%
Oral Augmentin use	-	85.7%	67.6%	80.9%	79.5%	83.9%
Inhaled Tobramycin use	-	4.7%	8.6%	12.2%	27.6%	44.4%
Ciprofloxacin use	-	0%	2.8%	6.1%	5.4%	9.9%

<sup>#</sup>% of patients reporting to have used the medication in the year prior to the annual assessment.

**Figure E1.** Coinfection rate according to age of patient at the time of the annual assessment and the specific infection cultured (n – number of annual assessments with a positive culture for the specified infection).

Figure E1

