Original Article

Review of a 7-Year Record of the Bacteriological Profile of Airway Secretions of Children with Cystic Fibrosis in North India

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Abstract

Background: Cystic fibrosis (CF) is now a recognised entity in India, with prevalence rates between 1/10,000 and 1/50,000. However, no data were available with regard to the profile of respiratory pathogens in the Indian setting. **Materials and Methods:** The records of respiratory secretion bacterial cultures of children with CF in a tertiary care hospital in North India from January 2010 to December 2016 were reviewed. Culture data were evaluated; the organisms were noted and their antimicrobial susceptibilities were analysed. The microbiological profile and antimicrobial susceptibility pattern of CF patients were evaluated. **Results:** A total of 445 samples from 146 children were processed, of which 246 (55%) samples showed bacterial growth. Mixed infections 48 (19.5%) were common in older children. Children aged 3–6 months (62.5%) showed the highest culture positivity. The most commonly isolated organisms were *Pseudomonas aeruginosa* (52.6%) and *Staphylococcus aureus*. Children with initial cultures positive for *P. aeruginosa* had 55% of their subsequent cultures showing polymicrobial infections. *P. aeruginosa* was most susceptible to ciprofloxacin (89%) and piperacillin-tazobactum (88%). Among the staphylococcal isolates, 38% were methicillin-resistant *S. aureus* (MRSA). The percentage of MRSA increased from 66% in 2010 to 75% in 2012, followed by a decline to 24% in 2016. **Conclusions:** The pattern of airway colonisation in the Indian setting is different from the Caucasian population, and *P. aeruginosa* and *Burkholderia cepacia complex* appear early. Colonisation with *P. aeruginosa* benefits from therapy. In case of infection, care must be taken while initiating empiric therapy. It should be based on local antibiograms to prevent the emergence of resistant microbes.

Keywords: Bacteriological profile, cystic fibrosis, India

Introduction

Cystic fibrosis (CF) is a multisystem disease inherited in an autosomal recessive manner. Mutations in the CF transmembrane regulator gene lead to CF.[1] The disease is characterised by an increase in the viscosity of secretions. The mucoid secretions clog the airways of the respiratory tract, increasing the number and severity of infections and decreasing the penetration of antimicrobials.[2] In addition, a dysfunctional immune system and impaired mucociliary defence mechanisms allow persistent bacterial colonisation.[3] The injudicious and excessive use of antimicrobials leads to the selection of multidrug-resistant organisms (MDRO). These children bear this dual brunt which leads to structural lung disease predisposing them to infections, thus snowballing into a vicious cycle of chronic lung disease and persistent infections with MDRO.[4,5]

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Although CF was initially thought to be a disease of the Caucasians, it has now been identified around the world including Asians and African–Americans.[6] There is a dearth of literature regarding CF in India. The first case of CF in India was reported from our centre 1968, and several cases have been reported over the past few decades.[7] Studies revealed that in the migrant Indian population, the incidence of CF is 1/40,000[8] in the USA and 1/10,000 in the UK.[9,10] It

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is estimated that the prevalence of CF in Indians is between 1/40,000 and 1/100,000.[11] With increased awareness about the presence of this disease in India and better availability of diagnostic tests, the number of CF patients identified is on the rise. Therapeutic advances and increase in the life expectancy of CF patients lead to a number of challenges, especially related to recurrent respiratory infections. Colonisation or infection risk increases with age.[12] The bacteriological profile of lung infections can play a vital role in preventing the spread of MDRO. The antibiograms of CF children can further aid in the management of these patients allowing the selection of appropriate empirical therapy.

