



Aetiological agents for pulmonary exacerbations in children with cystic fibrosis: An observational study from a tertiary care centre in northern India

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Background & objectives: Pulmonary disease is the main cause of morbidity and mortality in cystic fibrosis (CF). The infection occurs with a unique spectrum of bacterial pathogens that are usually acquired in an age-dependent fashion. The objective of this study was to find out the aetiological agents in respiratory specimens from children with CF during pulmonary exacerbation and relate with demographic variables.

Methods: In this observational study, airway secretions from children (n=104) with CF presenting with pulmonary exacerbations were collected and tested for bacteria, fungi, mycobacteria and viral pathogens using appropriate laboratory techniques. The frequencies of isolation of various organisms were calculated and associated with various demographic profiles.

Results: Bacteria were isolated in 37 (35.5%) and viral RNA in 27 (29.3%) children. *Pseudomonas* was the most common bacteria grown in 31 (29.8%) followed by *Burkholderia cepacia* complex (Bcc) in three (2.8%) patients. Among viruses, Rhinovirus was the most common, identified in 16 (17.4%) samples followed by coronavirus in four (4.3%). Fungi and mycobacteria were isolated from 23 (22.1%) and four (3.8%) children, respectively. *Aspergillus flavus* was the most common fungus isolated in 13 (12.5%) children.

Interpretation & conclusions: *Pseudomonas* was the most common organism isolated during exacerbation. Non-tuberculous mycobacteria were not isolated, whereas infection with Bcc and *Mycobacterium tuberculosis* was observed, which could probably have a role in CF morbidity. Polymicrobial infections were associated with severe exacerbations.

Key words Acute exacerbation - children - cystic fibrosis - microbiology - *Pseudomonas* - polymicrobial infection

Cystic fibrosis (CF) is a common life-limiting inherited disease in the western population. In India, CF was first reported in 1968¹. The exact prevalence

of the disease among Indian population is not known, but the prevalence has been estimated to be 1/43,321 to 1/100,323 based on carrier frequency studies².

Pulmonary involvement is the predominant cause of morbidity in CF. The disease course is punctuated by periods of acute worsening of the pulmonary disease, termed exacerbations. The aetiological agents responsible for these pulmonary exacerbations are different from those causing pneumonia in normal children. Children with CF are also at risk of acquiring mycobacterial, viral and fungal infections³.

The unique spectrum of bacterial pathogens is usually acquired in a time-dependent fashion. Early in the course of the disease, the organisms commonly isolated include *Staphylococcus aureus* and non-typeable *Haemophilus influenzae*⁴. *Pseudomonas aeruginosa* is more commonly isolated later in the course of the disease and infects approximately 80 per cent of the patients with CF. *Burkholderia cepacia* complex (Bcc) is another organism unique to CF and patients harbouring these have a worse prognosis⁵. Early and aggressive treatment with appropriate antibiotics may eradicate infection, prevent colonization, reduce the degree of inflammation and help delay progression of pulmonary disease⁶. Non-tuberculous mycobacteria (NTM) infection in CF is a serious concern. Multiple factors such as malnutrition, CF related diabetes mellitus, steroid treatment and chronicity of illness predispose these children to mycobacterial infection^{3,7}.

The incidence of viral infections in children with CF though is not elevated; the severity of infection is increased⁸. *In vitro* studies have suggested that viral infection increases bacterial adhesion to epithelial cells and impairs macrophage immune responses to bacterial products⁹. Various species of fungi have also been isolated from respiratory specimens of CF patients presenting with exacerbations¹⁰. There is a scarcity of data from India, therefore, this study was conducted to document aetiological agents of pulmonary exacerbation in Indian children with CF.

Material & Methods

This was a single-centre observational study conducted in the Paediatrics department, All India Institute of Medical Sciences (AIIMS), New Delhi, India, over a period of 18 months, from October 2013 to March 2015. Sample size was calculated to be 94 (95% CI and 10% precision) based on a retrospective study of 120 patients conducted at our center¹¹, where *Pseudomonas* was detected in 42 per cent of CF patients. The study protocol was approved by the Institutional Ethics Committee of AIIMS and written

informed consent was obtained from the parents of the children. Respiratory specimens were collected, either expectorated or induced sputum, nasopharyngeal aspirates or throat swabs, from 104 children who were diagnosed with CF, based on sweat chloride levels, aged up to 18 yr, who presented with pulmonary exacerbation either to the Pediatric outpatient department or admitted to the Pediatric ward. Children presenting within six months of the previous exacerbation were excluded. Pulmonary exacerbation was defined as per the criteria suggested by Fuchs *et al*¹².

