Original Article

# The emergence of multidrug-resistant *Pseudomonas aeruginosa* in cystic fibrosis patients on inhaled antibiotics

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## ABSTRACT

**Introduction:** Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) is an important and growing issue in the care of patients with cystic fibrosis (CF), and a major cause of morbidity and mortality. **Objective:** The objective of the study was to describe the frequency of MDR-PA recovered from the lower respiratory samples of pediatric and adult CF patients, and its antibiotic resistance pattern to commonly used antimicrobial agents including β-lactams, aminoglycosides, and fluoroquinolones. **Materials and Methods:** The lower respiratory isolates of *P. aeruginosa* were obtained from inpatients and outpatients CF clinics from a tertiary care teaching hospital for the period from October 2014 to September 2015. The identification and antimicrobial susceptibility for all the isolates were performed by using the BD Phoenix™ and E-test in compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** A total of 61 *P. aeruginosa* samples were isolated from thirty CF patients from twenty families. Twelve sputum samples were positive for MDR-PA (seven nonmucoid and five mucoid isolates) from five CF patients (five families) with moderate-to-very severe lung disease given MDR-PA frequency of 19.7%. The median age of the study group was 20 (range 10–30) years. Three CF patients were on chronic inhaled tobramycin and two on nebulized colistin. The antimicrobial patterns of isolates MDR-PA showed the highest rate of resistance toward each gentamycin, amikacin, and cefepime (100%), followed by 91.7% to ciprofloxacin, 75% to tobramycin, 58.3% to meropenem, and 50% to piperacillin-tazobactam. None of the isolates were resistant to colistin during the study period. **Conclusion:** The study results emphasize that the emergence of a significant problem in the clinical isolates of *P. aeruginosa* in CF patients that dictate appropriate attention to the antibiotic management after proper surveillance.

**KEY WORDS:** Cystic fibrosis, inhaled antibiotics, multidrug-resistant *Pseudomonas aeruginosa*

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## INTRODUCTION

About 70,000 people have cystic fibrosis (CF) worldwide, with prevalence varying by location and ethnic background.[1]

CF is caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene, leading to altered chloride ion exchange and hyperviscous mucus in the affected organs.[2,3]

Pulmonary complications resulting from CF currently account for up to 85% of CF mortality.[4] CF pulmonary disease begins early in life and is characterized by

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progressive airway obstruction, as measured by the expiratory volume over the first second of a forced exhalation (FEV1).[5]

In the lung, the CFTR gene produces a protein whose basic function is regulation of ion transport on the surface of the airways that contributes to mucus hydration and periciliary transport, which are altered on mucosal surfaces in CF patients resulting in air defective mucociliary clearance with the production of viscous and sticky bronchial mucus airway obstruction.[6] This provides a good environment for microbial growth, and as a consequence, CF patients suffer from recurrent infections of the respiratory tract and chronic inflammation, thus leads to tissue remodeling and finally to premature death caused by respiratory insufficiency.[7] The respiratory tract of the CF patients is colonized by pathogenic microorganisms early in childhood, and in the vast majority of cases, chronic infections are established.[8-10]

*Pseudomonas aeruginosa* is the main bacterial pathogen that infects the lungs of patients; with the prevalence of *P. aeruginosa,* infection increases with age and becomes the most frequently identified pathogen in adulthood.[11] Initial pulmonary infections with *P. aeruginosa* in patients with CF are typically intermittent and caused by nonmucoid environmental strains that are susceptible to anti-*P. aeruginosa* antibiotics.[12-14] Eventually, a single strain becomes established and develops a mucoid phenotype, which makes eradication from the airways difficult.[15] The alginate-containing matrix of the mucoid strain is thought to allow the formation of protected microcolonies and provide increased resistance to opsonization, phagocytosis, and destruction by antibiotics. As a result, conversion to mucoid phenotype is associated with a significant increase in morbidity and mortality and demonstrated a greater loss of lung function in comparison to CF patients with intermittent or no colonization.[16-19] Eradication strategies have emerged over the past several years that target nonmucoid strains and can delay the development of chronic infection.[20-23] Patients with CF are at very high risk of developing infections with multidrug-resistant (MDR) pathogens, particularly *P. aeruginosa*, owing to the frequent and often prolonged courses of oral, intravenous, and aerosolized antibiotics that are used to treat the chronic lung disease of CF.