Microorganisms responsible for respiratory tract infections in CF children were initially presumed to be *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*. Other pathogens were considered extremely rare and infections caused by them were thought to be nosocomially acquired.[13] Recent studies have shown that in addition to the above-mentioned organisms, non-fermenters such as *Burkholderia cepacia* complex (Bcc) and *Stenotrophomonas maltophilia*, non-tuberculous mycobacterium species, *Achromobacter xylosoxidans* and Gram-negative bacteria such as *Klebsiella pneumoniae* are also important airway pathogens in CF patients.[14]

The microbiota of the airways in CF children is dynamic and changes with time. To determine the epidemiology of respiratory tract infections in these children, a comprehensive study needs to be done over a long period of time.[5] Since data of this sort from India are lacking, we have attempted to study the microbiological profile and trends in antibiograms of respiratory tract infections in CF children over the past 7 years.

Materials and Methods

Study design

We have reviewed the records of respiratory secretion bacterial cultures of children with CF being followed up in a tertiary care hospital in North India. The samples included sputum, cough swab, induced sputum, bronchoalveolar lavage (BAL) and endotracheal aspirate (ETA) from patients. The objective was to study the bacteriological profile of respiratory specimen cultures in children with CF in a resource-limited setting. Laboratory records from January 2010 to December 2016 were examined, and culture data of all children with a diagnosis of CF during this period were evaluated. All data with respect to the patients enrolled at our cystic fibrosis clinic were analysed using Microsoft Excel 2014. The patient's details, including age, sex, patient identity number, date of investigation, sample sent and culture results were noted. In case of positive cultures, organisms isolated were noted and their antimicrobial susceptibilities were analysed to determine drug resistance among CF patients. All samples were matched with their respective patient identity numbers to identify the patients' subsequent cultures. All cultures from each patient were sequentially studied to see the trend in infections over the years. Informed consent of patients was taken at the time of examination and testing.

Data analysis

Data were tabulated using Microsoft Excel® 2014 (Microsoft®, USA), graphs were prepared using MATLAB and the analysis was performed to evaluate the microbiological profile and antimicrobial susceptibility pattern of CF patients.

Results

Children with CF are managed in the paediatric pulmonology unit of our institution, in collaboration with the department of medical microbiology, radiodiagnosis and imaging, as well as paediatric gastroenterology and nutrition services. Standard guidelines recommended by the CF foundation (CFF) are followed, with some local adaptations to offset cost and accessibility issues. During the period before 2013, cultures were obtained from children presenting with CF exacerbations or diagnosed for the first time. From 2013 onwards, samples were obtained for culture during follow-up visits (usually once in 2–3 months) even in asymptomatic children (surveillance culture), during acute exacerbation and at the end of therapy for acute exacerbations. In addition, culture samples were obtained from children showing poor weight gain.

A total of 445 samples from 146 children were processed during the 7-year period. Nearly half (51.6% [230/445]) of the samples were throat swabs, 28% (125/445) were sputum, 7.4% (33/445) were induced sputum, 6.5% (29/445) were ETA, 5.5% (24/445) were BAL and 1% (4/445) were cough swabs. Maximum positivity was seen in 87.5% (21/24) of ETAs followed by BAL 75% (3/4) and 75% (3/4) in cough swabs. Throat swabs gave the lowest positivity of 39% and were the least preferred specimen; sputum samples showed a good positivity rate of 68%. Considering the difficulty in obtaining sputum or BAL samples in the paediatric population, collection of cough swabs would be a suitable alternative. Samples from 95 (65%) males and 51 (35%) females were received, of which 199 (45%) samples were sterile and 246 (55%) samples showed bacterial growth (including polymicrobial) in 63/95 (66%) males and 32/51 (63%) females. The highest culture positivity was observed for the age group of 3–6 months (62.5%), and majority of them, i.e., 27/44 (61%) showed culture positivity at the very first hospital visit [Table 1]. This was followed by 9/16 (56%) of the children aged 12–24 months showing culture positivity at the first hospital visit. Patients between 6 and 12 months, 12–24 months and >24 months showed 5/16 (31%), 9/53 (55%) and 9/18 (50%) culture positivity at the first visit.