Microbiological methods: The samples were sent for bacteria, fungi, mycobacteria culture and viral RNA detection in the microbiology laboratory. Samples for bacterial isolation were smeared for Gram stain and also inoculated on MacConkey, blood, chocolate and *B. cepacia* Selective Agar. If any colonies were grown, these were further processed for phenotypic identification and antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute guidelines¹³. Samples were also mounted on 10 per cent potassium hydroxide and Sabouraud dextrose agar for fungus identification. In the mycobacteria laboratory, samples were examined for acid-fast bacilli and then inoculated to Löwenstein-Jensen medium and BD BACTEC™ Mycobacteria growth indicator tube (Becton, Dickinson and Company, NJ, USA), after decontamination with N-acetyl L-cysteine - 2% NaOH and 2.5 per cent oxalic acid solution, for mycobacterial identification¹⁴. These samples were subjected to real-time multiplex PCR using the commercial assay platform provided by Fast-track Diagnostics® (FTD) respiratory kit (FTD, Luxembourg) for detection of viral nucleic acid.

Statistical methods: The frequencies of detection (single as well as multiple) of each pathogen were calculated and related with various demographic profiles. Significance of difference between two related proportions was assessed using the McNemar's test.

Results

A total of 104 patients (10.42±3.2 yr age; 71 male, 33 female) were enrolled in the study. The mean age at the onset of illness was 2.0±1.1 yr and at the time of diagnosis was 4.2±1.8 yr. Of the 104 patients, 41 (39.4%) were inpatients and 63 (60.5%) were outpatients.

Among the criteria suggested by Fuch *et al*¹², increase in cough was the most common symptom

[100 (96.1%)], followed by increased dyspnoea [86 (82.7%)] and change in sputum volume or colour [79 (75.9%)]. Of the enrolled children, 68 (65.3%) were positive for at least one of the organisms, bacteria, fungi, mycobacteria or viruses. Table I summarizes all the groups of organisms isolated in these children.

Bacteria: Bacteria were isolated from 37 of the 104 samples; *P. aeruginosa* was the predominant species, isolated from 31 (29.8%) samples. *Burkholderia* was grown from three (2.8%), *Staphylococcus aureus* from two (1.9%) and *Acinetobacter* spp. from one (0.9%) sample.

***Pseudomonas* and clinical variables:** Though insignificant, children who grew *Pseudomonas* in their respiratory specimens were relatively younger (9.66 ± 1.86 yr) compared to children in whom *Pseudomonas* was not isolated (10.45 ± 1.32 yr). They became symptomatic at a younger age (1.13 ± 0.51 yr) and were diagnosed at an early age (3.65 ± 1.26 yr) and required frequent outpatient department visits or hospital admissions for antibiotic administration (either oral or intravenous). A significant observation was that a higher proportion of children, who were admitted, requiring hospital care, grew *Pseudomonas* than those who received treatment as outpatient basis (Table II).

Other microorganisms: Fungi were grown in 23 specimens, and filamentous fungus was the more common one (17%) (*Aspergillus flavus* - 12.5% and *A. fumigatus* - 4.5%). *Candida* sp. was grown in five (4.7%) specimens (Table I). *Mycobacterium tuberculosis* was cultured from four of the specimens, whereas NTM was not identified in any of the samples. All of the four children from who grew *M. tuberculosis* were boys ($P=0.01$) and were older than 10 yr (12.6 ± 5.8 yr). Viral nucleic acid was found in 27 of the 92 specimens (29.3%) subjected to PCR; 29 viruses were detected in these 27 individuals as two children were tested positive for two viruses each. Rhinovirus was the most common virus, positive in 16 (17.4%) individuals, followed by coronavirus (4.3%) (Table I).

