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Original Work

Resistance patterns of *Pseudomonas aeruginosa* isolated from HIV and Non-HIV patients with lower respiratory tract infections

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ABSTRACT: The increase in occurrence of infections due to opportunistic gram-negative bacilli in patients with impaired host defences emphasizes the need for information on the antibiotic susceptibility of the organisms that infects such patients. *Pseudomonas aeruginosa* are becoming increasingly resistant to antimicrobial agents, and serious infections caused by these organisms often require immediate attention as they cause treatment failures. *In vitro* antimicrobial susceptibility data are required for successful therapy because acquired resistance to such antimicrobials as β-lactams, fluoroquinolones and aminoglycosides is so prevalent in *P. aeruginosa*. The study was carried out in Chennai during the period May 2007 and March 2009. 69 isolates of Pseudomonas were isolated from HIV and 24 isolates were isolated from Non-HIV populations with lower respiratory tract infections. The antibiotic susceptibility pattern of all the isolates was studied for 12 antibiotics to find the multi drug resistant (MDR) isolates for which the minimum inhibitory concentration (MIC) were studied according to CLSI (2009).

KEY WORDS: ***Antibiotic resistance; P. Aeruginosa; MDR; HIV; Lower respiratory tract infection; MIC***

INTRODUCTION

A natural consequence of infectious agent is the antimicrobial resistance which occurs by adaptation of antimicrobials due to exposure in medicine used in farms and households[1-4].The effectiveness of the existing antimicrobials have declined which become difficult and expensive to treat the infection.[5-6]. *Pseudomonas aeruginosa* have emerged as an opportunistic multidrug resistant pathogens which is a growing problem worldwide[7,8].

Lung infections caused by *P. aeruginosa* are limited to patients who are immunocompromised, or who have defective mucociliary clearance, previous epithelial injury or foreign body placement. Given its ubiquitous presence in our environment and pathogenic potential, it is clear that a normally functioning host defence is very well adapted to prevent *P. aeruginosa* infection. Despite this, *P. aeruginosa* infections can be devastating in the hospitalized or sick. Understanding the failures of the host defence in these patients will help us understand how *P. aeruginosa* is converted from a common environmental exposure to a deadly pathogen[9].

Pseudomonas infection remains one of the untreatable and uncontrollable infection of the hospitals. Clinically it has been shown that *P. aeruginosa* has the capacity to develop resistance rapidly during the course of antimicrobial therapy by several mechanisms[10-13]. Therefore, sequential accumulation of resistance may result in emergence of multidrug resistance in *P. aeruginosa*. Factors influencing the emergence and spread of acquired

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resistance in *P. aeruginosa* include inadequate and overuse of antimicrobials[14].

The steady rise in adaptive and mutational resistance is increasingly impacting on therapeutic success and new antimicrobial therapeutic options are needed for resistant strains, some of which have developed resistance to virtually every type of antibiotic and have thus become hospital 'superbugs'[15]. So this study was undertaken in order to assess the current level of susceptibility of widely used antipseudomonal antibiotics against *P. aeruginosa.*

METHODOLOGY

Bacterial strains

Sixty-nine consecutive, *Pseudomonas spp.* isolates were collected between May 2007 and March 2009 from sputum samples from HIV patients was collected from Government hospital of thoracic medicine, Tambaram sanatorium, Chennai. Sputum samples from Non-HIV patients were collected from Government Stanley hospital, Chennai and Dr.Kamashi Memorial hospital, Chennai. All isolates were identified according to standard protocol of CLSI 2009. All cultures were incubated at 37^0C for 24 - 48 h for isolation of *Pseudomonas spp.* Of these isolates, 45 were from HIV and 24 from non-HIV patients. The study was approved by the institutional ethical committee and informed consent was obtained from patients.

Antibiotic susceptibility testing-disc diffusion method

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines 2009. The following antipseudomonal antibiotics were used to screen for multidrug resistance among the isolates. The antibiotics included were piperacillin (100µg), cefotaxime (30µg), ceftazidime (30µg), cefoperazone (75µg), tobramycin (30µg), ceftriaxone (30µg), amikacin (30µg), netilmicin (30µg), ofloxacin (5µg), ciprofloxacin (5µg), imipenem (10µg), mezlocillin (75µg), azlocillin (75µg), ticarcillin (75µg).