P. aeruginosa was the most commonly isolated organism across all age groups over all the years [Figure 1]. The second most commonly isolated organism was *S. aureus*. Bcc was the next major pathogen, although it was not isolated from any of the samples during 2011–2012. The prevalence of methicillin-resistant *S. aureus* (MRSA) and *S. maltophilia* was scattered throughout the years without any age predilection [Figure 1]. Mixed infections were also reported during all

Table 1: Number of samples received each year as well as the percentage of culture positivity

Age (months)	Year, *n*/*d**							Total (%)
	2010	2011	2012	2013	2014	2015	2016	
0-3	4/4	0	0/1	1/1	1/7	0/3	0/2	6/18 (33.3)
3-6	8/8	3/6	4/5	3/3	3/10	7/10	7/14	35/56 (62.5)
6-12	16/16	3/7	2/2	7/7	10/25	12/21	12/26	62/104 (59.6)
12-24	6/6	12/16	1/3	3/3	11/24	3/7	9/14	45/73 (61.6)
>24	2/2	1/7	15/20	6/6	17/26	22/33	38/67	101/161 (56.5)

*Numerator (*n*) represents positive culture and denominator (*d*) represents the total number

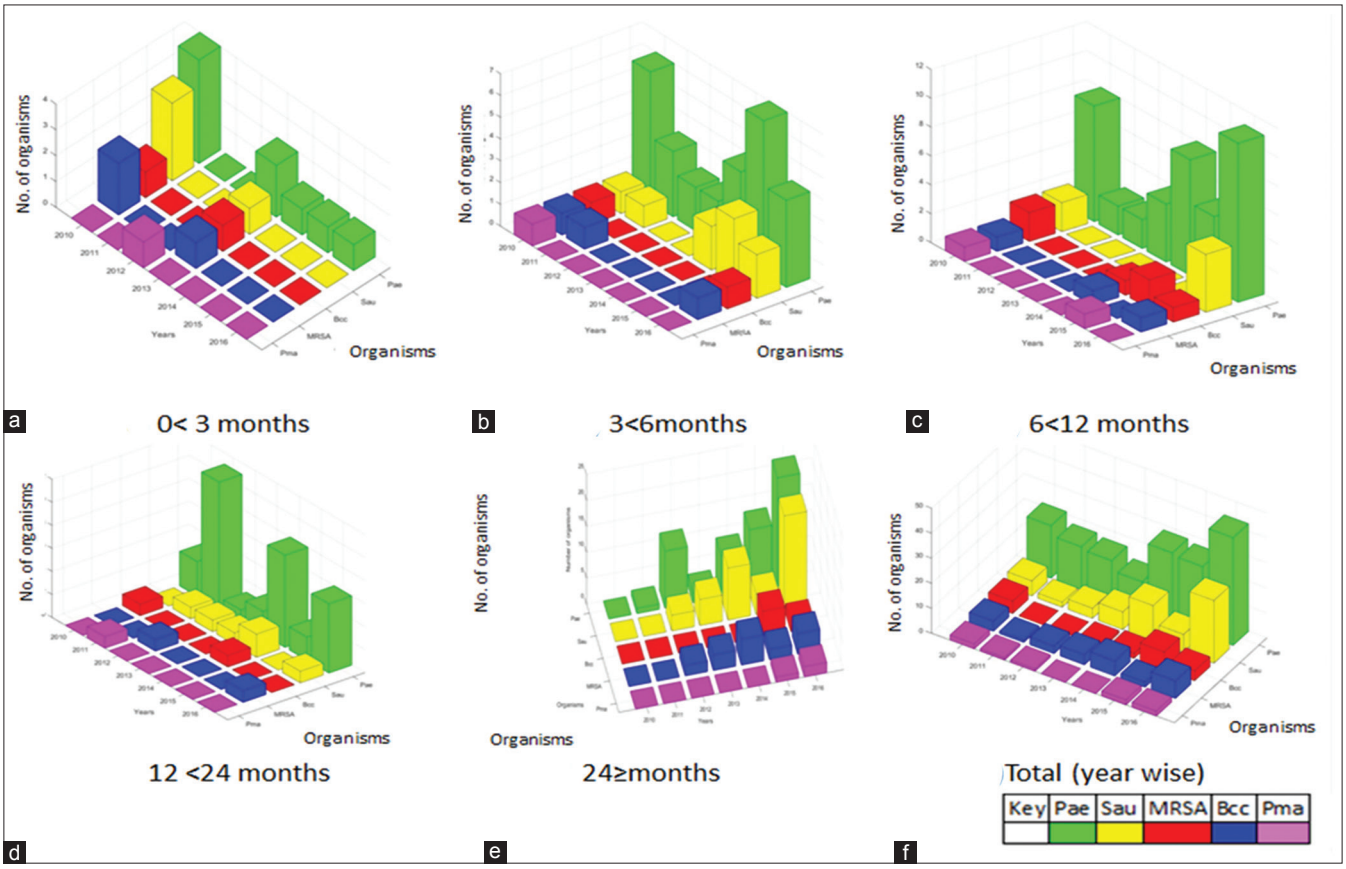

Figure 1: Microbial profiles of respiratory samples of cystic fibrosis children in various age groups over 7 years: (a-e) The number and type of organisms isolated in each age group over 7 years whereas (f) the total number and type of organisms isolated during the 7 years. The x-axis represents years from 2010 to 2016. The y-axis represents the number of organisms isolated and the z-axis shows the various organisms, here were have shown only the 5 most commonly isolated ones. **Burkholderia cepacea* complex, methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Staphylococcus aureus* (methicillin-sensitive *Staphylococcus aureus*)

the years, being more common in older children (24.4% in 12–24 months and 21.7% for an age ≥24 months) [Figure 2].

