ORIGINAL ARTICLE

Unveiling the Microbial Landscape: Chronic Airway Colonization in Egyptian Children with Cystic Fibrosis

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ABSTRACT

Background: *Cystic fibrosis (CF) is a genetically recessive disease marked by frequent, chronic lung infections that impair lung function and cause early death.* ***Objectives:*** *our aim was to identify the bacteria colonizing the airways among a cohort of Egyptian children with CF and to assess their antimicrobial susceptibility and biofilm formation capacity.* ***Methodology:*** *In this cross-sectional study, we assessed the prevalence of bacterial species colonizing airways of 34 Egyptian pediatric patients with CF. The isolated pathogens underwent antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method and biofilm formation analysis via a microtiter plate assay.* ***Results:*** *In a total of 43 isolated pathogens, the most frequently isolated microorganisms were Staph aureus (S. aureus) (51.2%) followed by Pseudomonas aeruginosa (P. aeruginosa) (34.9%). Other less prevalent bacterial isolates included E. coli, Klebsiella spp., and Acinetobacter spp. (4.7% each). Regarding antibiotic susceptibility profiles, S. aureus demonstrated high resistance to penicillin and cefoxitin (95.5% and 81.8% respectively) followed with erythromycin and tetracycline, with resistance rates of 68.2% and 45.5% respectively. Alarmingly, a high prevalence of Methicillin-Resistant S. aureus (MRSA) was observed among our tested isolates (81.8%). Among P. aeruginosa isolates, cefepime and imipenem exhibited the highest resistance rates (26.7% each), followed by tobramycin (20%). All isolated pathogens produced biofilm with varying degrees. Among them 50% of S. aureus and 66.7% of P. aeruginosa were strong biofilm producers.* ***Conclusion:*** *There was a significantly higher prevalence of S. aureus chronic colonization among our CF patients. Meanwhile chronic colonization with P. aeruginosa was less prevalent. Unfortunately, there was a high prevalence of MRSA and biofilm formation among the isolated bacteria. Implementing strict preventive measures and infection control can help prevent MRSA-related exacerbations in these patients.*

INTRODUCTION

Cystic fibrosis (CF) is a prevalent recessive genetic disease that can shorten life expectancy and potentially be fatal [1]. While the disease is predominantly found among European, North American, and Australian populations, it can affect people from diverse racial and ethnic backgrounds [2]. In the Middle East, occurrence is predicted to be 1 in 30,000–50,000, with an incidence rate of 1 in 2,000 to 5,800 live births [3]. CF is triggered by a mutation in the transmembrane conductance regulator (CFTR) gene, that encodes for an ion channel, its malfunction leads to increased mucus density in the lungs of CF individuals [4]. The dehydrated, sticky mucus and impaired mucous clearance create a perfect setting for bacterial colonization, leading to persistent pulmonary infections [5]. The ongoing cycle of infections, inflammation, and tissue damage in cystic fibrosis patients leads to a gradual deterioration of lung function. This is the main reason for illness and death among people with CF [6].

Approximately one-third of CF patients suffer from pulmonary complications that emerge during early years of life and exhibit radiological signs of bronchiectasis [7]. In the early stages of the disease, *Staphylococcus aureus* (*S. aureus*) and *Hemophilus influenzae (H. influenzae)* are the predominant pathogens; however, as patients age and disease progresses, *Pseudomonas aeruginosa (P. aeruginosa*) becomes the most common and problematic bacteria in CF lungs [8,9]. Pathogens continuously evolve both physically and genetically in response to the hostile lung conditions, the body's immune defenses, and antibiotic treatments, as long as the infection endures [10]. Once become established in the CF lungs, certain pathogens, like *Pseudomonas aeruginosa* and MRSA, can be hard to eliminate. Early detection and aggressive treatment may effectively clear these infections, improving outcomes for CF patients [11]

While chronic infection in the CF airway is often termed 'airway colonization,' the presence of these bacteria is far from harmless. For instance, *P. aeruginosa* infection is a known risk factor linked to a faster deterioration of lung function and shorter lifespan, with the shift to its mucoid form worsening the outlook [12]. MRSA infection is also recognized for its potential to worsen lung function [11]. Several factors can help bacteria survive in the lungs of CF patients, even with aggressive treatment. These include poor antibiotic penetration into thick airway mucus, antibiotic resistance (either inherent or acquired), defects in CF-related mucosal defenses, and the presence of bacterial biofilms that can neutralize antibiotics or hinder host defenses [13].

