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MULTIDRUG RESISTANCE IN *PSEUDOMONAS AERUGINOSA* ISOLATED FROM DIFFERENT SOURCES

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ABSTRACT

Pseudomonas aeruginosa has become an important cause of infections for example bacteremia, urinary tract infections and pneumonia etc. Antibiotic resistance rates in *Pseudomonas aeruginosa* are increasing worldwide. For treatment of infections caused by *P. aeruginosa*, beta-lactam and aminoglycoside antibiotics are widely used. The present study was undertaken to assess the current level of susceptibility to the most common antipseudomonal antibiotics (meropenem**,** timethoprim-sulfamethoxazole, piperacillin/tazobactum, amikacin, ciprofloxacin, gentamicin, polymyxin B, aztreonam, cefoperazone, azithromycin, ampicillin, amoxicillin/clavulanic acid and ceftazidime) against different strains of *P. aeruginosa* isolated from pus, water, blood, sputum, urine, ear swab, throat swab and fluids. The antibiotic sensitivity test of each isolate was performed by the Kirby-Bauser disc diffusion method as per recommendation of National Committee for Clinical Laboratory Standards. Among all tested antibiotics aztreonam, amoxicillin/clavulanic acid and ampicillin were found to be the most effective antibiotics against *P. aeruginosa* with the resistant rates of 1.0, 2.1, and 2.4 % respectively. The highest resistant rates were observed against ceftazidime (39.7%), gentamicin (33.9%) and ciprofloxacin (29.5%).

Key words: Antibiotic resistance, *Pseudomonas aeruginosa*, ceftazidime, aztreonam, amoxicillin/clavulanic acid, ampicillin

INTRODUCTION

Among Gram-positive bacteria, the most common antibiotic-resistant organisms include *Streptococcus pneumoniae*, *Enterococcus* species and *Staphylococcus aureus*. While in case of Gram-negative organisms multidrug-resistant bacteria include *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Lister *et al*., 2009). Among them multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) is an important pathogen causing hospital acquired infections with high mortality rate (Bonfiglio *et al*., 1998). It is also an important cause of infections, specifically in immuno-compromised hosts. *P. aeruginosa* also frequently causes of hospital acquired infections for example bacteremia, urinary tract infections and pneumonia (Shahriar and Akter, 2011).

Infections caused by *P. aeruginosa* can be complicated and even life threatening (Shahriar and Akter, 2011). Because rate of antibiotic resistance in *P. aeruginosa* is increasing worldwide. Limited numbers of antibiotics are available which are effective against *P. aeruginosa*. These include carbapenems, cephalosporins, penicillins and fluoroquinolones especially ciprofloxacin (Carmeli *et al*., 1999). For the treatment of infections caused by *P. aeruginosa*, aminoglycoside (amikacin), beta-lactams (ceftazidime), or some times combination of two different antibiotics belonging to different chemical classes is widely used (Oie *et al*., 1999). Amikacin, ciprofloxacin, ceftazidime and imipenem are widely used in hospital settings (Neves *et al*., 2010). For the above mentioned antibiotics, increasing of resistance during treatment has been well documented. This emergence of resistance has been identified as a cause of treatment failure in case of pseudomonal infections (Carmeli *et al*., 1999; Oie *et al*., 1999). An interesting question is to what extent the use of these agents is responsible for the emergence and spread of MDR-PA (Neves *et al*., 2010).

In view of above, the present study was undertaken to evaluate the current level of antibiotic resistance against commonly used anti-pseudomonal antibiotics.

MATERIALS AND METHODS

Bacterial strains

Five hundred and eighty four (584) identified bacterial strains of *Pseudomonas aeruginosa* were collected from Microbiology Laboratory of Abbasi Shaheed Hospital and PNS Shifa Hospital Karachi Pakistan between August 2008 and March 2011, isolated from different samples including pus (n=254), water (n=184), blood (n=28), sputum (n=14), urine (n=30), ear swab (n=30), throat swab (n=04) and fluids (n=40).