Children who were tested positive for virus were younger (9.1 ± 6.2 yr) than the children who were not (10.8 ± 5.3 yr) and had only mild exacerbation, receiving ambulatory treatment rather than in-hospital care. Table III describes the clinical variables in children

Table I. Summary of microbial pathogens demonstrated in respiratory tract secretions of children with cystic fibrosis and pulmonary exacerbations

Organisms	Positive (%)	Remarks
Bacteria		
Total positive	37	-
<i>Pseudomonas aeruginosa</i>	31 (29.8)	-
<i>Burkholderia cepacia</i>	3 (2.8)	-
<i>Staphylococcus</i> spp.	2 (1.9)	-
<i>Acinetobacter</i> spp.	1 (0.9)	-
Fungi		
Total positive	23	-
<i>Aspergillus flavus</i>	13 (12.5)	-
<i>Aspergillus fumigatus</i>	5 (4.5)	-
<i>Candida albicans</i>	4 (3.8)	-
<i>Candida tropicalis</i>	1 (0.9)	-
Mycobacteria		
<i>Mycobacterium tuberculosis</i>	4 (3.8)	Multiple samples from same patient was obtained only in case of inpatients [41 (39.4)], whereas only one sample was obtained from patients receiving ambulatory treatment
Non-tuberculous mycobacteria	0	
Viruses		
Total positive	29	Viral identification assay could be performed with total RNA isolated from 92 of 104 patients
Rhinovirus	16 (17.4)*	
Human corona virus	4 (4.3)*	
Influenza A virus	2 (2.1)*	
Adenovirus	2 (2.1)*	
Influenza B virus	1 (1.0)*	
Respiratory syncytial virus	1 (1.0)*	
Parainfluenza 4	1 (1.0)*	
Human metapneumovirus	1 (1.0)*	
Echovirus	1 (1.0)*	

*Percentage was calculated with n=92

who had grown various groups of microorganisms compared to those who had not.

Polymicrobial infection: Multiple microbial agents were identified in airway specimens from 16 children (15.3%). The most common combination observed was that of virus + bacteria seen in nine children (8.6%), followed by viral and fungus infection in six (5.7%). Infection with more than two groups of organisms, *i.e.*, bacteria, virus and mycobacteria was found in one patient. With regard to individual organism,

co-infection with Rhinovirus and *Pseudomonas* was the most common, seen in three children (2.8%), all of whom required hospitalized care.

Association between the different age groups and the prevalence of various bacterial species showed that *S. aureus* was grown in relatively older age group than *Pseudomonas* sp. Hospitalized care was required in children who had co-infection with bacteria + virus or bacteria + fungus.

Discussion

At least one of the microbial agents was identified in 68 children (65.3%). In studies from various parts of the world, *Pseudomonas* was the predominant bacteria¹⁵⁻¹⁸. The percentage of children who grew *Pseudomonas* in our study was 29.8 per cent, which was lesser than the results of a study (36%) from Brazil¹⁵ and (40%) Italy¹⁶. In a retrospective study Razvi *et al*¹⁹ reported a significant decline in the annual prevalence of *Pseudomonas* from 60.4 per cent in 1995 to 56.1 per cent in 2005 ($P<0.001$). In an earlier study on 120 CF children between 1995 to 2002 at our centre, *Pseudomonas* was documented in 42 per cent¹¹, which has declined to 29.8 per cent in 2015 in the current study. Possible explanations include increased awareness about infection control methods, use of long-term immunosuppressive therapy with azithromycin and rational use of antimicrobials for early eradication of *P. aeruginosa*.

Table II. Comparison of baseline characteristics of children who grew *Pseudomonas* as compared to those who did not

Characteristics	<i>Pseudomonas</i>	
	Grown (n=31)	Not grown (n=73)
Age (yr) (mean±SD)	9.66±1.86	10.45±1.32
Sex		
Male, n (%)	16 (51.6)	55 (75.3)
Female, n (%)	15 (48.4)	18 (24.7)
Age at onset of illness (yr) (mean±SD)	1.13±0.51	1.27±0.32
Age at diagnosis (yr) (mean±SD)	3.65±1.26	3.73±0.77
Treated as		
Inpatient, n (%)	21 (67.7)***	19 (26)
Outpatient, n (%)	10 (32.2)	54 (74)

*** $P<0.001$ compared to 'Not grown' group

Table III. Comparison of clinical variables of children who had grown various microorganisms and children who had not