In line with Cystic Fibrosis Foundation (CFF) guidelines, it is recommended that periodic surveillance cultures of sputum or throat swabs from CF patients be conducted. This helps monitor the acquisition of *P. aeruginosa*, allowing for early treatment with an eradication protocol, and supports the initiation of chronic inhaled antibiotic therapy for those with persistent colonization [14]. Additionally, these surveillance cultures are useful in guiding antibiotic selection during acute exacerbations, recognizing that most exacerbations are not linked to the emergence of novel bacterial species or strains. Investigating the occurrence and antimicrobial resistance patterns of these colonizing bacteria within the country's context is crucial for effectively managing lung infections in CF patients [15]. Due to the limited available research on the types of bacteria infecting CF patients in the Middle East, especially Egypt [16]. In this study we aimed to identify the bacteria colonizing the airways among a cohort of Egyptian children with CF. Additionally, we assessed bacterial antimicrobial susceptibility and biofilm formation capacity of the isolated bacteria.

METHODOLOGY

Study design and patients:

This study was carried out over a period between August 2023 and March 2024 on pediatric patients admitted or following up at the CF Center of Allergy and Pulmonology Unit, Children's Hospital, Faculty of Medicine, Cairo University. Prior to initiating the study, ethical approval was obtained from the institutional review board (IRB) (code: MS-181-2023). Written informed consent was secured from the parents of the participating children. Patients were included in this study if they met the criteria for CF, which included characteristic clinical symptoms, a sweat chloride level of 60 mmol/L or higher, and/or mutations in the CFTR gene [17].

Sample collection and culture:

Sputum samples were collected from patients in sterile containers provided by the lab. Parents were asked to ensure their children did not eat for an hour before providing samples. Children were asked to perform a water mouth rinse, cough, and then expectorate sputum into the container. Samples with excessive saliva were discarded [18]. The collected samples were immediately transferred to the microbiology lab within the same facility and incubated for 24 hours at 37°C on various culture media, including blood, chocolate, and MacConkey agar. Following 24-hour incubation period, colonies were recognized, and subjected to biochemical reactions in accordance with conventional microbiological methods [19].

Antimicrobial Susceptibility Testing:

The susceptibility of bacteria to antibiotics was determined using the Kirby-Bauer method, in compliance with the Clinical and Laboratory Standards Institute (CLSI, 2023) recommendations [20]. The antibiotic susceptibility of *S. aureus* strains was determined by testing the following drugs: penicillin (10µg), cefoxitin (30µg), trimethoprim sulfamethoxazole (1.25/23.75µg), clindamycin (2 µg), erythromycin (15µg), tetracycline (30µg), doxycycline (30µg), levofloxacin (5µg), linezolid (30µg), and The following antibiotics were tested for Gram-negative isolates: ampicillin-sulbactam (20µg), piperacillin-tazobactam (100/ 10µg), cefoxitin (30µg), ceftriaxone (30µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), imipenem (10µg), meropenem (10µg), gentamicin (10µg), tobramycin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), doxycycline (30µg), and TMP/SMX (1.25/23.75µg). All the antibiotic discs were purchased from (Biomaxima, Poland). The strain of *E. coli* ATCC 25922 was employed as the control. In compliance with CLSI 2023 guidelines, 30 µg cefoxitin antibiotic discs (Biomaxima, Poland) were used to identify isolates of MRSA. Bacteria were classified as multidrug-resistant (MDR) if they were resistant to at least one antibiotic in three or more different antibiotic groups.

Minimum inhibitory concentration (MIC) of vancomycin by agar dilution method [21]:

Following the protocols advised by the CLSI, MIC of vancomycin for each MRSA strain was determined using the agar dilution method [20]. Muller Hinton Agar (MHA) plates containing vancomycin concentrations from 0.25 to 32 µg/ml were prepared. MRSA isolates were inoculated in nutrient broth and incubated at 37 °C for 24 h. Bacterial suspensions of each isolate were prepared equivalent to 0.5 McFarland and a 10 µL drop of each suspension was inoculated on the prepared plates containing different vancomycin concentrations and incubated for 24 hours at 35 °C. MIC was defined as the highest concentration of the antibiotic that visibly prevented the growth of tested organism. The results were interpreted according to CLSI 2023 guidelines.