The objectives of this study described the frequency of MDR *P. aeruginosa* (MDR-PA) recovered from the lower respiratory samples of CF patients, and its antibiotic resistance pattern to the commonly used antimicrobial agents.

## MATERIALS AND METHODS

### Patients and sampling

Respiratory specimens such as sputa, deep pharyngeal swab, or bronchoalveolar lavages were collected from CF patients during visits to the CF clinic and as part of inpatient care from October 2014 to September 2015 at Hamad Medical Corporation, in the state of Qatar. The diagnosis of CF was based on one or more clinical features consistent with CF, positive family history of CF in siblings and close relatives, pathologically elevated sweat chloride (>60 mmol/L) on two separate occasions, in addition to the presence of two disease-causing mutations in the CFTR gene. Screening for CFTR mutations was performed initially by mutation detection enhancement heteroduplex analyses and currently coding DNA Sanger sequences method.[24]

Routine culture for *P. aeruginosa* was performed on blood agar and MacConkey agar. The media were incubated for 18–24 h at 37°C and *P. aeruginosa* was identified by biochemical tests.

### Drug susceptibility testing

Antibiotic susceptibility testing for all *P. aeruginosa* isolates was performed using BD Phoenix automated system and *E*-test (previously known as Epsilometer test) methods. BD Phoenix™ Automated Microbiology System in compliance with Clinical and Laboratory Standards Institute. The identification and antimicrobial susceptibility analysis were performed, as described previously.[25,26]

The range of antimicrobial agents was routinely evaluated in two or more of the following groups: Aminoglycosides (tobramycin, gentamicin, and amikacin), fluoroquinolones. (ciprofloxacin), and beta-lactams (ceftazidime, meropenem, piperacillin, ticarcillin-clavulanate, and aztreonam). This method is internationally accepted, simple, and allows ease of use when screening a large number of isolates. This allowed the production of a large antibiogram including multiple classes of antibiotic. The United States CF Foundation consensus guidelines definition of MDR was defined as resistance to all agents tested in two or more of the following antibiotic categories: aminoglycosides, β-lactam antibiotics, and/or the fluoroquinolone, ciprofloxacin, according to the CF Foundation Microbiology and Infectious Disease Consensus Conference.[25]

## RESULTS

A total of 61 *P. aeruginosa* samples were isolated from thirty CF patients from twenty families. The mean age of the study group was 20.56 ± 8.95 years, 11 males and 19 females. Twenty CF patients (66.7%) were above 18 years old. Twenty-five (83.3%) CF patients from 15 families were with homozygous *CFTR* I234V mutation, and the other five have other types of CFTR mutations (including three CF patients with homozygous delta F508 mutation, 1CF patient with homozygous Y569D mutation, and unidentified CFTR mutation in one CF patient).

*P. aeruginosa* isolates from all siblings (two families with four CF siblings, two families with three CF siblings, and 13 families contributed one sibling each). The remaining three families were two families with two CF siblings

where *P. aeruginosa* isolates in one CF sibling and one family with three CF siblings where *P. aeruginosa* isolates in one CF sibling.

*P. aeruginosa* in lower respiratory samples of thirty CF patients showed the highest sensitivity to piperacillin/tazobactam (90.2%) followed by meropenem (88.5%), ciprofloxacin (77%), cefepime (70.5%), amikacin (67.2%), and gentamicin (59%). All the isolates were susceptible to colistin during the study period [Figure 1]. Twelve sputum samples were positive for MDR-PA (seven nonmucoid and five mucoid isolates) from five CF patients of five families with moderate-to-very severe lung disease given MDR-PA frequency of 19.7%. This includes one family with four CF siblings harbor *P. aeruginosa;* only one sibling had MDR-PA and one family with two CF siblings, only one had MDR-PA. The rest of three families were with one CF sibling in each. The demographic characteristic of CF patients with MDR-PA is shown in Table 1.