P. aeruginosa was the most commonly isolated organism across all age groups, over all the years [Figure 1]. The second most commonly isolated organism was *S. aureus*. Bcc was the next major pathogen, although it was not isolated from any of the samples during 2011–2012 [Table 2]. The prevalence of MRSA and *S. maltophilia* was scattered throughout the years without any age predilection [Figure 1]. Mixed infections were also reported during all the years, being more common in older children (24.4% in 12–24 months and 21.7% for an age ≥24 months) [Figure 2].

A total of 246 culture-positive samples from 96 children could be followed up for assessing the pattern during subsequent respiratory infections and surveillance cultures [Figure 3]. The most commonly isolated pathogen at the first visit was *P. aeruginosa* (52.6%) followed by *S. aureus* (15.7%). Forty-seven per cent of second cultures had organisms different from the first episode and 45% of third cultures had organisms different from the second culture. The incidence of mixed infection was 16.6%, 5.7%, 19.3%, 24.4% and 21.7% for the age groups 0–3, 3–6, 6–12, 12–24 and ≥24 months, respectively. The methicillin-sensitive isolates became resistant during subsequent episodes of colonisation/infection in 61.5%

of the participants. Of the children with initial cultures positive for *P. aeruginosa*, 55% of their subsequent cultures showed polymicrobial infections.

P. aeruginosa was most susceptible to ciprofloxacin (89%) and piperacillin-tazobactam (88%) followed by amikacin (85%). Susceptibility to imipenem and ceftazidime was nearly 75%. Among the *S. aureus* isolates, 38% were MRSA. The percentage of MRSA increased from 66% in 2010 to 75% in 2012. From 2012, the percentage of MRSA showed a significant decline to 24% in 2016. In 2017, it was noted that 40% of *S. aureus* isolates were resistant to methicillin.