Characteristics	Bacteria		Fungi		Mycobacteria		Viruses	
	Grown (n=37)	Not grown (n=67)	Grown (n=23)	Not grown (n=81)	Grown (n=4)	Not grown (n=100)	Isolated (n=27)	Not isolated (n=65)
Age (yr) (mean±SD)	9.9±5.5	10.4±5.5	10.6±5.8	10.1±5.4	12.6±5.8*	10.1±5.4	9.1±6.2	10.8±5.3
Sex								
Male, n (%)	18 (48.6)*	52 (77)	17 (73.9)	54 (66.6)	4 (100)*	67 (67)	18 (66.6)	46 (70.7)
Female, n (%)	19 (51.4)*	15 (23)	6 (26.1)	27 (33.4)	0 (0)*	33 (33)	9 (33.3)	19 (29.3)
Age at onset of illness (yr) (mean±SD)	1.2±0.8	1.8±1.1	0.8±0.8	1.1±1.4	1.3±1.8	1.0±1.3	1.0±1.3	1.0±1.4
Age at diagnosis (yr) (mean±SD)	3.6±3.3	3.8±3.2	4.3±3.2	3.5±3.3	3.7±3.3	3.5±3.3	3.1±3.2	3.8±3.2
Treated as								
Inpatient, n (%)	24 (64.8)	16 (23.8)	10 (43.4)	31 (38.2)	2 (50)*	39 (39)	10 (37)	23 (35.3)
Outpatient, n (%)	13 (35.2)	51 (76.2)	13 (56.6)	50 (61.8)	2 (50)*	61 (61)	17 (63)	42 (64.7)

* $P<0.05$ compared to 'Not grown' in the respective group

Bcc was another important pathogen, isolated from three (2.8%) patients. Bcc is associated with a poor prognosis and a rapid deterioration of pulmonary function as evidenced by frequent exacerbations and worse lung capacities in children who had grown Bcc^{5,20}. The youngest children from whom *Pseudomonas* and *Burkholderia* were isolated were three and nine months old, respectively. This is a matter of concern since younger age of acquisition has been associated with accelerated deterioration of lung function and frequent exacerbations leading to a poor quality of life. The multicentric Standardized Treatment of Pulmonary Exacerbations (STOP) study conducted in 11 US centres has identified *P. aeruginosa* in 71 per cent of the 220 participants of all age groups and Bcc were cultured in three per cent²¹.

The yield of *Staphylococcus* spp. in our study was only 1.9 per cent whereas other studies reported as high as 78 per cent^{15,22-24}. In the STOP study, methicillin-resistant *S. aureus* was detected in 39 per cent whereas methicillin-susceptible *S. aureus* was detected in 36 per cent of study population²¹. The *Staphylococcus* isolated in our study was methicillin resistant.

Paugam *et al*²⁵ conducted a study in 201 adult patients with CF, and showed *Aspergillus fumigatus* in 56.7 per cent and other *Aspergillus* (non-fumigatus) species in 10.4 per cent patients. In another study conducted by Güngör *et al*¹⁰ in Istanbul, 48 CF patients were followed up. Contrary to the present observation they reported *Candida albicans* as the most common isolate (62.5%), followed by *A. fumigatus* (10.4%). Paugam *et al*²⁵ also reported increased percentage recovery of *Pseudomonas*, NTM and *Stenotrophomonas maltophilia* in patients colonized with *A. fumigatus*, however no such association was observed in the present study.

Growth of NTM was not seen in any of the patients, whereas *M. tuberculosis* was grown in four patients. Seddon *et al*²⁶ in their multicentre questionnaire based study reported a NTM prevalence of 3.3 per cent among 3,317 children. *M. tuberculosis* has been rarely associated with CF and there are only a few case reports available in literature^{27,28}. In the STOP study, the positivity for NTM was seven per cent²¹. In our study, samples at multiple occasions could be obtained only for those patients who received in-hospital treatment which may explain the nil yield of NTM.

Hoek *et al*²⁹ reported presence of viruses in 33 per cent of their adult population of CF patients, and Asner *et al*³⁰ showed in 60.5 per cent, whereas in our study, 29.3 per cent of patients were tested positive for at least one of the respiratory viruses. Hospitalized care was required in all the children who had co-infection suggesting increased severity of exacerbations in these patients. Our study has reiterated the role of non-bacterial microorganisms causing pulmonary exacerbations in children with CF.

In conclusion, *Pseudomonas* was the most common organism isolated during exacerbation. Infection with Bcc and *M. tuberculosis* was observed in our study whereas NTM were not isolated. *Aspergillus* was the commonest fungus isolated. Viruses resulted in exacerbations in a significant number of CF children. Polymicrobial infections were associated with severe exacerbations.

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Conflicts of Interest: None.

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