

ANTIMICROBIAL SENSITIVITY PATTERN OF PEDIATRIC CYSTIC FIBROSIS PATIENTS IN PAKISTAN

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Abstract: Cystic fibrosis (CF) is a complex genetic disorder affecting the respiratory and digestive systems, causing recurrent infections. This study aimed to evaluate the microbiological cultures and identify the patterns of antimicrobial sensitivity among young CF patients in Pakistan. This prospective observational study was conducted at the Pakistan Institute of Medical Sciences (PIMS) from December 2021 to November 2022, involving comprehensive assessments, interviews, clinical tests, MTs, CBCs, CXRs, sputum cultures, RBS measurements, microbiological analysis of sputum and oropharyngeal swabs, antimicrobial susceptibility testing, and statistical analyses ($p \leq 0.05$) using SPSS for both continuous and categorized data. *Pseudomonas Aeruginosa* was the most common respiratory pathogen ($n=21$; 36%), and most CF-afflicted children ($n=46$; 77.96%) had infections with a single microorganism. Significant associations ($p < 0.05$) were observed between *Streptococcus pneumoniae*, *Klebsiella Pneumoniae*, *Staphylococcus Aureus*, and *Acinetobacter* with their antibiotic sensitivity patterns. In contrast, *Pseudomonas Aeruginosa* and *Escherichia Coli* showed no statistically significant associations. Ciprofloxacin demonstrated high sensitivity, followed by Levofloxacin, while several first-generation antibiotics, including rifampicin, erythromycin, and amoxicillin, exhibited nearly complete resistance against bacteria collected from children with CF. Ciprofloxacin and Levofloxacin emerge as the foremost effective therapeutic agents for CF patients, advocating the perpetual promotion of active surveillance to monitor resistance patterns.

Keywords: Antimicrobial Sensitivity, Pediatric Cystic Fibrosis, Microbial Prevalence, Respiratory Infections

Introduction

Cystic fibrosis (CF) is a complex and inherited genetic disorder primarily affecting the respiratory and digestive systems. It is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, producing thick and sticky mucus, which obstructs airways and promotes bacterial colonization, resulting in recurrent respiratory infections (Almughem et al., 2020; Dhooghe et al., 2013; Florescu et al., 2014; Gbian and Omri, 2021; Manik et al., 2023; Martínez-Alemán et al., 2017). Antimicrobial therapy is a cornerstone in treating CF-related infections, aiming to control microbial growth and improve patients' overall health outcomes. However, the emergence of antimicrobial resistance has become a growing concern, compromising the effectiveness of these therapeutic agents and limiting treatment options (Junkins et al., 2014; Klinger-Strobel et al., 2015; Reece et al., 2017).

In Pakistan, where the burden of CF is rising, understanding common pathogens' susceptibility to antimicrobial agents is crucial for optimizing therapeutic approaches (Okeke et al., 2005; Redondo et al., 2016). Unfortunately, limited research exists on this vital aspect of CF treatment in Pakistan. Hence, this study aimed to evaluate the microbiological cultures and identify the patterns of antimicrobial sensitivity among young CF patients in Pakistan.

Understanding the antimicrobial sensitivity pattern in pediatric CF patients can empower healthcare practitioners to tailor treatment approaches more effectively, leading to improved clinical outcomes, shorter hospital stays, and an enhanced quality of life for affected children. Additionally, the data obtained from this study may contribute to the formulation of targeted antimicrobial stewardship programs, which are crucial for preserving the efficacy of existing antibiotics and addressing the rising tide of antimicrobial resistance in Pakistan. Ultimately, this research aims to make a valuable and impactful contribution to pediatric CF care in Pakistan while providing valuable insights to tackle the broader global challenge of antimicrobial resistance.

Methodology

This study was conducted as prospective observational research at Divisional Headquarters Teaching Hospital KDA Kohat and Lady Reading Hospital Pulmonology Department Peshawar from December 2021 to November 2022.

Ethical standards were strictly followed, and the Kohat University of Science and Technology, Kohat, Pakistan, research ethics committee approved the study. Every patient

who participated in the study provided a written, signed consent form before the study.

Patients aged 0-18 years diagnosed with CF based on clinical criteria and measured sweat chloride level ≥ 90 mmol/L were considered for the study. Patients who provided written informed consent or whose legal guardians provided written informed consent were included in the study.