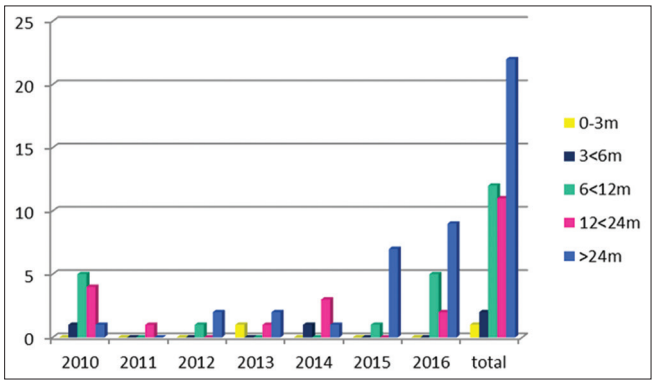

Figure 2: Mixed infections among the various age groups over 7 years

All *S. aureus* were susceptible to vancomycin, teicoplanin and linezolid during all the years. Increasing resistance to clindamycin and erythromycin was noted with only 54.5% and 40.5% of the isolates being susceptible to them [Figure 4]. *Acinetobacter baumannii* showed high levels of resistance to all drugs except for colistin, where 100% *in vitro* susceptibility was noted. Among Bcc isolates, 100% susceptibility was observed for cotrimoxazole and minocycline. *Escherichia coli* isolates were most susceptible to amikacin, cefepime, piperacillin-tazobactam and imipenem (71% each).

Discussion

P. aeruginosa was the most commonly isolated organism across 7 years and age groups included in the present study. The CFF Patient Registry of United States reported the isolation of *P. aeruginosa* in 52.5% sputum culture specimens in 2008.[13] However, in a recent retrospective analysis of 2006–2012 data from the CFF Patient Registry, methicillin-sensitive *S. aureus* (MSSA) was found to be the most prevalent organism (52.3%) followed by *P. aeruginosa* (49.6%).[15] They further showed there was a significant reduction in the incidence and prevalence of *P. aeruginosa* across most age groups from 2006 to 2012. The decline in *P. aeruginosa* infection was attributed to improvements in clinical care, infection prevention and control,[15] which are

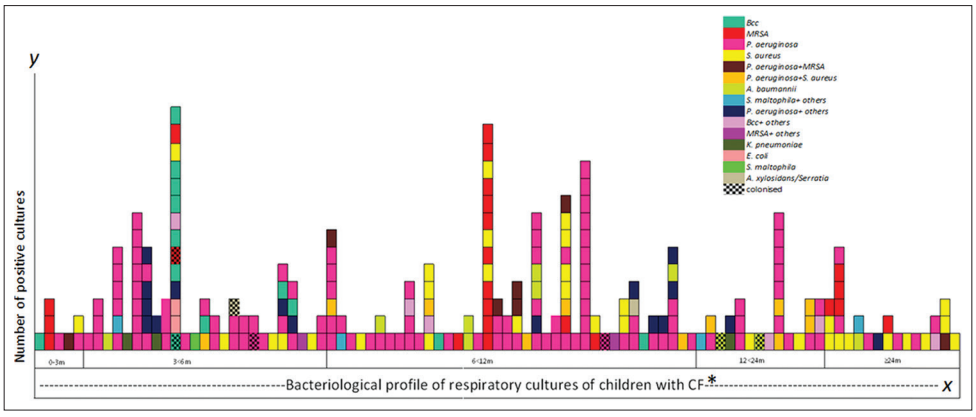

Figure 3: Temporal culture positivity and organisms isolated from each child with cystic fibrosis: The stacked bar graphs represent the multiple infections encountered by each child during the study period. The colour of each block represents the organism causing infection/colonisation. The lowest block shows the first positive culture, and the topmost shows the last positive culture. Children have been grouped agewise taking into account age at first culture positivity. *The graph shows the culture of 95 children

