Original Article

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Microbiology Section

Changing Trend in the Antibiotic Resistance Pattern of *Pseudomonas Aeruginosa* Isolated from Wound Swabs of Out-Patients and in-Patients of a Tertiary Care Hospital

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ABSTRACT

Context: *Pseudomonas aeruginosa* is the most common gram negative bacteria associated with nosocomial infections. Active surveillance of trends in antibiotic resistance of *P. aeruginosa* is necessary for the selection of appropriate antimicrobial agent for empirical therapy.

Aim: To assess the rates of antibiotic resistance and multidrug resistance among *P. aeruginosa* isolates and to observe the trend in its resistance pattern over a period of 5 years.

Materials and Methods: *Pseudomonas aeruginosa* isolated from wound swabs during January to June 2007 and January to June 2012 were included in the study. Isolates were identified by conventional tests and antibiotic susceptibility was determined by disc diffusion method according to CLSI guidelines.

Results: A total of 307 *Pseudomonas aeruginosa* isolates were included in the study. Among these isolates, 165 were isolated during Jan-June 2007 and 142 were isolated during Jan-June 2012. Among in-patients, there was a significant reduction in resistance rates of the isolates to ciprofloxacin (49% to 33%), ceftazidime (50% to 33%), meropenem (35% to 19%) and imipenem (28% to 14%) in 2012. Similarly, the rate of MDR *Pseudomonas aeruginosa* among the in-patients decreased from 37.9% in 2007 to 23.7% in 2012 (p value 0.0241). There was no significant difference in the resistance rates of the isolates from out-patients during the two study periods.

Conclusion: There was a significant decreasing trend in the resistance rates of the isolates to ciprofloxacin, ceftazidime, meropenem and imipenem. Reduction in the use of ciprofloxacin could be probable reason for the decreased resistance among *P. aeruginosa* isolates, which needs to be further investigated.

Key words: *Pseudomonas aeruginosa*, Antibiogram, Trend

INTRODUCTION

Pseudomonas aeruginosa is the most common gram negative bacteria associated with nosocomial infections [1,2]. It is also being increasingly implicated in community acquired infections. *P. aeruginosa* is an important cause of wound infections in diabetic individuals and burns patients [3,4]. Resistance to various anti-Pseudomonal agents is on the rise, challenges the selection of appropriate treatment. The 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* [1]. The emergence of multidrug resistant *P. aeruginosa* remains an issue of public health concern globally, as it is associated with increased morbidity in those who are infected by this pathogen [2,5]. Patients with *P. aeruginosa* wound infections have increased need for debridement and they frequently require re-grafting due to loss of skin grafts or allografts [3]. Skin and soft tissue infections caused by *P. aeruginosa* are also associated with prolonged hospital stay and increased mortality [4]. The morbidity and mortality associated with *P. aeruginosa* are mainly attributed to inadequate empirical therapy and/ or delay in the initiation of appropriate therapy [1,2].

The resistance rates of *P. aeruginosa* are known to vary widely in different settings. Active surveillance of trends in antibiotic resistance of *P. aeruginosa* is necessary for the selection of appropriate antimicrobial agent for empirical therapy. The objectives of this study were to assess the rates of antibiotic resistance and multidrug resistance among *P. aeruginosa* isolates and observe the trend in its resistance pattern over a period of 5 years.

MATERIAL AND METHODS

This study was conducted in the Department of Clinical Microbiology of a 600-bedded tertiary care multi-specialty hospital and teaching institute, located in south India. It serves as a referral center for tertiary specialist care for a catchment population of approximately 10 lakh people from the adjoining areas. This study was approved by the institute's ethics committee.

This was an observational study used aggregate data from January to June 2007 and January to June 2012. *Pseudomonas aeruginosa* isolated from wound swabs of both the in-patients and out-patients of our tertiary care teaching hospital were included in the study. Repeat isolates from the same patients were excluded. The isolates were identified as *P. aeruginosa,* based on standard bacteriological techniques [6]. The susceptibilities of the isolates to gentamicin, amikacin, ciprofloxacin, piperacillin, ceftazidime, meropenem, imipenem, piperacillin-tazobactam, cefoperazone-sulbactam were determined by the Kirby-Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines [7]. *P. aeruginosa* ATCC 27853 was used for quality control in Kirby Bauer disc diffusion method.

