ABSTRACT:

Background: Cystic fibrosis (CF) is a chronic autosomal recessive disease caused by CF transmembrane conductance regulator (CFTR) gene mutations, which encodes the CFTR protein. Impaired function of this protein leads to increased mucus thickness with subsequent chronic infections. The fungal biota in CF is dominated by Aspergillus and Candida species (spp).

Aim of the Work: To determine the common lower respiratory tract infections among CF pediatric patients and to correlate culture results with the patient clinical status.

Patients and Methods: A Cross Sectional Study was applied on 32 lower respiratory samples collected from CF pediatric patients admitted to Chest department, Pediatric Hospital, Ain Shams University. Samples were tested through bacterial and fungal culture.

Results: Bacterial growth was detected in five samples (15.6%), where Pseudomonas spp was the most common bacteria. Fungal growth was detected in three samples (9.4%), all showed growth of Candida spp. Two samples showed mixed growth of Pseudomonas spp and Candida non albicans.

Conclusion: Pseudomonas spp was the most frequent pathogen isolated from CF patients. Candida spp was the predominant fungal species detected.

Keywords: Cystic fibrosis, pediatrics, respiratory tract infections, fungal infections, Candida

INTRODUCTION:

Cystic fibrosis (CF) is a chronic autosomal recessive hereditary potentially life-threatening disease caused by mutations of the CF transmembrane conductance regulator (CFTR) gene on chromosome 7, which encodes the CFTR protein\(^1\). This protein functions as an anion channel that is responsible for negatively charged chloride ion transport across cells in the body. This protein is present in various organs of the body, including respiratory tract, gastrointestinal tract, liver, pancreas as well as male reproductive tract\(^2\). The incidence of CF varies according to the ethnic origin. Case reports from several Arabic countries and Egypt have suggested estimates ranging from 1 in 2,560 to 1 in 15,876 individuals, and from one in 2,000 to one in 3,500 Caucasians born in Europe, and the United States\(^3\).

In the airways, impaired function of this protein leads to increased mucus thickness, which fails to be cleared by the mucociliary system\(^4\). This in turn leads to colonization, chronic infection of the respiratory tract and often associated with ineffective treatment, inflammatory cytokines accumulate, leading
to lung damage and bronchiectasis. Airway infections are associated with progressive lung function decline and ultimately, with respiratory failure, which is the leading cause of mortality in CF\(^5\).

The fungal biota in CF is dominated by filamentous fungi such as *Aspergillus fumigatus*, *Aspergillus terreus*, *Scedosporium apiospermum*, and yeasts such as *Candida albicans* (*C. albicans*) and *Candida glabrata*. Therapy can be challenging as some of the moulds are multi-drug resistant, for example azole-resistant *Aspergillus* spp which have been described in the recent years\(^6\).

Being caused by genetic mutation, neonatal screening of CF is essential for early detection of cases and is done by measuring Immunoreactive trypsinogen (IRT)\(^7\). Suspected cases (positive screening test), child with suggestive history / symptoms/ examination or sibling of a known case (even if asymptomatic) will undergo sweat testing where, Sweat Chloride value > 60 mmol/L confirm the diagnosis in 98% of cases. Culture-based studies suggest that lower airway infection is a key trigger for inflammation)\(^8\). Different respiratory sampling methods exist to identify lower airway pathogens in patients with CF such as sputum, Endotracheal aspirates (on ventilated patients) and bronchoscopically obtained specimens, including bronchoalveolar lavage (BAL) specimens of which expectorated sputum and bronchoalveolar lavage (BAL) are considered the “gold standard.” Results for sensitivity, specificity and predictive values of BAL samples are all high (>75.0%). Lower airway samples from infants and young children can only be reliably obtained via bronchoscopy\(^9\).

Despite chronic and acute bacterial respiratory infections usually remain predominant; the frequency of isolation of fungi from the respiratory tract is increased in children compared to adult. *Aspergillus* spp and *Pseudomonas aeruginosa* (*P. aeruginosa*) are commonly present in the lower airways from infancy\(^10\). The decrease in prevalence of *P. aeruginosa* and *Staphylococcus aureus* (*S. aureus*) since 2000, coinciding with the aggressive therapeutic approach, has resulted in *Aspergillus* becoming the most commonly isolated pathogen in children\(^11\).