Biofilm Formation Assay:

By employing the tissue culture plate approach, the ability of bacterial isolates to produce biofilms was quantified as previously described [22]. In brief, fresh overnight cultures of each isolate were added to 10 ml of trypticase soy broth (TSB), and the tubes were incubated overnight at 37°C. After adjusting broth's turbidity to meet the 0.5 McFarland standard, fresh TSB was added to dilute the mixture to a ratio of 1:100. A 96-well polystyrene microtiter plate with a flat bottom was then inoculated in triplicate with 200 µL of each bacterial suspension. Each well's contents was disposed of after an overnight incubation period at 37°C, and the wells were rinsed three times using phosphate-buffered saline (PBS). After that, fixation was achieved by adding around 200 microliters of methanol. Each well was allowed to air dry before being stained with 200 µl of a 0.1% crystal violet solution for 30 minutes. The plates were then washed with sterile distilled water and allowed to dry naturally. Eventually, the stain was dissolved by adding 200 microliters of 33% glacial acetic acid. Using the Micro ELISA auto reader State Fax-2100 (GMI, Germany), the optical density (OD) of each well was measured at 570 nm. Uninoculated media was regarded as a negative control sample, while *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 were employed as positive controls. A cutoff value was determined based on the average OD of the negative control samples plus three standard deviations. This cutoff was used to categorize the bacteria into four biofilm formation groups: non-biofilm producers (OD test less than ODC), weak biofilm producers (ODC<OD test < 2ODC), moderate biofilm producers (2ODC <OD test < 4ODC), and strong biofilm producers (4ODC less than OD test).

Statistical analysis:

Data were entered and organized using SPSS statistical software. Descriptive statistics were employed. Mean, standard deviation, median, minimum, and maximum values were calculated for numerical data. For categorical data, frequencies and percentages were determined. To compare groups, non-parametric tests (Mann-Whitney and Kruskal-Wallis) were utilized for numerical data. To compare different groups based on categorical data, we used the Chi-square test. However, if the expected number of cases in any category was fewer than five, we employed an exact test instead. A p-value of less than 0.05 was used to establish statistical significance.

RESULTS

Study population:

A total of 34 children with cystic fibrosis were enrolled in the study. The mean age of participants was 7.59 ± 3.69 years. Females constituted 58.8% of the cohort, while males comprised 41.2%. The predominant age group was 6-10 years, accounting for 50% of the sample, whereas the least represented age group was less than two years, comprising 5.9%. Among 34 children with CF patients, 41.1% of their parents had consanguinity of marriage (**Table 1**).

Table 1: Demographics of pediatric cystic fibrosis patients

Variable	**n (%)**
Age (years)	
Median (min-max)	7 (1-14)
Age group	
<2	2 (5.9%)
2-5	7 (20.6%)
6-10	17 (50%)
11-14	8 (23.5%)
Gender	
Male	14 (41.2%)
Female	20 (58.8%)
Parental consanguinity	
Present	14 (41.1%)
Absent	20 (58.9%)

Prevalence of microbial isolates:

A total of 43 bacterial isolates were retrieved from 34 respiratory samples. Gram-positive bacteria constituted 51.2% of isolates (n=22), all of them were *S. aureus*. Of the *S. aureus* samples, 81.8% were identified as MRSA and 18.2% as MSSA. Gram-negative bacteria accounted for 48.8% (n=21).

Among different species, *S. aureus* was the predominant species, representing 51.2% (n=22) of all isolates, followed by *P. aeruginosa* at 34.9% (n=15). Less frequently isolated organisms included *Klebsiella spp.*, *Escherichia coli (E. coli)*, and *Acinetobacter spp.*, each comprising 4.7% (n=2) of the total. The distribution of bacterial species across age groups is illustrated in **Figure 1.**