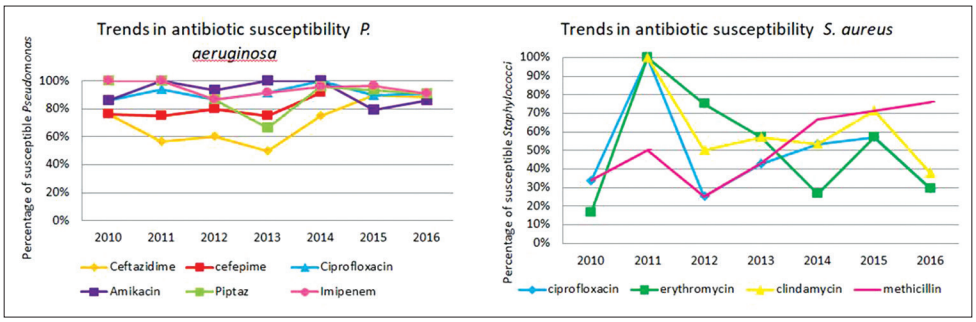

Figure 4: Trends in antimicrobial susceptibility

Table 2: Trends in bacteriological profile over the various age groups over the years

Organism (months)	*N*/yr							Total
	2010	2011	2012	2013	2014	2015	2016	
Pseudomonas								
0-3	4	0	0	2	1	1	1	9
3-6	6	3	2	2	4	7	4	28
6-12	8	2	2	4	8	5	11	40
12-24	3	11	1	2	8	2	6	33
>24	0	1	11	2	8	12	21	45
Staphylococcus								
0-3	3	0	0	1	0	0	0	4
3-6	1	1	0	0	2	3	2	9
6-12	2	0	0	0	0	0	4	6
12-24	0	1	1	1	2	0	1	6
>24	0	0	3	5	10	4	18	40
Bcc								
0-3	1	0	0	1	0	0	0	2
3-6	1	0	0	0	0	0	1	2
6-12	2	0	0	0	1	2	1	6
12-24	1	0	0	0	1	0	0	2
>24	0	0	0	0	0	5	2	7
MRSA								
0-3	2	0	0	1	0	0	0	3
3-6	1	1	0	0	0	0	1	3
6-12	1	0	0	0	1	0	1	3
12-24	0	0	1	0	0	0	1	2
>24	0	0	2	3	5	2	4	16
Stenotrophomonas								
0-3	0	0	1	0	0	0	0	1
3-6	1	0	0	0	0	0	0	1
6-12	1	0	0	0	0	1	0	2
12-24	0	1	0	0	0	0	0	1
>24	0	0	0	0	0	1	2	3

N/yr: Number of organisms/year, *Bcc*: *Burkholderia cepacia complex*, MRSA: Methicillin-resistant *Staphylococcus aureus*

still suboptimal in India. Germany also reported *S. aureus* as the most prevalent pathogen in CF in 2008 (63%) followed by *P. aeruginosa* (50%) and *Haemophilus influenzae* (17%).[16] Studies on CF from the USA have also shown *H. influenzae* to vary from 15% to 30%.[13,15] *H. influenzae,* however, was not isolated from any patient in the present study. The reason for the absence of *H. influenzae* from patients with CF in our study could not be determined, although we are routinely isolating this organism from respiratory specimens of non-CF patients.

There is always a dilemma of whether or not to treat a patient of CF who is colonised with pathogens which are likely to cause infection. In this study airway samples were colonised with *P. aeruginosa* (2), *A. baumannii* (2), MRSA (1), *Achromobacter xylosoxidans* (1) and Bcc (1). Studies have shown that treating *P. aeruginosa* colonisation in non-chronically infected CF children helps prevent chronic colonisation and also allows early eradication in 80%–100% of cases. Contrary to earlier beliefs that mucoid variants of *Pseudomonas* cannot be eradicated, recent evidence shows that early treatment allows for eradication and reduces the morbidity. Oral therapy is preferred as there is better compliance and intravenous therapy does not offer any additional benefit.[17] Colonisation with Bcc may become chronic if left untreated and can affect the lung function.[18] A recent study by Garcia *et al.* suggest that treatment can prevent chronic colonisation,[19] whereas another study by de Souza *et al*. on CF patients with Bcc colonisation undergoing lung transplant and found that colonisation did not increase the morbidity or mortality.[20]

