Research Article

Evaluation of Fluoroquinolone Resistance and Its Role in the Emergence of Multidrug Resistant Phenotypes in *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa are gram negative bacilli that exhibit inherent resistance to several structural classes of antibiotics. Active surveillance of trends in antibiotic resistance is necessary for the selection of appropriate antibiotics for empirical therapy. The objective of this study was to assess the *in vitro* activity of seven commonly prescribed antibiotics against local multidrug resistant *P. aeruginosa* isolates. A prospective descriptive study was conducted at a tertiary care hospital in South India on 106 isolates of *P. aeruginosa* obtained from 1240 clinical samples. *P. aeruginosa* showed highest resistance to ciprofloxacin (73.8%) followed by gentamicin (61.3%) and 23.6% of these were multidrug resistant. We observed that fluoroquinolone resistance was more commonly associated with ceftazidime and gentamicin resistance. The concurrent resistance to other antibiotics among fluoroquinolone resistant *P.aeruginosa* suggests that fluoroquinolone resistance may be an important driver of multidrug resistance. The drug resistance rates are largely determined by the pattern of antibiotic usage in the hospital setting. Reduced rates of resistance observed against piperacillin, piperacillin-tazobactum and imipenem suggest that these antibiotics could still be prescribed.

Keywords: *P. aeruginosa,* multidrug resistance, fluoroquinolones.

INTRODUCTION

Pseudomonas aeruginosa is a nonfermentative gram-negative bacteria that has minimal nutritional requirements and can survive on a wide variety of surfaces and in aqueous environments. It rarely causes serious infections in otherwise healthy persons and is infrequently identified as normal microbial flora in healthy individuals.[1] They are of greatest concern as opportunistic pathogens among hospitalized, critically ill and immunocompromised patients. Patients with cystic fibrosis, neutropenia, iatrogenic immunosuppression, or disrupted anatomical barriers are commonly at risk of infection with this organism.[1,2] High rates of colonization with *P. aeruginosa* are observed in hospitalized patients, particularly in those who have been hospitalized for extended periods of time and/or have received broad-spectrum antimicrobial therapy or cancer chemotherapy.[1] The spectrum of human infections caused by *P. aeruginosa* ranges from superficial skin infections to fulminant sepsis and includes nosocomial urinary tract infections, wound infections, septicaemia etc .[1,3]

P. aeruginosa is inherently resistant to several structural classes of antibiotics either intrinsically or through acquisition of genetic determinants for resistance over time. There are a limited number of antimicrobial agents with reliable activity against *P. aeruginosa*, and some of them are antipseudomonal penicillins, cephalosporins, carbapenems, and fluoroquinolones, particularly ciprofloxacin. Aminoglycosides are frequently used as a part of combination regimens for treatment of serious pseudomonal infections but are generally not recommended as single drugs because of its toxicity in high doses.[4] For each of these agents, emergence of resistance during the course of therapy has been described and has been recognized as a major cause of treatment failure.[5-10] Resistance to various anti-pseudomonal agents is on the rise, and this challenges the selection of appropriate treatment. Currently carbapenems are generally considered as the most reliable agents for treating *P. aeruginosa* infections. However, there is a steady increase in the occurrence of carbapenem-resistant *P. aeruginosa.*[11] New antimicrobial agents with activity against *P. aeruginosa* will not be available in the near future, making ongoing surveillance of the activities of currently available agents very important. The present study investigated *in vitro* activities of seven commonly prescribed antimicrobial agents against *P.* aeruginosa. This study was taken up as a preliminary activity prior to assessing the nature of biofilm production among multidrug resistant phenotypes and its susceptibility to antibiotics and disinfectants.

MATERIALS AND METHODS

In a prospective descriptive study, wound swabs or exudates were screened for *P. aeruginosa* at a tertiary care hospital in South India between August 2011 and July 2012. The primary isolation was performed on blood agar and Mac Conkey agar. *P. aeruginosa*, isolated from wounds, was identified by standard bacteriological methods including: colony morphology, gram staining, pyocyanin pigment production, growth at 42°C, oxidase and oxidative-fermentative (OF) tests. The antibiotic susceptibility testing of all the isolates was done by Kirby Bauer disc diffusion test on Mueller-Hinton agar plates and zones of inhibition were measured in accordance