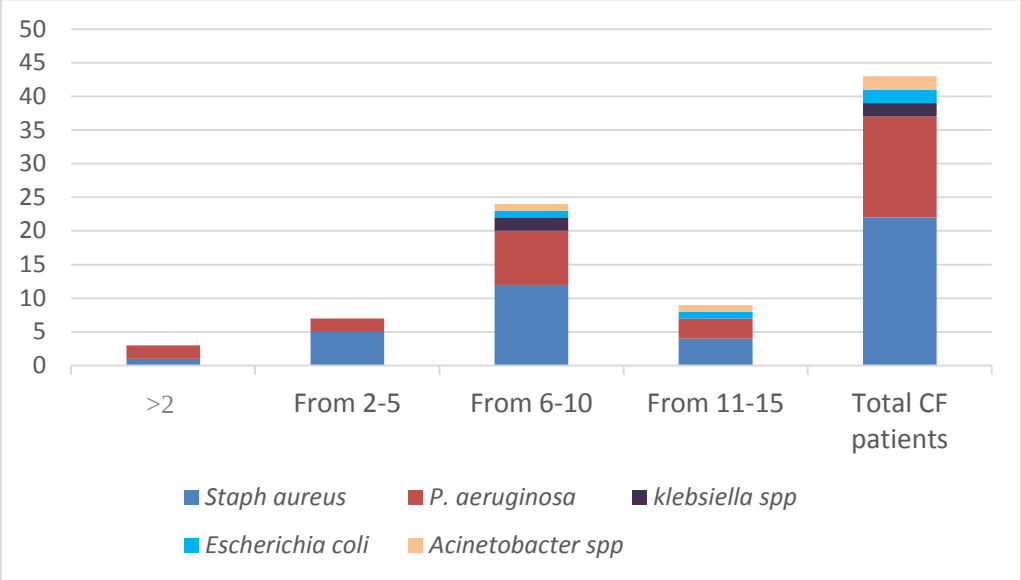

Fig 1: Prevalence of bacterial strains among all patients and within different age groups.

Monomicrobial and polymicrobial infections were observed in 52.9% (n=18) and 35.3% (n=12) of cases, respectively. On the other hand, no pathogenic bacteria were recovered from respiratory specimens of 11.8% of patients (n=4). Co-infection with *S. aureus* and *P. aeruginosa* occurred in 26.5% (n=9) of patients, predominantly affecting the 6-10 age group (88%). Other less prevalent microbial co-existence include (*S. aureus and Acinetobacter spp.,)* which occurred in two patients while only one patient had co-infection with *S. aureus and klebsiella spp.*

Antimicrobial susceptibility testing:

Antibiotic testing revealed that *S. aureus* bacteria were most resistant to penicillin and cefoxitin; (95.5%, 81.8% respectively) followed by erythromycin, and tetracycline (68.2%, 45.5% respectively) (**Figure 2**). None of the isolates showed resistance to vancomycin by agar dilution method. Among *P. aeruginosa* strains, Resistance rates were highest for cefepime and imipenem (26.7% each), followed by tobramycin (20%). (**Figure 3**). Antibiotic susceptibility rates among less prevalent bacteria isolated from CF patients are presented in **Table 2.**

A total of 20 isolates (46.5%) were classified as MDR, showing resistance to at least one antibiotic from three distinct drug groups, most of them were *S. aureus* (80%).

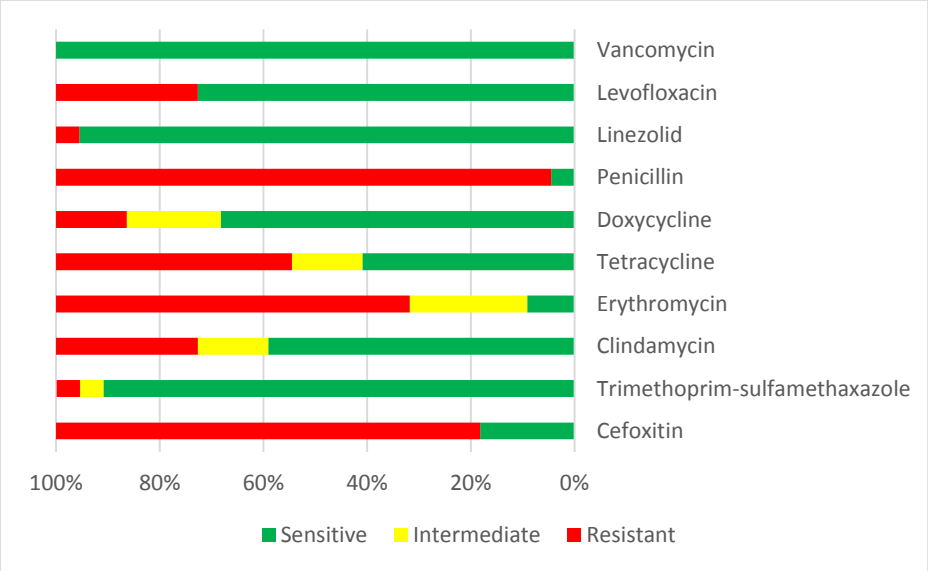

Fig 2: Rate of *in vitro* susceptibility (%) of *S. aureus* isolates against different antibiotics.