**AIM OF THE WORK:**

This study aims to detect lower respiratory tract infections among CF pediatric patients in Chest department at Ain Shams University Pediatric Hospital and to correlate culture results with the patient clinical status.

**PATIENTS AND METHODS:**

The study included 32 pediatric patients with CF admitted to Chest department, Pediatric Hospital, Ain Shams University. Lower respiratory samples were collected in the period from January 2022 to February 2023 and sent to the Main Microbiology Laboratory, Clinical Pathology Department, Ain Shams University Hospitals for processing.

**Inclusion criteria:**
- Confirmed CF patients by clinical symptoms and signs (most frequently chronic respiratory symptoms and malabsorption) and positive sweat chloride (> 60 mmol/L)\(^12\).
- CF patients aged less than 18 years.

**Exclusion Criteria:**
- Children with other known chronic lung disease (Childhood interstitial lung disease and TB).

All cases were subjected to:
**Complete history taking including:**
- Demographic data: Name, age, sex and consanguinity, birth order, residence
- Respiratory and gastrointestinal symptoms
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- Medication history

Laboratory investigations:
- Complete blood count (CBC)
- C-reactive protein (CRP)
- Sweat chloride test
- Serum Na, K, Ca and PO4.
- Cystic fibrosis Genotyping.

Imaging:
- High-Resolution Computed Tomography (HRCT) scan.

Bronchoalveolar lavage specimen Collection and processing was carried out using pediatric flexible fiberoptic bronchoscope, samples were collected in sterile container and transferred to the Main Microbiology Lab within 1 hour, where, 5 ml of each sample was centrifuged at 3000 rpm for 20 minutes, supernatant was discarded, and the sediment was tested through Direct Gram stain examination then culture was done.

Bacterial culture of bronchoalveolar lavage specimens:
Each specimen was cultured on Blood agar, Chocolate agar and MacConkey agar plates (Oxoid, UK) by semi-quantitative technique, plates were incubated aerobically for 24-48 hours at 37°C. Positive bacterial cultures were identified through manual identification using biochemical reactions and Vitek 2 Compact (BioMérieux, France).

Fungal culture of bronchoalveolar lavage specimens:
Each specimen was cultured on two Sabouraud dextrose agar plates supplemented with chloramphenicol (SDA) plates (Oxoid, UK), one plate was incubated at 25-28°C and the other at 36°C ±1°C, plates were examined every other day for growth up to 4-6 weeks.

Positive fungal cultures, were identified through colony morphology together with microscopic examination by Germ tube test, methylene blue stain using scotch tape technique and Gram stain.

Cystic fibrosis DNA analysis:
Cystic fibrosis transmembrane conductance regulator gene (CFTR) mutation analysis was done by Multiplex polymerase chain reaction (PCR). A panel of 32 CFTR mutations was tested which include; p.F508del; p.G551D; c.3120+1G→A; p.G85E; p.R34P; c.394delTT; p.1507del; p.R553X; p.R1162X; c.621+1G→T; p.R347H; c.2184delA; c.3876delA; p.R560T; c.3659delC; p.R117H; p.R334W; p.V520F; p.S549R; p.W1282X; c.2789+5G→A; p.A455E; c.1717-1G→A; p.S549N; p.N1303K; c.711+1G→T; c.1898+1G→T; p.G542X; c.3849+10kbC→T; c.3905_3906insT; 1078delT; c.2183_2184delinsG.

Data Management and Analysis:
The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 25). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Descriptive statistics:
- Mean, Standard deviation (± SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric numerical data.
- Frequency and percentage of non-numerical data.

