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# Antimicrobial susceptibility profiles of bacterial isolates from patients with cystic fibrosis cases: a cross-sectional study

## Abstract

**Background:** Cystic fibrosis (CF) is a severely progressive genetic disorder which is characterized by chronic pulmonary infections, often due to opportunistic bacterial colonization. Antimicrobial resistance (AMR) among these pathogens shows a significant therapeutic challenge, particularly in lower middle-income countries like Bangladesh.

**Objective:** To assess the antimicrobial resistance patterns of bacterial isolates from the pediatric CF patients visited in a tertiary care hospital in Dhaka, Bangladesh.

**Methods:** This cross-sectional study included 65 children aged between 2 months to 18 years with at least one inclusion criteria of CF. Specimens, including sputum and posterior pharyngeal swabs, were collected and cultured to identify bacterial pathogens. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

**Results:** A total of 65 bacterial isolates were collected and tested. Pseudomonas aeruginosa was the most frequently isolated organism (35.4%), followed by Klebsiella pneumoniae (23.1%), E. coli (18.5%), Acinetobacter spp. (7.7%) and Staphylococcus aureus (15.4%).

**Conclusion:** The detection of P. aeruginosa and E. coli showed that antimicrobial resistance should be kept under surveillance so that the treatment can be improved for patients with CF.

**Keywords:** Cystic fibrosis, antimicrobial resistance, Pseudomonas aeruginosa, antimicrobial susceptibility, Bangladesh

Volume 16 Issue 3 - 2025

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**Received:** October 30, 2024 | **Published:** May 6, 2025

## Background

A genetic condition that mostly affects the lungs is called cystic fibrosis (CF), distinguished by thick mucus that promotes bacterial colonization and chronic infection. Cystic fibrosis (CF) is a life-limiting autosomal recessive disorder resulting from a genetic mutation targeting the cystic fibrosis trans-membrane conductance regulator (CFTR) gene.[1] Patients typically exhibit symptoms at birth or shortly thereafter, with respiratory infections and inadequate weight gain being the most common presentations.[1,2] Other classic disease manifestations are failure to thrive, chronic cough, recurrent pneumonia, chronic mal-absorption, steatorrhea, rectal prolapse and azospermia.[1,3] Subsequently, the microbiology of cystic fibrosis evolves into a more intricate landscape as Pseudomonas aeruginosa and various gram-negative non-fermenting organisms, including Stenotrophomonas maltophilia, members of the Burkholderia cepacia complex, and Alcaligenes xylosoxidans, establish themselves.[4-6]

Pseudomonas aeruginosa has traditionally been identified as the primary pathogen in cystic fibrosis, with chronic infections associated with increased morbidity and mortality related to the disease. New 'emerging' pathogens, such as fungi and uncommon bacteria, are showing up more frequently in adult and adolescent CF patients due to increased antibiotic therapy and longer patient survival rates.[8,9] However, the clinical effect of these pathogens on lung function is not well understood. More and more research is focusing on the role of certain bacteria and fungi in cystic fibrosis lung disease, including methicillin-resistant Staphylococcus aureus (MRSA), non-tuberculous mycobacteria (NTM), Stenotrophomonas maltophilia, and fungus.[1,10-13]

Both MRSA infections and Mycobacterium abscesses complex are clinically associated with a more severe progression of cystic fibrosis lung disease.[14] However, the specific impact of other emerging pathogens, including S. maltophilia, Aspergillus, Candida, and Scedosporium species, is still not clearly delineated.[8,14] The relationship between colonization by these species and its implications for cystic fibrosis (CF) lung disease is not well elucidated. It is unclear whether such colonization indicates a more advanced and vulnerable stage of the disease or if specific microbial patterns independently influence the decline in lung function. Additionally, the role of certain microbes in either facilitating or inhibiting the colonization

of other microbes during the progression of CF lung disease requires further investigation.[13] Ongoing microbiological monitoring of the involved pathogens, along with the assessment of resistance, must be prioritized highly. Therefore, studying the microorganisms isolated from posterior pharyngeal swab or sputum samples of cystic fibrosis patients with different age groups was the general objective of the study.