P. aeruginosa was commonly associated with mixed or polymicrobial infections in the current study with the most prevalent coinfection being that with *S. aureus*. Double colonisation is associated with frequent exacerbations of respiratory tract infections leading to worsening of lung function.[21] The coinfecting organisms, when present together, show enhanced survival even in the presence of antibiotics to which they are individually susceptible.[22] Further, both organisms form biofilms which hinder their eradication from the thick secretions of CF lung.[23]

P. aeruginosa species isolated in this study were most susceptible to aminoglycosides, fluoroquinolones and

piperacillin-tazobactam. Susceptibility to imipenem and ceftazidime was nearly 75% each. This is in contrast to a study by Courtois *et al.* where contemporary French isolates of *P. aeruginosa* were only 36.8% susceptible to ceftazidime and only 9.2% to imipenem.[24] Although we did not look for the mechanism of resistance in the present study, Courtois *et al.* reported that resistance to ceftazidime and imipenem was due to cephalosporinases and porin alterations, respectively, and not due to the production of extended-spectrum cephalosporinases or carbapenemases. Recently, Qin *et al.* have reported that during the process of adaptation to the CF lung milieu, several isogenic strains of *P. aeruginosa* emerge and coexist and these strains may have vastly different susceptibility patterns.[25]

Among *S. aureus* stains, an increasing trend in the incidence of MRSA from 2010 to 2016 was noted. This could be attributed to sustained parallel increase in MRSA prevalence among non-CF population in the community. A study conducted in 2013 showed the prevalence of MRSA in non-CF patients in the outpatient setting to be 28%.[26] Fall in the prevalence of MRSA is contrary to world data.[25] This fall could be attributed to several factors, including better antimicrobial stewardship and better personal hygiene. Data of nasal swabs of primary caregivers of CF children showed that very few of them harboured MRSA.[27] Possible mixing of hospital-acquired MRSA clones into the community through health-care workers cannot be ruled out.[28] Cross infectivity among children in CF clinics could also be a factor which has been controlled, to some extent by calling the patients in small numbers for follow-up. Treatment options for MRSA include vancomycin and teicoplanin as all isolates were susceptible to them. Clindamycin was not a suitable drug for treating MRSA infections since all methicillin-resistant isolates showed resistance to clindamycin as well. However, it was effective against MSSA.

No comment can be made about the susceptibility of Bcc, *A. baumannii* and *S. maltophilia* from the current study as the number of isolates were <30 (CLSI recommends a minimum of thirty isolates per organism to study an antibiogram). However, the data from a non-CF cohort from our institute revealed that the *S. maltophilia* isolates were 100% susceptible to levofloxacin and minocycline, 97% to cotrimoxazole, 64% to chloramphenicol and 50% to ceftazidime. In comparison, 75% Bcc isolates were susceptible to minocycline, 27% to levofloxacin and ceftazidime but only 13% to chloramphenicol.[29] Data from non-CF patients suggest that colistin may be appropriate either intravenously or inhalational for the treatment of *A. baumannii*.

Limitations

Being a retrospective study, some children may have been missed. Those who were asymptomatically colonised or had mild infections might not have visited the hospital for regular follow-up, and hence, this may not give a true picture of the actual burden. From the records, we were unable to judge whether the positive culture signified infection or colonisation as most of our children were below 2 years of age and at this age neither forced expiratory volume in 1 s is conclusive nor the Fuchs criteria for exacerbation are met.

Conclusions

Pseudomonas and *Staphylococcus* were the most common organisms and have continued to remain so. They have the highest incidence and prevalence among all age groups. To reduce the incidence of cross-infections, children with CF are called for follow-up in small numbers on different days. Hand hygiene may play a crucial role in reducing the risk of infections. Antimicrobial stewardship can definitely help us tackle the growing menace of drug-resistant bugs. Regular follow-up along with counselling of parents can help reduce undue antibiotic use as well as ensure treatment compliance. In case of suspected infection, care must be taken while initiating empiric therapy. It should be based on local antibiograms to prevent the emergence of resistant microbes.

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Conflicts of interest

There are no conflicts of interest.

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