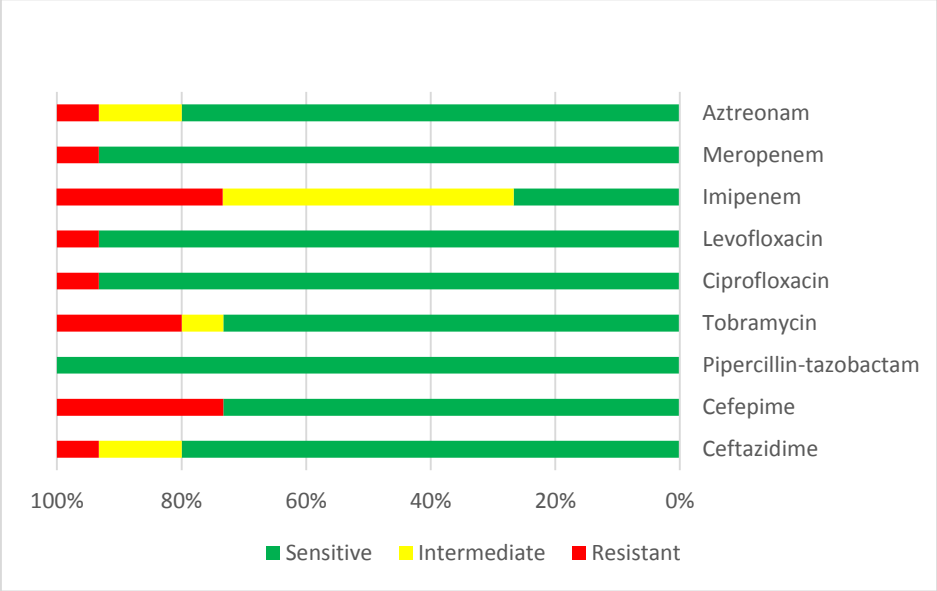

Fig 3: Rate of *in vitro* susceptibility (%) of *P. aeruginosa* isolates against different antibiotics.

Table 2: Antibiotic susceptibility rates among less prevalent bacteria isolated from cystic fibrosis patients

Bacteria **Antibiotic**	***Klebsiella spp.*** **N (%)**			***Escherichia coli*** **N (%)**		***Acinetobacter spp.*** **N (%)**	
	S	**I**	**R**	***S***	***R***	***S***	***R***
Ampicillin-sulbactam	2 (100%)	0 (0%)	0 (0%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)
Piperacillin-tazobactam	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)
Cefoxitin	2 (100%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	-	
ceftriaxone	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	0 (0%)	2 (100%)
Ceftazidime	0 (0%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)
Cefepime	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	0 (0%)	2 (100%)
Aztreonam	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Imipenem	0 (0%)	2(100%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Meropenem	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Gentamicin	0 (0%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Tobramycin	2 (100%)	0 (0%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Ciprofloxacin	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)
Doxycycline	0 (0%)	0 (0%)	2 (100%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Trimethoprim-sulfamethoxazole	0 (0%)	0 (0%)	2 (100%)	1 (50%)	1 (50%)	2 (100%)	0 (0%)

Quantitative biofilm formation assay:

The microtiter plate assay revealed biofilm formation in all bacterial isolates tested. For the 22 *S. aureus* isolates, 11 isolates (50%) were strong biofilm producers and 11 (50%) were moderate biofilm producers. For *P. aeruginosa* isolates, out of the 15 isolates, 10 (66.7%) produced strong biofilm, 3 (20%) moderate biofilm, and 2 (13.3%) weak biofilm (**Table 3**). No significant correlation was detected between biofilm formation pattern and antibiotic susceptibility profile of the isolated organisms (p=0.079).