The antimicrobial susceptibility pattern of MDR-PA isolates showed the highest rate of resistance (100%) toward each gentamycin, amikacin, and cefepime, followed by 91.7% to ciprofloxacin, 75% to tobramycin, 58.3% to meropenem, and 50% to piperacillin-tazobactam [Figure 2].

## DISCUSSION

Antibiotic resistance is a hallmark of chronically colonizing pathogens generally and particularly, in those associated with CF infections. The problem of antibiotic resistance in *P. aeruginosa* CF is on the increase.[27,28]

Several studies worldwide have reported of MDR-PA in patients with CF. There an increased prevalence of patients with an MDR-PA infection has been reported at Texas Children's Hospital in Houston, Texas.[29] The Epidemiologic Study of CF assessed data from 110 United States and Canadian CF centers and found that approximately one-third of patients aged 18 years or over with a percentage forced expiratory volume in 1 s predicted to be <40% harbored MDR strains.[30] In the Arabian Gulf region, Saudi Arabia has the largest CF population in the Arabian Gulf region,[31] and in the state of Qatar, CF is common in a large kindred Arab tribe, consisting of some families who share a common ancestry and culture.[24]

*P. aeruginosa* was the most common bacteria isolated from the first culture samples in 44% of CF patients in Saudi Arabia,[32] and the prevalence of *P. aeruginosa* in lower respiratory cultures of CF patients from our institute

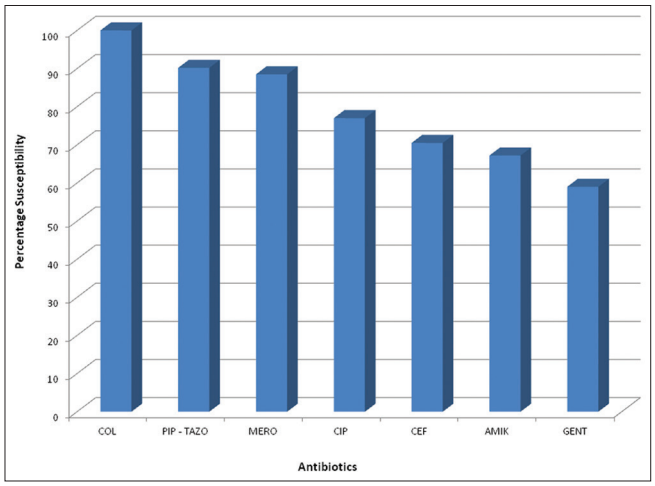


**Figure 1:** The antimicrobial susceptibility of *Pseudomonas aeruginosa* from lower *respiratory* samples of thirty cystic fibrosis patients. COL: Colomycin, PIP-TAZO: Piperacillin-tazobactam, MERO: Meropenem, CIP: Ciprofloxacin, CEF: Cefepime, AMK: Amikacin, GENT: Gentamicin

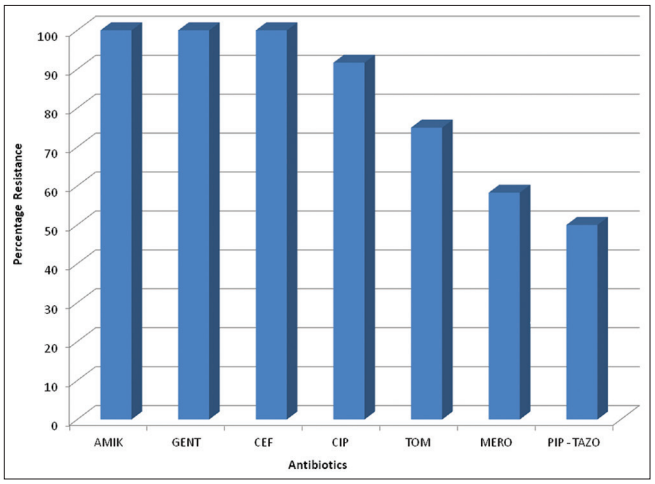


**Figure 2:** The antimicrobial pattern of MDR-PA isolates from lower respiratory samples of five cystic fibrosis patients. AMK: Amikacin, GENT: Gentamicin, CEF: Cefepime, CIP: Ciprofloxacin, TOB: Tobramycin, MERO: Meropenem, PIP-TAZO: Piperacillin-tazobactam