Patients with clinical features suggestive of aspiration pneumonia, congenital diaphragmatic hernia, foreign body aspiration, congenital lobar emphysema, pulmonary tuberculosis, asthma, primary immunodeficiency, congenital heart disease, and pulmonary abscess were excluded from the research. Patients unable or unwilling to provide informed consent or cooperate with the study procedures were also excluded.

This study's diagnostic evaluation and data collection process involved comprehensive assessments to ensure a thorough understanding of the pediatric CF patients' health status. Parents were actively engaged in the study as they were interviewed, providing valuable insights into the patient's medical history. A structured questionnaire was utilized to systematically gather essential information for each case. Additionally, clinical tests and investigations were conducted, including Mantoux tests (MTs), complete blood counts (CBCs), chest X-rays (CXRs), cultures of antibiograms from oropharyngeal swabs, and sputum and random blood sugar (RBS) measurements.

Sputum samples were gathered, placed in sterilized containers supplied by the lab, secured with screws, and tagged. Parents were told to keep their kids off meals for an hour before pick-up. Oropharyngeal swabs were taken from participants who were unwilling to produce saliva on their own with the help of their parents or by requesting the kid to open their lips while a sterilized cotton swab stick was softly rubbed over the wall of the pharynx and then placed in a sterilized plastic bottle. After being collected, samples were delivered to the same institution's microbiological lab within an hour. Incubation occurred at 37 °C for a full day after samples had been inserted into Blood agar, Mac-Conkey's, and chocolate agar culture. After 24 hours,

colonies were detected, subculture, and exposed to biochemical processes for microbe confirmation.

To assess the effectiveness of antimicrobial agents against isolated pathogens, the Kirby Bauer modified disc diffusion technique was employed. A wide range of antibiotic discs, including aztreonam, levofloxacin, tobramycin, vancomycin, ciprofloxacin, piperacillin, timentin, cotrimoxazole, gentamicin, imipenem, meropenem, clindamycin, erythromycin, ceftazidime, moxycillin, rifampicin, linezolid, cefepime, and amikacin, were utilized in the testing process. Careful maintenance of the proper concentrations of these drugs was ensured during the experimentation.

The statistical analysis was performed using SPSS software version 26. Continuous variables were presented as the mean and standard deviation, which provides an average value along with the variability in the data. Categorical variables were expressed as counts and percentages, showing the frequency and proportion of each category. Nonparametric Fischer exact tests were employed to analyze certain data sets. The level of statistical significance was set at $p \leq 0.05$, indicating that results with a p-value less than or equal to 0.05 were considered statistically significant.

Results

The study included a total of 80 children diagnosed with CF. among these patients, 54 were boys (67.5%) and 26 were girls (32.5%). The age distribution revealed that 13 patients (16.25%) were aged ≤ 1 year, 45 patients (56.25%) fell between 1 and 12 years old, and 22 patients (27.50%) were in the age range of 12 to 18 years. Concerning the consanguinity of marriage between parents, 59 patients (73.75%) had parents with a consanguineous marriage, while 21 patients (26.25%) had a non-consanguineous marriage. Regarding family history, 51 patients (63.75%) had a positive family history of CF, while 29 patients (36.25%) did not have a family history of the condition (table 1).

Table 1: Demographic Characteristics of Pediatric CF Patients in Pakistan

Characteristics	Number of Patients (n)	Percentage (%)	P-Values
Gender Distribution			
Boys	54	67.5	0.002
Girls	26	32.5	
Age Groups			
≤ 1	13	16.25	0.466
$>1-12$	45	56.25	
$>12-18$	22	27.50	
Consanguinity of marriage between parents			
Present	59	73.75	0.0003
Absent	21	26.25	
Family history of CF			
Yes	51	63.75	0.022.
No	29	36.25	

The analysis of respiratory specimens from pediatric CF patients demonstrated a positive microbial presence in 73.75% of children (n=59), as depicted in Figure 1. Out of the 59 participants, 46 (77.96%) displayed the presence of a single microorganism, while 13 patients (22.04%) had the

identification of two distinct microorganisms. Specifically, among infants aged ≤ 1 year, 84.61% of participants (n=11) tested positive for microorganisms, while 68.89% (n=31) of children aged >1 and ≤ 12 years, and 77.27% (n=17) of those aged >12 and ≤ 18 years tested positive (Fig. 1). However,

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the differences in microorganism presence across different age groups were not statistically significant (p=0.362). Among the respiratory specimens obtained from pediatric

CF patients, the most frequently identified microorganism was *Pseudomonas Aeruginosa* (n=21; 36%), followed by *Klebsiella pneumonia* (n=13; 22%), as shown in Figure 2.