Table 3: Biofilm-forming ability of bacteria from cystic fibrosis patients

Bacterial isolates	Biofilm formation pattern		
	Weak *% (n)*	**Moderate** *% (n)*	**Strong** *% (n)*
S. aureus	0% (0)	50% (11)	50% (11)
P. aeruginosa	13.3% (2)	20% (3)	66.7% (10)
Klebsiella spp.	50% (1)	50% (1)	0% (0)
E. coli	50% (1)	50% (1)	0% (0)
Acinetobacter spp.	50% (1)	0% (0)	50% (1)

DISCUSSION

CF patients experience an endless cycle of airway infection and inflammation as a consequence of inadequate airway moisture and compromised cilia function [23]. Chronic infection in CF lungs is initiated by a few bacterial types colonizing the airway [23]. The proper management of lung infections among patients with CF depends on a clear understanding of the prevalent bacteria colonizing their airways and monitoring their antibiotic susceptibility patterns within the national population [24]. There is scarce data on the microbiological profile of children with CF in Egypt. Thus, this research investigated the microbiological makeup of bacteria colonizing the airways, antibiotic susceptibility patterns, and biofilm formation among a cohort of pediatric patients with CF following up at the CF Center of Children's Hospital, Faculty of Medicine, Cairo University.

S. aureus (51.2%) was the most prevalent species among the isolated pathogens, followed by *P. aeruginosa* (34.9%). Our findings are consistent with earlier Middle East research. In Jordan, ***Alshraiedeh et al***. [25] reported that *S. aureus* accounted for 42% of bacteria colonizing airways of their CF patients, while *P. aeruginosa* came in second with 40%. In a similar vein, ***Erfanimanesh et al.*** [18] in Iran examined sputum samples from CF patients during pulmonary exacerbations, they stated that *S. aureus* was the most prevalent isolated species (55.3%), followed by *P. aeruginosa* (41.7%). Previous researchers from different countries worldwide have revealed comparable results [26–28]. On the other hand, previous studies from Egypt and the Middle East revealed that *S. aureus* was less prevalent (6%, 15%, respectively) while *P. aeruginosa* was the most common bacterial colonizer among their studied CF patients (22%, 55%, respectively) [29,30]. The CFF patient registry evaluated the age-group-specific trends in CF airway pathogens over time in their 2021 annual data report. According to their findings, the frequency of *P. aeruginosa* chronic colonization has been declining since 2003, with the biggest declines seen in people under the age of 18. They stated that this could have something to do with the broad use of eradication tactics during the first acquisition. Additionally, they observed that over 60% of patients had positive *S. aureus* cultures, and that children aged 6 to 10 and adolescents aged 11 to 15 were the most afflicted age groups [14], which corresponds to our study, in which these age groups made up the majority of the study population (50% and 23.5%, respectively). Co-infection with *S. aureus* and *P. aeruginosa* occurred in 26.5% of our patients. The average global rate of *S. aureus/P. aeruginosa* coinfection is 28.3%, with patients in their mid-twenties having the greatest rate, ranging from 8.6% to 60% [31].

There is a correlation between prolonged MRSA infection and poorer survival rates, as well as a rapid pace of lung function reduction among CF patients [32]. Total MRSA prevalence of our study was 41.9% which is similar to that of an earlier investigation carried out in Iran, where MRSA colonization rate was 43% among their isolated organisms [32]. However, the CFF Patient Registry's yearly report for 2021 revealed a lower percentage, with a mean colonization rate of 16% [14]. The higher prevalence of MRSA observed in our study aligns with the broader trend in Egyptian hospitals. A recent systematic review reported a 63% prevalence of MRSA among clinical isolates nationwide [33]. There could be distinct explanations for the high prevalence of MRSA in Egypt. First, there are insufficient infection control programs. with common issues including high workload, limited resources, deficient infection control training and understaffing, which hinder healthcare personnel from implementing basic infection prevention strategies [34]. Second, self-medication with antibiotics and inappropriate antibiotic usage are very common in Egypt [35].

It is quite disturbing that our analysis revealed an alarming increase in the MRSA rate. Lung infections in CF patients exhibit increasing resistance to treatment over time, resulting in diminished therapeutic efficacy of antibiotic regimens. Therefore, it is crucial to perform routine antibiotic susceptibility testing (AST) in order to track antibiotic resistance profiles [25]. Of the pathogens we investigated, 20 isolates (46.5%) had MDR phenotype; 80 percent of these isolates were *S. aureus*. Nonetheless, most of our *S. aureus* remained susceptible to vancomycin (100%), linezolid (95.5%), TMP/SMX (90.9%) and doxycycline (68.2%). As of right now, there is no agreement on the optimum approach to manage MRSA [36]. A prophylactic MRSA regimen