For purpose of study analysis, any antibiotic displaying intermediate susceptibility according to the CLSI guidelines was considered as resistant. *P. aeruginosa* isolates were considered to be multidrug resistant if they were resistant to at least three drug classes.

Data entry and analysis were done using SPSS for Windows, version 16.0 (SPSS Inc, Chicago, IL, USA). Percentages were calculated for categorical variables. Chi-square test or Fisher's exact test was

used to compare two groups. All p values < 0.05 were considered as statistically significant.

RESULTS

A total of 307 *Pseudomonas aeruginosa* were included in the study. Of these 307 isolates, 165 were isolated over a period of 6 months from January to June 2007 and the remaining 142 were isolated from January to June 2012. The isolates were obtained from in-patients with surgical site infections, secondary infections of bedsore and diabetic ulcers and out-patients with traumatic ulcers and diabetic foot infections. The age and sex distribution of the study patients have been shown in [Table/Fig-1]. There was no statistically significant difference among the study population during the two periods (i.e., 2007 and 2012).

The comparison of the antibiotic resistance of *Pseudomonas aeruginosa* isolated from in-patients in 2007 and 2012 has been shown in [Table/Fig-2]. There was a significant reduction in resistance of the isolates to ciprofloxacin, ceftazidime, meropenem and imipenem in 2012. Similarly, the rate of MDR *Pseudomonas aeruginosa* among the in-patients decreased from 37.9% (50/132) in 2007 to 23.7% (27/114) in 2012 (p value 0.0241).

In-patients	2007 (n=132)	2012 (n=114)	p value
Male	104 (78.8)	98 (86.0)	0.1943
Female	28 (21.2)	16 (14.0)	
Age	43.9 ± 18.0 (4 to 85)	47.1 ± 17.5 (2 to 78)	0.1603
Out-patients	2007 (n=33)	2012 (n=28)	p value
Male	28 (84.8)	26 (92.6)	0.4367
Female	5 (15.2)	2 (7.1)	
Age	37.6 ± 15.3 (18 to 79)	35.5 ± 12.8 (21 to 55)	0.5674

[Table/Fig-1]: Age and sex distribution of the patients

	2007 (n=132)	2012 (n=114)	p value
Gentamicin	67 (50.8)	68 (59.6)	0.2044
Amikacin	40 (30.3)	30 (26.3)	0.5827
Ciprofloxacin	65 (49.2)	38 (33.3)	0.0167
Piperacillin	57 (43.2)	42 (36.8)	0.3785
Ceftazidime	66 (50.0)	38 (33.3)	<0.0001
Meropenem	46 (34.8)	22 (19.3)	0.0100
Imipenem	37 (28.0)	16 (14.0)	0.0122
Piperacillin-tazobactam	43 (32.6)	38 (33.3)	0.9921
Cefoperazone-sulbactam	58 (43.9)	52 (45.6)	0.8927

[Table/Fig-2]: Comparison of the resistance of *Pseudomonas aeruginosa* isolated from in-patients in 2007 and 2012

	2007 (n=33)	2012 (n=28)	p value
Gentamicin	14 (42.4)	11 (39.3)	0.9897
Amikacin	6 (18.2)	7 (25.0)	0.7382
Ciprofloxacin	14 (42.4)	11 (39.3)	0.9897
Piperacillin	8 (24.2)	4 (14.3)	0.5146
Ceftazidime	14 (42.4)	7 (25.0)	0.2473
Meropenem	0	3 (10.7)	0.0910
Imipenem	0	0	-
Piperacillin-tazobactam	3 (9.1)	4 (14.3)	0.6927
Cefoperazone-sulbactam	5 (15.2)	4 (14.3)	1.0000

[Table/Fig-3]: Comparison of the resistance of *Pseudomonas aeruginosa* isolated from out-patients in 2007 and 2012

The comparison of the antibiotic resistance of *Pseudomonas aeruginosa* isolated from out-patients in 2007 and 2012 has been summarized in [Table/Fig-3]. There was no significant difference in the resistance of the isolates from out-patients during the two study periods. The rate of MDR *Pseudomonas aeruginosa* among the out-patients showed an apparent increase from 18.2% (6/33) in 2007 to 25% (7/28) in 2012. However, this difference was not statistically significant (p value 0.7382).