with the recommendations of clinical and laboratory standards institute (CLSI 2012).[12] The antimicrobial agents used in this test were as follows: amikacin (30 ug), ceftazidime (30 ug), ciprofloxacin (5 ug) gentamicin (10 ug), imipenem (10 ug), piperacillin (100 ug), and tobramycin (10 ug). (Hi-Media, Mumbai). *Pseudomonas aeruginosa* ATCC 27853 was used as the quality control strain. The results were interpreted as susceptible or resistant by measuring the diameter of inhibition zone, according to the criteria designated by CLSI. For purpose of study analysis, any antibiotic displaying intermediate susceptibility according to the CLSI guidelines was considered as resistant. In this study, multi-drug resistant (MDR) isolates were defined as those resistant against at least one agent in 3 or more of the following 5 antimicrobial categories: (1) Aminoglycosides (2) Antipseudomonal carbapenems (3) Antipseudomonal cephalosporins (4) Antipseudomonal fluroquinolones (5) Antipseudomonal penicillins + β-lactamase inhibitors.[13]

RESULTS AND DISCUSSION

P. aeruginosa is an important cause of nosocomial skin and soft tissue infections. In this study, out of 1240 wound swabs and exudates received at the diagnostic microbiology laboratory, 512 samples showed bacterial growth. Out of this only 106 (20.7%) samples showed pure growth of *P.aeruginosa*. The other isolates are shown in table 1.

P. aeruginosa is inherently resistant to several structural classes of antibiotics. The potential for antimicrobial resistance is an important concern for clinicians treating patients with confirmed or suspected *P. aeruginosa* infections. Knowledge of the local resistance pattern is necessary for selection of appropriate antibiotics for treatment. In this study, 73.8% of the isolates were resistant to ciprofloxacin, and 61.3% isolates were resistant to gentamicin. (table 2).

The antibiogram revealed a total of 25 multidrug resistant (MDR) *P. aeruginosa* as shown in table 3.

Seven isolates of *P. aeruginosa* were resistant to all the tested antibiotics and 18 isolates were resistant to at least 3 of the following five groups: (1) imipenem (2) ceftazidime; (3) piperacillin-tazobactum (4) ciprofloxacin (5) amikacin, gentamicin or tobramycin. The different MDR phenotype patterns observed, stratified by the number of antimicrobial classes to which isolates were resistant, are presented in table 4. Among MDR isolates resistant to 3 antimicrobial classes, the most common phenotype was resistance to ciprofloxacin, aminoglycosides, and ceftazidime. For MDR isolates resistant to 4 antimicrobial classes, the most common phenotype was resistance to ciprofloxacin, aminoglycosides, imipenem, and ceftazidime. Colistin was not used in the classification of MDR isolates. All the MDR isolates evaluated in this study retained susceptibility to colistin.

Table 1: Percentage incidence of bacterial wound infections

Name of the isolate	Percentage
Staphylococcus aureus	31.3
Coagulase negative staphylococci	29.3
Other Pseudomonas spp	12
Klebsiella spp	2.3
Proteus spp	2
Acinetobacter spp	2
Escherichia coli	0.4

Table 2: The antibiotic resistance pattern of *P.aeruginosa* isolates

Name of the antibiotic	Percentage resistance (%)
Amikacin	26.4
Ceftazidime	35.8
Ciprofloxacin	73.8
Imipenem	8.5
Gentamicin	61.3
Piperacillin	16.9
Piperacillin-tazobactum	8.5
Tobramycin	35.8
Colistin	0

Table 3: Antibiotic resistance pattern of 25 MDR *P. aeruginosa* isolates (N=25)

Name of the antibiotics	Percentage resistance (n) (%)
Aminoglycosides (Amikacin, Gentamicin, Tobramycin)	(24) 96%
Ceftazidime	(25) 100%
Ciprofloxacin	(25) 100%
Imipenem	(9) 36%
Piperacillin- tazobactum	(9) 36%

Several studies conducted across the country have shown high rates of resistance to antibiotics in *P. aeruginosa* isolated from hospitalized patients. In a study conducted by Joseph *et al* high rates of resistance were observed to gentamicin (50.8%), ceftazidime (50%), ciprofloxacin (49.2%), cefoperazone-sulbactam (43.9%), piperacillin (43.2%), meropenem (34.8), and imipenem (28%).[15] A study conducted by Chaudhari *et al* from central India has revealed a high rate of resistance to ciprofloxacin followed by gentamicin.[16] Karlowsky *et al* have suggested that the cumulative use of fluoroquinolones is a greater selector of resistance or may be that the fluoroquinolone resistant strains are more easily spread than are strains to other agents.[17]