MIC (Micro-Broth Dilution Method)

The MIC was done for the following antibiotics: ceftazidime, cefoperazone, cefotaxime (Orchid Pharmaceuticals Ltd), amikacin and ciprofloxacin (Hi Media laboratories ltd.). MICs were determined by micro broth dilution method as per CLSI guidelines using cation adjusted Mueller Hinton broth (CMHB, Difco) at pH 7.0. The antibiotics with various dilutions were prepared from the stock solutions and the inocula were prepared according to CLSI standards. Minimal inhibitory concentration (MIC) was visually read after 24 hrs of incubation at 37^0C. MIC was defined as the lowest drug concentration resulting in 90% reduction in turbidity when compared to the drug free control. Minimum bactericidal concentration (MBC) was also done for further confirmation.

The final concentrations of the antibiotics ranged from 0.25µg/ml to 256µg/ml. *P. aeruginosa* ATCC 27853 was included as the quality control strain. Interpretive criteria for resistance to various antibiotics according to CLSI guidelines were as follows: ceftazidime (≥ 32µg/ml), cefotaxime (64µg/ml) cepoferazone (≥ 64µg/ml), amikacin (≥ 64µg/ml), and ciprofloxacin (≥ 4µg/ml).

RESULTS

A total of 71 respiratory isolates of pseudomonas spp. were isolated both from HIV and Non-HIV patients with lower respiratory tract infection over the period of May 2007-March 2009. Among the 71 isolates 44 were *Pseudomonas aeruginosa* 19 were *Pseudomonas stutzeri*, 8 were *Pseudomonas putida* 1 were *Alcaligenes faecalis* Out of 71/45 were from HIV population, 24 were from Non-HIV and 2 were environmental isolates. A standard strain of ATCC was used as control. All the 71 (22.76%) isolates were examined for the antibiotic sensitivity against 15 antipseudomonal antibiotics.

Table 1 shows that among the 45 HIV isolate tested, 37 (82.2%) showed resistance to ceftazidime, 30/45 (66.7%) showed resistance for mezlocillin. Ceftriaxone and cefotaxime resistance was shown by 29/45(64.4%), resistance to cefoperazone was shown by 27/45 (60%), whereas 21/45 (46.7%) showed resistance to ticarcillin. 10/45 (22.2%) showed resistance to, ciprofloxacin and piperacillin. 9/46 (20%) showed resistance to amikacin and azlocillin. 7/45 (15.6%) isolates showed resistance towards tobramycin and ofloxacin, 5/45 (11.1%) showed resistance to netilmicin. All the 45 isolates showed 100% sensitivity to imipenem.

Table 2 shows that non HIV isolates showed highest resistance to cefotaxime and mezlocillin 21/24(87.5%), they showed intermediate resistance to ceftazidime and ticarcillin 18/24 (75%) followed by resistance to cefoperazone16/24 (66.7%). Azlocillin and ceftriaxone showed 11/24 (45.8%) resistance. 8/24 (33.3%) isolates showed resistance to piperacillin, while 3/24 (12.5%) showed resistance to amikacin followed by 2/24 (8.3%) resistance towards ciprofloxacin and imipenem. The least resistance was observed for ofloxacin 1/24 (4.17%).

Table 1: Resistance pattern exhibited by *Pseudomonas spp*. among HIV patients to various antibiotics

Antibiotics group	Antibiotics in µg	Number of strains resistant to antibiotics (%)
Carbapenems	Imipenem (10µg)	0 (0)
Cephalosporin	Ceftazidime (30µg)	37 (82.2)
	Cefotaxime (30µg)	29 (64.4)
	Cefoperazone (75µg)	27 (60.0)
	Ceftriaxone (30µg)	29 (64.4)
Aminoglycosides	Amikacin (30µg)	9 (20.0)
	Tobramycin (30µg)	7 (15.6)
	Netilmicin (30µg)	5 (11.1)
Fluoroquinolones	Ofloxacin (5µg)	7 (15.6)
	Ciprofloxacin (5µg)	10 (22.2)
Penicillin group	Piperacillin (100µg)	10 (22.2)
	Mezlocillin (75µg)	30 (66.7)
	Azlocillin (75µg)	9 (20.0)
	Ticarcillin (75µg)	21 (46.7)

Table 2: Resistance pattern exhibited by *Pseudomonas spp*. among Non-HIV patients to various antibiotics

Antibiotics group	Antibiotics in µg	Number of strains resistant to antibiotics (%)
Carbapenems	Imipenem (10µg)	2 (8.3)
Cephalosporin	Ceftazidime (30µg)	18 (75.0)
	Cefotaxime (30µg)	21 (87.5)
	Cefoperazone (75µg)	16 (66.7)
	Ceftriaxone (30µg)	11 (45.83)
Aminoglycosides	Amikacin (30µg)	3 (12.5)
	Tobramycin (30µg)	4 (16.7)
	Netilmicin (30µg)	4 (16.7)
Fluoroquinolones	Ofloxacin (5µg)	1 (4.2)
	Ciprofloxacin (5µg)	2 (8.3)
Penicillin group	Piperacillin (100µg)	8 (33.3)
	Mezlocillin (75µg)	21 (87.5)
	Azlocillin (75µg)	11 (45.8)
	Ticarcillin (75µg)	18 (75)