Ethical Consideration:
This research was approved by Ethical Research committee, faculty of Medicine, Ain Shams university, Date: 25/1/2022, No. FMASU MS53/2022

RESULTS:
In our study, the patients age ranged from 1.7 to 15 years (mean 5.64± 3.6) while age of onset of CF disease ranged from
newborn to 84 months (mean 9.12± 20.75). As regard clinical picture, 18 (56.3 %) cases presented with productive cough, 13 (40.6 %) cases presented with failure to gain weight, nine (28.1 %) cases presented with steatorrhea, eight (25 %) cases presented with dyspnea and five (15.6 %) cases presented with hepatomegaly.

All patients were positive for sweat chloride test (> 60 mmol/l). Gene mutation test was done for 19 cases (59.3 %), of whom 14 cases (73.7%) were positive and five patients (26.3 %) were negative. In positive cases, F508 del mutation was the most common mutation detected in nine cases (64.3 %) and mutations other than F508 del were detected in five cases (35.7%) as shown in table (1).

As regard bronchoalveolar lavage samples, bacterial growth was detected in five samples (15.6%), where Pseudomonas spp was the most commonly isolated bacteria; detected in four samples and one sample showed mixed growth of Pseudomonas spp and MRSA. Fungal growth was detected in three samples (9.4%), all showed growth of Candida spp; one sample showed growth of C. albicans and two samples showed growth of Candida non albicans (CNA), two samples showed mixed growth of Pseudomonas spp and CNA as shown in table (2).

Fungal growth was detected in three cases, where one patient showed mixed growth of Pseudomonas spp and CNA presented by productive cough, dyspnea, steatorrhea, elevated CRP and TLC, positive chest CT findings and positive F508del mutation. The second patient showed mixed growth of Pseudomonas spp and CNA presented with positive chest CT findings and negative CFTR mutations. The third patient showed growth of C. albicans had productive cough, dyspnea, elevated CRP and TLC.

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**Table 1: Specific cystic fibrosis lab tests**

<table>
<thead>
<tr>
<th>CFTR gene mutation</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
<td>73.7%</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>26.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of CFTR gene mutation (in positive cases)</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>F508del</td>
<td>9</td>
<td>64.3%</td>
</tr>
<tr>
<td>Mutations other than F508del</td>
<td>5</td>
<td>35.3%</td>
</tr>
</tbody>
</table>

**Table 2: Positive microbiological cultures in CF patients**

<table>
<thead>
<tr>
<th>Type of microorganism</th>
<th>N</th>
<th>%</th>
<th>CFTR mutation</th>
<th>Type of CFTR mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure bacterial growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>2</td>
<td>6.3%</td>
<td>Positive in one patient only</td>
<td>F508del</td>
</tr>
<tr>
<td>Pseudomonas spp and MRSA</td>
<td>1</td>
<td>3.1%</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Pure fungal growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>1</td>
<td>3.1%</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Bacterial and fungal coinfection</td>
<td>2</td>
<td>6.3%</td>
<td>Positive in one patient only</td>
<td>F508del</td>
</tr>
<tr>
<td>Total number</td>
<td>6</td>
<td>18.8%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Bacterial growth was detected in five samples (15.6%); two samples showed mixed fungal and bacterial infection as previously mentioned, two samples with *Pseudomonas* spp had productive cough, steatorrhea, abdominal distension, underweight, elevated CRP, one of them had positive F508del mutation and the other was negative for CFTR mutation. Fifth sample showed mixed growth of *Pseudomonas* spp and MRSA had productive cough, dyspnea, steatorrhea, elevated CRP, elevated TLC and positive chest CT findings.

DISCUSSION:

This study was done on 32 patients diagnosed as CF admitted to Chest department, Pediatric Hospital, Ain Shams University. Lower respiratory samples were collected and sent to the Main Microbiology Laboratory, Clinical Pathology Department, Ain Shams University Hospitals for routine microbiological cultures.