## Materials and methods

This cross-sectional study was carried out in a tertiary hospital in Dhaka, Bangladesh over a period of 12 months among the patient visited. Sixty-five patients were enrolled in the study in accordance with the inclusion criteria. Patients were interviewed with a structured questionnaire to fill out the required variables for all the cases. Ethical approval was obtained from the Ethical Review Committee (ERC) before conducting the study. Following an explanation of the study to the parent or legal guardian, a signed informed permission was obtained from the individual of the participant.

### Specimen collection, culture and antimicrobial susceptibility test

Sputum samples were collected in disposable containers and stored in ambient temperature which is later transferred to the microbiology laboratory of the hospital. All the specimens that were collected were inoculated and cultured in blood-agar, chocolate agar & Mac-Conkey's agar media.[15] Then streaking (spreading) was done to find out the isolated colony. Specimens were incubated overnight for 18-24 hr. at 37°C. After 24 hr. we observed for any colony growth & gram staining was done. On the 2nd day identification as well as antibiotic sensitivity were done in disk diffusion method according to the institutional protocol.[16] Additional biochemical tests for bacterial identification were performed whenever necessary. Bacterial organisms were identified by using standard laboratory cultivation protocol, morpho type analysis, growth behavior on plates.[17]

### Susceptibility testing of the bacterial strains

All bacterial strains underwent susceptibility testing using the disk diffusion technique, adhering to the criteria established by CLSI, previously recognized as NCCLS. Detection of MBL-producing P. aeruginosa strains was conducted using Etest MBL strips (AB BIODISK, Solna, Sweden), following the methodology outlined in prior studies.[18]

### Statistical analysis

SPSS software version-24 was employed to conduct the analyses. The mean and standard deviation were used to represent continuous variables. Categorical variables were represented as counts (percentages).

## Results

A total of 65 children visited the out-patient department, fulfilling inclusion and exclusion criteria, were included in this study. The mean age of all patients was 76.63±41.08 months (2-216 months) with majority belonged to 61-120 months of age (47.7%). About 45(69.2%) respondents were male and 20(30.8%) were female. Also, about 10.8% study children had family history of cystic fibrosis (Table 1).

In this study, 4 types of gram-negative bacteria were found including Pseudomonas aeruginosa (35.4%), Klebsiella pneumonae (23.1%), E. coli (18.5%), Staphylococcus aureus (15.4%), Acinetobacter spp. (7.7%) and Streptococcus pneumonae (4.6%). Besides, only one type of fungal microorganism, Candida albicans (6.2%), was also found in this study (Table 2).

**Table 1** Distribution of bacterial and fungal microorganisms in respondents with CF (n=65)

| | No of colonized patients | Percentage |
|---|---|---|
| Bacterial strains | 61 | 93.8 |
| Pseudomonas aeruginosa (mucoid and non-mucoid isolates) | 23 | 35.4 |
| Klebsiella pneumonae | 15 | 23.1 |
| Staphylococcus aureus (Methicillin resistant) | 10 | 15.4 |
| E. coli | 12 | 18.5 |
| Acinetobacter spp | 5 | 7.7 |
| Streptococcus pneumoniae | 3 | 4.6 |
| Fungal strains | | |
| Candida albicans | 4 | 6.2 |

**Table 2** Rate of susceptibility (%) of non-mucoid and mucoid P. aeruginosa isolates against different antibacterial agents

| Antibacterial agents | Rate of susceptibility (%) of P. aeruginosa | |
|---|---|---|
| | Mucoid | Non-mucoid |
| Piperacillin | 72.1 | 55.3 |
| Imipenem | 57.6 | 46.2 |
| Ceftazidime | 74.4 | 58.9 |
| Meropenem | 79.1 | 63.4 |
| Aztreonam | 78.3 | 65.3 |
| Gentamycin | 62.1 | 58 |
| Tobramycin | 73.7 | 61.2 |
| Amikacin | 49.1 | 37.8 |
| Ciprofloxacin | 56.8 | 60.5 |
| Fosfomycin | 45.1 | 33.1 |

*Susceptibility test was done according to standard laboratory protocol

*P. aeruginosa* for both mucoid and non-mucoid showed a consistent increase in different age groups. In the study, mucoid P. aeruginosa isolates showed much higher antimicrobial susceptibility than the non-mucoid strains (Table 3&4).