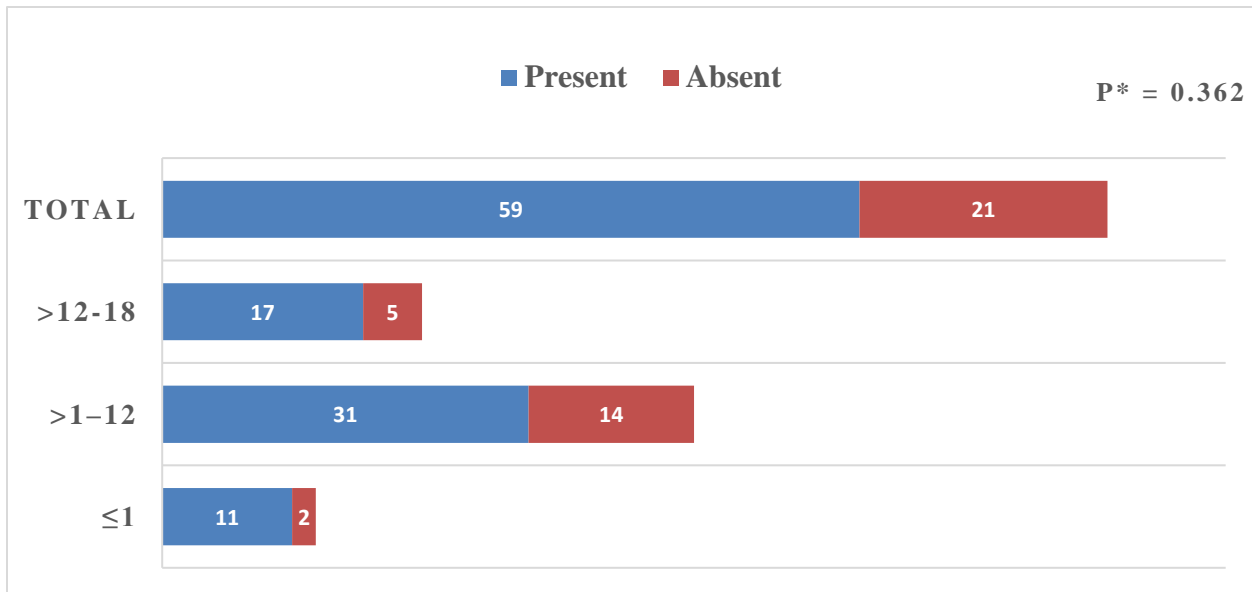


Figure 1: Age-wise distribution of microbial presence in respiratory specimens of pediatric CF patients (n=80); Fisher's test was used to obtain the p-value.