Table 4: Resistance pattern observed among 25 MDR *P.aeruginosa* isolates[14]

MDR phenotype	Antibiotic resistance profile					
	Ceftazidime	Piperacillin-tazobactum	Imipenem	Aminoglycosides (Ak=Amikacin, G=Gentamicin, Tob=Tobramycin)	Ciprofloxacin	No. of isolates
MDR3	R	R			R	1
	R			$R_{Ak,G,Tob}$	R	11
	R			R_{Tob}	R	1
	R			R_{Gen}	R	2
MDR4	R		R	$R_{Ak,G,Tob}$	R	2
	R	R		$R_{Ak,G,Tob}$	R	1
MDR5	R	R	R	$R_{Ak,G,Tob}$	R	7

(Resistance interpreted according to current CLSI breakpoints (CLSI, 2012).; MDR= Multidrug-resistant—The number that appears after the MDR designation indicates the number of different antimicrobial classes to which isolates are resistant (e.g., MDR 3 indicates *P. aeruginosa* isolates that are resistant to at least 1 antimicrobial agent from 3 different classes).

The possible loss of the fluoroquinolones to treat *P. aeruginosa* infections implies that injectable therapy with alternative agents and possibly hospitalization may be required to treat these infections in the future. In this study, we observed that resistance to a fluoroquinolone was always associated with resistance to at least one other class of antimicrobial agent more commonly ceftazidime, and gentamicin (table 4), suggesting that perhaps fluoroquinolones may be an important driver of multidrug resistance. This concurrent resistance to other antimicrobial agents has also been reported in a study conducted by Sahm *et al*.[18] Given that combination treatment is generally recommended for suspected *P. aeruginosa* infections, there is a risk that this approach too may encourage development of resistance to multiple agents or selection of resistant strains. Data from a surveillance study conducted by Jones *et al* suggest that the choice of a carbapenem, cefepime, or piperacillin-tazobactam in combination with amikacin or tobramycin would give the widest potential empirical antibiotic coverage against *P. aeruginosa*.[19] Rigorous monitoring for multidrug resistance among *P. aeruginosa* isolates is very important because outbreaks of strains resistant to potentially useful agents, including carbapenems (32.2% resistance), have been reported by Castanheira *et al* in a study conducted from 10 different Indian hospitals.[20]

CONCLUSION

Antimicrobial resistance rates among *P. aeruginosa* are increasing slowly for most agents. However, fluoroquinolone resistance appears to be increasing more rapidly than is resistance to other agents and is the most common resistance component found among MDR isolates. The lack of any new compounds in the near future indicates that national, regional, and local surveillance efforts are imperative to provide clinicians with relevant information for choosing empirical or directed therapy. Although treatment with imipenem could result more often in the emergence of resistant *P. aeruginosa* than treatments with other antipseudomonal agents, this tendency may not translate into a higher prevalence of imipenem resistance among hospital isolates. This apparent discrepancy might be related to differences in the frequency of use of various agents and to the different likelihoods of persistence of resistant strains.[4] In cases where imipenem is selected as the antipseudomonal antibiotic, the potential for emergence of resistance should be anticipated, and in appropriate circumstances, routine culturing and susceptibility testing should be performed to detect the emergence of resistance in *P. aeruginosa* as soon as possible. Since all the MDR isolates evaluated in this study retained susceptibility to colistin, it could be still thought of a reserve to treat severe *P. aeruginosa* infections inspite of its nephrotoxicity, if not alone, at least in combination. Since combination treatment promises hope to treat gram negative bacilli infections, the effect of combination antibiotics will be evaluated on the multi drug resistant *P. aeruginosa* isolates. In view of the present scenario of the resistance pattern of *P.aeruginosa* in our hospital, we recommend that amikacin, piperacillin, piperacillin – tazobactam could still be prescribed alone. Imipenem could be used more judiciously for treatment of patients with serious infections such as sepsis. Fluoroquinolones could be rested for sometime based on the principal of antibiotic cycling and monitored continuously to help regain sensitivity when the antibiotic pressure is withdrawn. This could help in reviving fluoroquinolones for future use, lest we lose them.

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