DISCUSSION

P. aeruginosa is an important nosocomial pathogen associated with increased morbidity and mortality. In a recent study performed in a burns unit of Cape Town, patients with clinically significant wound infections caused by *P. aeruginosa* had increased loss of allografts and skin grafts [3]. It also was observed that on an average, 12 days of vigorous dressing with debridement was needed to achieve negative cultures [3]. Infection with *P. aeruginosa* was shown to be a significant independent risk factor for increased mortality rates in diabetic patients with skin and soft tissue infections [4]. *P. aeruginosa* wound infections were also observed to increase the length of hospital stay and costs. [4] Emergence of multi-drug resistance in *P. aeruginosa* is being reported globally, due to the indiscriminate use of antibiotics [1]. The increase in occurrence of multidrug resistant strains is caused by a continuous selective pressure of regularly used antibiotics. This selective antibiotic pressure leads to development of bacterial resistance by favouring rapid evolution of the bacterial genome [8]. Treatment of infections caused by this pathogen is becoming difficult, because of the increased rate of drug resistance. Knowledge on the resistance pattern of the local microbial flora is necessary for selection of appropriate antibiotic therapy. In this study, increased resistance to gentamicin, ciprofloxacin, ceftazidime, cefoperazone-sulbactam and meropenem was observed among the in-patients during the first study period. Several studies have reported such high rates of antibiotic resistance in *P. aeruginosa* isolated from hospitalized patients [9-11]. The drug resistance rates are largely determined by the pattern of antibiotic usage in the hospital setting. Ciprofloxacin use has been reported to be an independent risk factor for development of fluoroquinolone and carbapenem resistance [12-14]. Therefore, several interventions aimed at modifying the antibiotic prescription pattern have been tried, to decrease the resistance rates. One such approach is antibiotic cycling, which refers to scheduled substitution of a class of antibiotics with a different class exhibiting a comparable spectrum of activity [15]. However, the usefulness of antibiotic cycling in controlling drug resistance has not been proved conclusively [16,17].

We studied the changing trend in the resistance rates of *P. aeruginosa* in our hospital. Although there was no significant difference in the resistance of the isolates from out-patients, a significant reduction in resistance to ciprofloxacin, ceftazidime, meropenem and imipenem was observed among the isolates from in-patients, over a period of five years. In a similar study done by Lewis et al., a significant improvement in the susceptibility of *P. aeruginosa* to carbapenems and ciprofloxacin was noticed during a 7 year study period [1]. We did not study the exact cause for the decrease in the resistance to ciprofloxacin, ceftazidime and carbapenems. However, in the study of Lewis et al., the reduction in drug resistance was attributed to the restriction of ciprofloxacin use as a part of antibiotic stewardship program [1]. Similarly, in another study done by Messadi et al., a decrease in the resistance of *P. aeruginosa* to ciprofloxacin and group-2 carbapenem was observed following restriction of ciprofloxacin [18]. These studies suggest that ciprofloxacin use is an important factor in selection of both ciprofloxacin and carbapenem resistance.

Exposure to ciprofloxacin is believed to mediate development of resistance to both fluoroquinolones and carbapenems, by selecting mutations that upregulate MexEF-OprN efflux system and decrease levels of outer membrane porin protein OprD [19,20]. In the present study, we could not show any significant association between ciprofloxacin usage and resistance to fluoroquinolones

and carbapenems, as the data regarding ciprofloxacin usage was lacking in our hospital. However, personal communications with clinicians in our hospital revealed their increased preference for oral cephalosporins over ciprofloxacin for treatment of most gram negative infections, which suggested a decline in ciprofloxacin use. Further studies are needed to prove causal relationship between restriction of ciprofloxacin use and improvement in susceptibility to carbapenems.

In conclusion, a decreasing trend was observed in the resistance of *P. aeruginosa* to ciprofloxacin, ceftazidime and carbapenems among the isolates from the in-patients in our hospital. In the present era of antibiotic resistance, with emergence of multi-drug resistance globally, it is interesting to note a favourable trend in the susceptibility of *P. aeruginosa*. This study therefore paves way for further studies on factors promoting drug susceptibility. It is also necessary to establish the role of antibiotic cycling in reduction of antibiotic resistance by selection of susceptible strains.

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