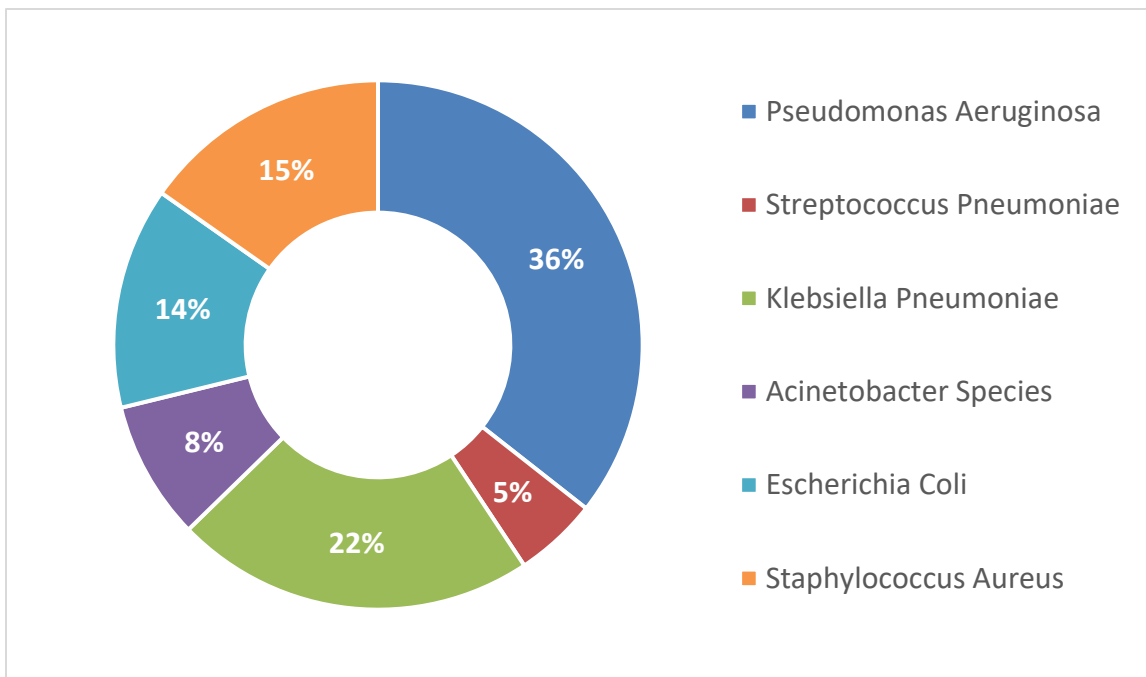


Figure 2: Microbial identification from respiratory specimen cultures in pediatric CF patients

The antibiotic sensitivity patterns of microbes collected from samples of children having CF's respiratory system are shown in Table 2. Ciprofloxacin and Levofloxacin showed the highest levels of antimicrobial sensitivity across all bacterial strains, while Amoxicillin, Erythromycin, and Rifampicin showed pronounced resistance. Additionally, vancomycin showed consistent sensitivity against *Staphylococcus Aureus* and *Streptococcus Pneumoniae*,

while Meropenem and Ceftazidime displayed variable sensitivity across bacterial species, showing particularly notable effectiveness against *Pseudomonas Aeruginosa*. Imipenem and cotrimoxazole also maintained relatively favorable sensitivity profiles across various species, particularly *Streptococcus Pneumoniae* and *Acinetobacter Species*.

Table 2: Antimicrobial sensitivity patterns across various bacterial species

Antibiotic Sensitivity pattern		<i>Pseudomonas Aeruginosa</i>	<i>Streptococcus pneumoniae</i>	<i>Klebsiella Pneumoniae</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Acinetobacter Species</i>
Ceftazidime	Sensitive	14	0	5	2	2	2
	Resistant	5	3	8	5	5	3
	Intermediate	2	0	0	1	2	0
Vancomycin	Sensitive	8	3	2	3	7	1
	Resistant	11	0	9	5	2	4
	Intermediate	2	0	1	0	0	0
Amoxicillin	Sensitive	0	0	0	0	0	0
	Resistant	21	3	13	8	9	5
	Intermediate	0	0	0	0	0	0
Imipenem	Sensitive	7	3	5	2	3	3
	Resistant	9	0	6	5	5	0
	Intermediate	5	0	2	1	1	2
Cefepime	Sensitive	6	0	2	2	3	1
	Resistant	13	2	9	4	6	1
	Intermediate	2	1	1	2	0	3
Gentamicin	Sensitive	5	0	4	3	3	2
	Resistant	14	2	8	4	6	3
	Intermediate	2	1	1	1	0	0
Linezolid	Sensitive	7	3	4	5	4	2
	Resistant	13	0	6	3	4	3
	Intermediate	1	0	3	0	1	0
Meropenem	Sensitive	18	2	6	5	4	4
	Resistant	2	0	5	1	2	0
	Intermediate	1	1	2	2	3	1
Cotrimoxazole	Sensitive	6	3	2	3	0	5
	Resistant	11	0	7	2	4	0
	Intermediate	4	0	4	3	5	0
Erythromycin	Sensitive	0	0	0	0	0	0
	Resistant	19	3	12	8	9	4
	Intermediate	2	0	1	0	0	1
Amikacin	Sensitive	12	2	4	4	6	3
	Resistant	9	1	9	4	3	2
	Intermediate	0	0	0	0	0	0
Levofloxacin	Sensitive	20	3	11	6	8	4
	Resistant	1	0	2	2	1	1
	Intermediate	0	0	0	0	0	0
Ciprofloxacin	Sensitive	21	3	13	8	9	5
	Resistant	0	0	0	0	0	0
	Intermediate	0	0	0	0	0	0
Rifampicin	Sensitive	0	0	0	0	0	0
	Resistant	18	3	9	4	8	2
	Intermediate	3	0	4	4	1	3

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The table 3 exhibits the outcomes of chi-square tests, which demonstrate the statistical relationships between various bacterial species (*P. Aeruginosa*, *S. Pneumoniae*, *K. Pneumoniae*, *E. Coli*, *S. Aureus*, and *Acinetobacter*) and their corresponding patterns of antibiotic sensitivity. The Chi-Square values obtained for each bacterial species, namely 24.857, 25.429, 21.143, 11.714, 23.238, and 15.429, indicate the degree of connection between variables. The degrees of freedom (df) differ between species as follows:

17, 3, 12, 7, 9, and 5, respectively. The asymptotic significance values (0.098, 0.000, 0.048, 0.110, 0.006, and 0.009) correspond to the chi-square statistics and reflect the statistical significance of the association between bacterial species and antibiotic sensitivity patterns. These values indicate the p-values obtained from the asymptotic distribution, demonstrating the level of significance in the connection.