As regard clinical picture of the patients, the respiratory tract is the most commonly affected system including 18 (56.3 %) cases presented with productive cough, 13 (40.6 %) cases presented with failure to gain weight, nine (28.1 %) cases presented with steatorrhea. This was concordant with Guo *et al.* (13) who found that respiratory symptoms in the form of sinusitis was present in 33 (46.5%) cases, gastrointestinal symptoms in 42 (59.2%) cases. Also *Shi et al.* (14) reported that 54 (47.8%) cases had upper respiratory tract infections and 104 (92.0%) cases had lower respiratory tract infections. Respiratory tract illness is the common presentation and the main cause of morbidity and mortality in CF patients since the defect in airway epithelial ions-water transport result in viscid mucus with subsequent impaired clearance, and adherence to airway surfaces, forming mucus plugs, leading to air flow obstruction, infection and eventually chronic inflammation(15).

Among 19 (59.3%) cases tested for CF gene mutation, F508 del mutation was the most common CFTR mutation detected in nine (64.3 %) cases. This was concordant with *Sosnay and his co-workers* (16) who found that F508del was the most frequent mutation, detected in 39,696 (70%) cases. Also, *Shi et al.* (14) stated that c.2909G>A mutation was the most commonly detected; in 7 (9.15%) cases while F508 del was reported only once (1.26%), in many populations the most common pathogenic variant of the CFTR gene is F508del, which represent about two thirds of all CFTR alleles. The other third of alleles are heterogeneous, composed of 20 mutations occurring at a very minute frequency (17). The difference in CF mutations distribution is reported to be due to wide heterogeneity of mutation in the CFTR gene within the CF patients of different ethnic populations and geographic distribution (18).

Bacterial growth was detected in five samples (15.6%), where *Pseudomonas* spp was the most frequent pathogen detected in four samples and one sample showed mixed growth of *Pseudomonas* spp and MRSA. This was concordant with Guo *et al.* (13) who found that *P. aeruginosa* was the most common pathogen in Chinese CF patients, in 46 (78.0%) cases. Also *Nasri and his colleagues* (19) reported that *P. aeruginosa* had the highest rate, 24 (57.1%) cases followed by nine (21.4%) cases caused by *S. aureus*. Similarly, *Shi et al.* (14) found that the predominant organisms found in the airways were *Pseudomonas aeruginosa*, detected in 60 (64.5%) cases followed by *S. aureus* 13 (14.0%) cases. However, *Hatzigorous et al.* (20) stated that *S. aureus* had the highest prevalence; 25897 (38.8%) cases followed by *P. aeruginosa* 33832 (29.8%) (18). Also *Breuer et al.* (21) concluded that *S. aureus* was the most common detected pathogen; 199 (11%) cases followed by *P. aeruginosa* in 143 (8%) cases.
Fungal growth was detected in three samples (9.4%), where Candida spp was the fungal species detected in all; one sample (3.1%) showed growth of C. albicans and two samples (6.3%) showed growth of CNA. This was concordant with Ziesing and his co-workers, who detected the most prevalent species identified were Candida spp: 19481 (58.8) cases, including 7408 (38%) C. albicans and 2337 (12%) cases Candida dubliniensis. Also Nasri et al. found Candida spp was the most common fungi, isolated from 32 (73.8%) CF patients, with the predominance of C. albicans 24 cases (80%). This was discordant with Breuer et al., who reported that Aspergillus spp were the most common fungi in CF patients, isolated from 192 (11%) cases while Candida spp was not detected.

**Conclusion:**

Most common species implicated in lower respiratory tract infections in CF patients were Pseudomonas and Candida spp. Exacerbations in CF patients are not always associated with bacterial or fungal infections. Our study had limitations like the relatively small sample size, further studies that include more number of patients for better estimation of prevalence of infections as well as to find any correlation between genotype and clinical status among CF patients and other methods for detection of fungal and viral infections are recommended.

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**Authors' contributions:**

All authors contributed to the study conception and design. Material preparation and data collection were performed by Omnia Hendawy. Patient selection and management was done by Eman Mahmoud Fouda. Results analysis and discussion was written by Basma Sherif and Dalia Hosni Abdelhamid revised and approved the final manuscript.

**Declarations:** Our paper has not been published in its current form or substantially similar form elsewhere including on a website and also, it has not been accepted for publication elsewhere.

**Disclosure of potential conflicts of interest:** The authors declare they have no conflicts of interest.

**REFERENCES:**


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