**Table 3** Rate of susceptibility (%) of methicillin susceptible and methicillin resistant of S. aureus antibacterial agents

| Antibacterial agents | Rate of susceptibility (%) of S. aureus | |
|---|---|---|
| | Methicillin susceptible | Methicillin resistant |
| Penicillin | 39.1 | 0 |
| Amoxicillin/clavulanate | 97 | 0 |
| Cefuroxime | 95 | 0 |
| Gentamicin | 90 | 70 |
| Vancomycin | 93 | 95 |
| Teicoplanin | 96 | 100 |
| Ciprofloxacin | 81 | 73 |
| Linezolid | 100 | 100 |
| Fosfomycin | 95 | 100 |

Among the MRSA there is no resistance against Teicoplanin, linezolid, Fosfomycin occurred.

**Citation:** Farhana T, Mahmud S, Tasneem F, et al. Antimicrobial susceptibility profiles of bacterial isolates from patients with cystic fibrosis cases: a cross-sectional study. *Gastroenterol Hepatol Open Access*. 2025;16(3):73–76. DOI: 10.15406/ghoa.2025.16.00611

**Table 4** Rate of susceptibility (%) of E. coli isolates against different antibacterial agents

| Antibacterial agents | Rate of susceptibility (%) E. coli |
|---|---|
| Piperacillin | 75 |
| Imipenem | 75 |
| Ceftazidime | 58.3 |
| Meropenem | 66.7 |
| Gentamycin | 83.3 |
| Tobramycin | 75 |
| Amikacin | 100 |
| Ciprofloxacin | 66.7 |

All the E. coli isolates showed a higher percentage of susceptibility towards all the antibacterial agents where else 100% of the E. coli isolates were found to be susceptible to Amikacin.

## Discussion

The susceptibility of microbial organisms isolated from respiratory samples with CF patients were explained in different studies.[5,19-22] Other investigations have also indicated that chronic respiratory tract colonization in cystic fibrosis patients is attributable to bacteria such as Staphylococcus aureus and Pseudomonas aeruginosa. According to a previous study we also observed that S. aureus P. aeruginosa and were the two most found isolated bacteria from the sputa of CF patients in their first decade of life.[6] The rate of methicillin resistance in cystic fibrosis patients was reported to be as high as 18.8% in the United States,[5] 25.9% in Argentina,[19] 6% in Poland[20] and 18% in Spain.[21] The variation in MRSA rates among CF patients correlates with the nosocomial prevalence of MRSA in each country.

Our study indicates that mucoid P. aeruginosa isolates demonstrate significantly greater antimicrobial susceptibility compared to non-mucoid isolates. In terms of antimicrobial susceptibility, mucoid strains found in respiratory samples from cystic fibrosis patients are associated with a poor prognosis due to their production of exopolysaccharide/alginate, which confers resistance to phagocytosis. Moreover, they significantly contribute to the progression of lung disease in cystic fibrosis compared to non-mucoid P. aeruginosa.[23-26]

Future studies may be essential to determine whether the presence of these microorganisms correlates with the deterioration of clinical parameters in patients with cystic fibrosis.

## Conclusion

This study found that majority of cystic fibrosis children had bacterial infections and few with fungal infections. Pseudomonas aeruginosa, K. pneumonae, E. coli and S. aureus were the most common isolated bacteria which were highly susceptible to Levofloxacin, Imipenem, Amikacin and Ciprofloxacin respectively. Therefore, antimicrobial resistance patterns need to be put under the surveillance. Further similar studies might be helpful to better understand the influence of these microorganisms in CF patients.

## Availability of data and materials

The dataset used in the current study is available from the corresponding author on reasonable request.

## Funding

The study is self-funded.

## Conflict of interest

There was no conflict of interest.

## Acknowledgement

The authors thank all the people responsible whose feedback helped to improve the paper.

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**Citation:** Farhana T, Mahmud S, Tasneem F, et al. Antimicrobial susceptibility profiles of bacterial isolates from patients with cystic fibrosis cases: a cross-sectional study. *Gastroenterol Hepatol Open Access.* 2025;16(3):73–76. DOI: 10.15406/ghoa.2025.16.00611