Table 3: Chi-square test results for the association between bacterial species and antibiotic sensitivity patterns

	<i>Pseudomonas Aeruginosa</i>	<i>S. Pneumoniae</i>	<i>K. Pneumoniae</i>	<i>E. Coli</i>	<i>S. Aureus</i>	<i>Acinetobacter</i>
Chi-Square	24.857	25.429	21.143	11.714	23.238	15.429
df	17	3	12	7	9	5
Asymp. Sig.	.098	.000	.048	.110	.006	.009

Discussion

The research focused on assessing the antimicrobial sensitivity pattern among pediatric CF patients in Pakistan, addressing a critical gap in understanding CF-related infections in this population. CF is a genetically inherited disorder with significant implications for the respiratory and digestive systems, often leading to recurrent respiratory infections due to compromised mucus clearance and bacterial colonization. Given the importance of effective antimicrobial therapy in managing CF-related infections, understanding the susceptibility of common pathogens to antibiotics is crucial for guiding treatment strategies.

A fascinating aspect of CF was brought to light by the study's important observation of a high prevalence of consanguinity (n=59; 73.75%) among CF patients in the Pakistani community. According to comparisons with past studies (Aziz et al., 2017; Bhinder et al., 2019), Pakistan has greater consanguinity rates than several other nations. This data raises the possibility that cultural norms surrounding consanguineous marriages and genetic and hereditary factors may contribute to Pakistan's higher prevalence of CF.

Our research findings revealed *P. Aeruginosa* (n=21; 36%) as the predominant isolate among respiratory pathogens, with the added insight that more than three-quarters (n=46; 77.96%) of children with CF displayed infections attributed to a single microorganism. Our results are consistent with those reached by Kabir et al. (Kabir et al., 2020), who conducted a thorough clinical-epidemiologic examination among CF kids in Bangladesh and discovered a significant incidence of *P. aeruginosa* (27%). Furthermore, *S. aureus* isolates predominated in 54% of specimens, according to Nobandegani et al. (Nobandegani et al., 2016), whereas *P. aeruginosa* was found in 30% of cases. Similar results were made by Valenza et al. (Valenza et al., 2008), who found that *S. aureus* (63.3%) and *P. aeruginosa* (50%) were prevalent among their individuals.

The antimicrobial sensitivity patterns examined in our current study revealed ciprofloxacin to be the most sensitive, followed by Levofloxacin. But, other first-generation antibiotics, such as rifampicin, erythromycin, and amoxicillin, showed total resistance to bacteria isolated from kids with CF. Imipenem and cotrimoxazole also maintained relatively favorable sensitivity profiles across various species, particularly about *S. Pneumoniae* and

Acinetobacter Species. Our results support the findings of Chang et al. (Chang et al., 2019), Hamzah et al. (Hamzah et al., 2019), and Carroll et al. (Carroll et al., 2016). It's common knowledge that excessive antibiotic usage and poor patient compliance are to blame for the quick evolution of drug-resistant bacteria, some showing resistance to numerous medications. As a result, it becomes imperative to frequently update antibiotic guidelines based on continuously evolving research insights pertaining to a country's specific context.

Conclusion

In conclusion, this study provides crucial insights into the antimicrobial sensitivity patterns among pediatric CF patients in Pakistan. The prevalence of *P. Aeruginosa* as a dominant isolate emphasizes the need for tailored treatment approaches. While Ciprofloxacin and Levofloxacin exhibited notable sensitivity, several first-generation antibiotics showed high resistance, highlighting the urgency of judicious antibiotic use. This research underscores the significance of continuous surveillance and updating antibiotic guidelines to combat rising antimicrobial resistance. By guiding effective treatment strategies, these findings aim to enhance the clinical outcomes and quality of life for children with CF in Pakistan and contribute to the global efforts against antimicrobial resistance.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department Concerned.

Consent for publication

Approved

Funding

Not applicable

Conflict of interest

The authors declared absence of conflict of interest.

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