Minimum inhibitory concentration by micro broth dilution method (MIC): The MIC for the antibiotics was tested by micro broth dilution method according to CLSI guidelines 2008. Among HIV isolates tested, 37 isolates which showed resistance for ceftazidime were taken for MIC studies of third generation cephalosporin. 13 (35.13%) showed resistance in our MIC studies.

Thirteen out of 30 isolates (35.13%) showed resistance for cefotaxime and 17 out of 27 isolates (62.96%) were resistant to cefoperazone. 4 (40%)isolates showing ciprofloxacin resistance and 1(11.11%) out of 9 strains showed resistance to amikacin. (Table 3)

Table 3: MIC for HIV isolates

Antibiotics	S	I	R	MIC Break points		
				S	I	R
Ceftazidime (N=37)	21	3	13	≤8	16	>32
Cefotaxime (N=29)	4	12	13	8	16-32	64
Cefoperazone (N=27)	9	1	17	16	32	≥64
Ciprofloxacin (N=10)	-	7	4	≤1	2	≥4
Amikacin (N=9)	9	-	-	≤16	32	≥64

In non HIV isolates, 2 out of 16 isolates (12.5%) tested showed resistance by MIC for cefoperazone, 100% sensitivity was seen for ciprofloxacin, 4 (19.04%) out of 21 isolates showed resistance by MIC in our study for cefotaxime.16 out of 18 isolates (88.89%) showed resistance by MIC for ceftazidime. (Table 4)

Table 4: MIC for Non-HIV isolates

Antibiotics	S	I	R	MIC Break points		
				S	I	R
Ceftazidime (N=18)	2	-	16	≤8	16	>32
Cefotaxime (N=21)	10	17	4	8	16-32	64
Cefoperazone (N=16)	13	1	2	16	32	≥64
Ciprofloxacin (N=2)	1	1	-	≤1	2	≥4
Amikacin (N=3)	3	-	-	≤16	32	≥64

DISCUSSION

As resistance among *P. aeruginosa* continues to increase globally, novel dosage strategies will be needed to retain the effectiveness of currently available antibiotics. Pseudomonas is inherently resistant to many antimicrobial Agents. The rate of strains with acquired resistance to ceftazidime has been estimated to range from 10% to 40%[16]. Our rate of ceftazidime resistance was 35.13%.the development of antibiotic resistance is very common during the course of treatment. Our results demonstrate that half of our isolates are multiple resistant both in HIV and in non-HIV population. These data indicate that a high number of isolates probably have resistance due to impermeability or multi-drug efflux or a combination of multiple unrelated resistance mechanisms. Ciprofloxacin showed the highest in vitro antibacterial activity followed by amikacin among the non HIV population whereas in HIV population only amikacin showed the highest antibacterial activity than ciprofloxacin in our centre[11,17,18]. These data indicate that a high number of isolates probably have resistance due to impermeability or multi-drug efflux or a combination of multiple unrelated resistance mechanisms.

Although comparison between studies is difficult since patient populations of centres and methods of studying differ, interestingly, we found a higher level of resistance to cephalosporin group by disc diffusion method followed by penicillin group. Aminoglycosides and fluoroquinolones showed the least resistance. Whereas in MIC we noticed that in HIV isolates ciprofloxacin resistance was high compared to non-HIV isolates, in HIV cefoperazone showed the highest resistance by MIC followed by cefotaxime and ceftazidime compared to non-HIV where it was seen for ceftazidime followed by cefotaxime and cefoperazone[19-22]. The incidence of resistance is dependent on the patterns of antibiotic usage.

Our findings suggest that imipenem, amikacin and ciprofloxacin may be of significant value for the treatment of severe infections caused by *P. aeruginosa*. Though resistance to imipenem has developed in our study we did not find any imipenem resistance both in HIV and Non-HIV isolates tested. So imipenem remains the drug of choice for treatment in most of the severe cases and may be more useful than β-lactams for combined treatment.

CONCLUSION

The prevalence of ceftazidime resistance in our study was 35.13% compared to other studies. There is a higher level of resistance among the HIV population than non-HIV population which may be due to the varying usage of antibiotics to treat infections in immunocompromised hosts. Hence there is a need for periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy.

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