would raise serious concerns and could encourage the evolution of further resistance [37]. Treatment regimens that strike a balance between safety, efficacy, and treatment load should be investigated for these individuals in order to enhance their quality and duration of life [38]. Regarding AST patterns among *P. aeruginosa* isolated in our study, the overall resistance rate was low, only one isolate exhibited MDR phenotype (2.3% of all isolated organisms). The highest resistance was observed to cefepime and imipenem (26.7%), followed by tobramycin (20%) meanwhile, the lowest resistance rates were observed in by ceftazidime, aztreonam, ciprofloxacin, levofloxacin and meropenem (6.7% each). All isolates were susceptible to piperacillin-tazobactam. Our results are consistent with those reported by the CFF Patient Registry, which reported in their recent annual report that the prevalence of MDR *P. aeruginosa* (MDR-PA) infection decreased from 4.2% in 2020 to 3.5% in 2021 and that the highest rates of MDR-PA infection are found in older adults and adolescents with CF, potentially reflecting cumulative exposure to antibiotics by these individuals [14].

The development of biofilm may be closely related to the virulence and antibiotic resistance of bacterial infections in the CF lungs [39]. In CF patients, biofilm formation significantly prolongs infections and fosters antibiotic resistance; bacteria shielded by biomass are able to endure harsh environments and develop resistance against both host defenses and antimicrobials [25]. All isolated organisms in the current investigation produced biomass to varied degrees; of these, 50% of *S. aureus* isolates and 66.7% of *P. aeruginosa* isolates were significant biofilm builders. Our findings are consistent with earlier studies that found the majority of CF patient isolates had the ability to form a biofilm [18,25,30]. Biofilm-associated infections in cystic CF pose a significant therapeutic challenge, the enhanced antibiotic resistance of biofilm-embedded bacteria and the potential for secondary infections following treatment contribute to these difficulties. Even with our growing knowledge of how biofilms form and persist, effective therapies remain elusive, and most biofilm removal techniques are essentially mechanical, leaving little novel therapeutic choices for use in clinical settings. Thus, it is essential to conduct ongoing research and innovate to provide cutting-edge methods for disrupting biofilms and enhancing the prognosis of CF patients [40].

CONCLUSION

Our findings reveal a significantly higher prevalence of chronic *S. aureus* colonization among our CF patients compared to *P. aeruginosa*. One of the alarming findings of our study is the high prevalence of MRSA and biofilm formation among isolated bacteria. This observation underscores the importance of targeted interventions to address the burden of MRSA colonization in this vulnerable population. To prevent acute exacerbations caused by MRSA strains in CF patients, our hospitals should implement stringent preventive measures, judicious antibiotic use and infection control policies to improve the overall health outcomes and quality of life for CF patients. While the current study demonstrates low resistance rates among *P. aeruginosa* isolates, it is imperative to maintain vigilant surveillance of antimicrobial resistance patterns in CF patients. This ongoing monitoring will enable us to adapt treatment strategies and implement appropriate control measures in response to emerging resistance trends. Our study's shortcomings include a small patient cohort from a single CF center, along with the absence of comprehensive clinical data and previous culture results. Additionally, our investigation did not evaluate anaerobic bacteria, viruses, or fungi, which could be important in further worsening of respiratory parameters. Lastly, additional research is needed to verify the link between biofilm formation and CF patients' clinical status and explore the contribution of less common pathogens to disease progression.

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Competing Interests: All authors have no relevant financial or non-financial interests to disclose.

Ethical Approval
The current work was approved by the Research Ethics Committee, Faculty of Medicine, Cairo University (MS-181-2023).
Consent to participate: Informed consent was obtained from all individual participants included in the study.

Author Contributions:
Conceptualization: Mostafa Z. A., Mowafy H. L., Karas M. S., Fawzy E. F. **Methodology and validation:** Mostafa Z. A., Mowafy H. L., Karas M. S., Fawzy E. F. **Writing original draft and figure preparation:** Mowafy H. L., Karas M. S., **Writing review and editing:** Mowafy H. L., ElFeky D.S., Karas M. S., **Supervision:** Mostafa Z. A., ElFeky D.S., Mowafy H. L., Fawzy E. F. A. All authors reviewed the manuscript.

Data availability statement: All data used in the current study are available from the corresponding author on reasonable request.

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