**Table 1: Baseline demographic characteristics of cystic fibrosis patients with multidrug-resistant *Pseudomonas aeruginosa***

| Age (years) | Sex | Types of CFTR gene | Pancreatic insufficiency | CF related diabetes | Nebulize tobramycin | Nebulize aztreonam | Nebulize colistin | FEV1% | Previous MDR-PA colonization | Co-infection |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | Female | I1234V | No | No | No | No | Yes | 34 | No | No |
| 23 | Male | I1234V | No | Yes | No | No | Yes | 23 | Yes | *C. albicans* |
| 20 | Female | I1234V | No | No | Yes | No | No | 56 | Yes | *C. albicans* |
| 14 | Male | I1234V | No | No | Yes | No | No | 45 | Yes | *S. aureus* |
| 10 | Female | Y569D | Yes | No | Yes | Yes | No | 49 | Yes | *S. aureus* |

FEV1: Forced expiratory volume defined as the volume of air that can be forced out in 1 s after taking a deep breath, CFTR: Cystic fibrosis transmembrane conductance regulator, CF: Cystic fibrosis, *C. albicans*: *Candida albicans, S. aureus*: *Staphylococcus aureus, MDR-PA: Multidrug-resistant Pseudomonas aeruginosa*

was 60.9%.[33] In the present study, we reported small frequency (19.7%) of MDR-PA among CF patients with moderate-to-very severe airflow obstruction. There is no report in the Arabian Gulf region of MDR-PA in CF patients.

*P. aeruginosa* has the capability of adapting to the environmental conditions, developing resistance to antibiotics, and producing a variety of virulence factors. It has been reported multiple antibiotic-resistant *P. aeruginosa* is more likely to be a marker of more severe disease and more intensive therapy and is less likely to be contributing independently to more rapid lung function decline, suggesting that this may be an important potential pathogen across all stages of airflow limitation.[34]

*P. aeruginosa* is masterful at developing resistance by either spontaneous mutations or the acquisition of plasmids (extrachromosomal DNA) harboring resistance genes.[35] Two such mechanisms, efflux pumps and beta-lactamases, are among the most common mechanisms of resistance detected in *P. aeruginosa* isolate from patients with CF. Another less conventional mechanism of resistance noted in *P. aeruginosa* strains infecting patients with CF is the biofilm mode of growth.[36] The emergence of hypermutable *P. aeruginosa* strain that may contribute to increased resistance of *P. aeruginosa* to antibiotics and become more frequent in later stages or chronic infection.[37] Mutations in *mutS*, *mutL*, and *uvrD* genes encoding proofreading proteins which normally correct errors during DNA replication give rise to these hypermutable strains.[15] It is important to note that the increase in hypermutable *P. aeruginosa* isolates later in CF patients suggest that genetic and phenotypic diversification plays an essential role in the adaptation of *P. aeruginosa* to the hostile and diverse CF lung environment and plays a role in survival *in vivo* by selecting for less virulent phenotypes.[38]

We reported previously that clustering of *P. aeruginosa* isolate from CF patient with advanced lung disease and MDR *P. aeruginosa* isolates in different pulsed-field gel electrophoresis clusters suggests that the colonizing strain may occasionally be changed.[39]

There is now evidence that *P. aeruginosa* survives in cough aerosols up to 4 m and for as long as 45 min, suggesting cough bioaerosols as a possible transmission pathway, may be possible to certain specific *P. aeruginosa*.[40] In the present study, we found MDR *P. aeruginosa* isolates only in one sibling in the families having more than one sibling with CF suggesting that certain intrinsic mechanism of resistance and possible certain strain MDR *P. aeruginosa* with less transmissible.

## CONCLUSION

Hygiene regulations in CF clinics should prevent a further spread of resistant bacterial strains, as antibacterial treatment options are limited in CF patients. Finally, adequately powered studies should be performed to determine the clinical utility of synergy studies in patients with CF infected with MDR pathogens.

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### Conflicts of interest
There are no conflicts of interest.

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