Antibiotic susceptibility

Mueller-Hinton agar (MHA) (Oxoid) plates were used for the susceptibility testing. The antibiotic sensitivity test of each isolate was carried out by the Kirby-Bauser disc diffusion method as per recommendation of National Committee for Clinical Laboratory Standards (Cheesbrough, 2000). The discs used contained following antibiotics: meropeneme (MEM) (10 μg), trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 μg), piperacillin/tazobactum (TZP) (100/10 μg), amikacin (AK) (30 μg), ciprofloxacin (CIP) (5 μg), gentamicin (CN) (10 μg), polymyxin B (PB) (300 units), cefoperazone (SCF) (75 μg), aztreonam (ATZ) (30 μg), azithromycin (ATM) (15 μg), ampicillin (AMP) (10 μg), amoxicillin/clavulanic acid (AMC) (20/10 μg) and ceftazidime (CAZ) (30 μg). The inoculums were standardized by comparing MacFarland number 0.5. The antibiotic discs were then placed on the petridishes seeded with the bacterial inoculums over the surface MHA. The petridishes were incubated for 18-24 hours at 37°C and zones of inhibitions observed were measured. The susceptibility or resistance was interpreted on the basis of criteria mentioned in Table 1.

RESULTS AND DISCUSSION

The study was conducted on 584 strains of *P. aeruginosa* isolated from pus (n=254), water (n=184), blood (n=28), sputum (n=14), urine (n=30), ear swab (n=30), throat swab (n=04) and fluids (n=40). The present study revealed the extent and level of antibiotic resistance in *P. aeruginosa* in Karachi, Pakistan. Results of *in vitro* antibacterial activity of commonly used anti-pseudomonal antibiotics are presented in Table 2. The anti-biogram patterns of *P. aeruginosa* exhibited considerable variation in resistance rates against antibiotics used in the study. The overall highest resistance was observed against ceftazidime (39.7%; 232/584), followed by gentamicin (33.9%; 198/584). While the lowest rate of resistance was observed against aztreonam (1.0%; 06/584).

As far as isolates from different sources viz., pus water, blood, sputum, urine, ear swab, throat swab and fluids were concerned, the highest rates of resistance were observed against gentamicin (43.3.%; 110/254), ceftazidime (46.7%; 86/184), amikacin (57.1%; 16/28), ceftazidime (50.0%; 07/14), ceftazidime (36.7%; 11/30), ceftazidime (53.3%; 16/30), ceftazidime (75.0%; 03/04), and ceftazidime (77.5%; 31/40) respectively (Table 2). Whereas, consistent antibiotic resistance pattern could not be established for *P. aeruginosa* on the basis of different sources.

Recently, multi-drug-resistant strains of bacteria have developed due to inappropriate use of existing antibiotics used for the treatment of infections (Saeed *et al*., 2006). Multidrug resistant strains further complicate the therapy of infection (Afshan *et al*., 2006). People may sick longer due to the infections caused by resistant bacteria and sometimes these infected people are not able to recover at all particularly in case in of patients with weak immune system (Saeed and Tariq, 2009).

In the current study, the highest rate of resistance in *P. aeruginosa* was found against ceftazidime. I is a third generation antibiotic and is frequently used for the treatment of pseudomonal infections. However, the emergence of ceftazidime-resistant *P. aeruginosa* has limited the effectiveness of ceftazidime in the treatment of *Pseudomonas* infections (Lee *et al*., 1999).

Based on local antibacterial practices, considerable geographic variations in bacterial resistance patterns are there (Yuksel *et al*., 2006). The susceptibility pattern of bacteria to antibiotics also varies with time and place (Magalit *et al*., 2004). Bacterial resistance to antibiotics is also enhanced by antimicrobial selection pressure by antibiotics and crossed transmission (Ferroni and Zahar, 2006). Therefore, antimicrobial therapy should depend upon the findings of diagnostic laboratory studies (Modarres *et al*., 1998). Due to this reason it requires regular monitoring of resistance patterns in *P. aeruginosa*. Keeping in view the object of the present study was to examine the rate of resistance in *P. aeruginosa* isolated from different sources.

The findings of the present study are consistent with another study carried out in Karachi by Nadeem *et al*. (2009) in which *P. aeruginosa* showed resistance to amikacin (8%), ceftrixone (15%), cefotaxim (16%), sulzone (7%), meropenem (8%), ciprofloxacin (11%) and fosfomycin (18%). In a similar study in Karachi, Pakistan, the resistance pattern of *P. aeruginosa* showed that 79% isolates were resistant to ceftrixon, 58% to aztreonam, 24% to imipenem, 19% to gentamicin, 15% to ciprofloxacin, 11% to ceftazidime and 4 % isolates were resistant to amikacin (Mansoor *et al*., 2009).

In contrast to these studies, the findings of other study in Peshawar, Pakistan revealed significantly high rates of resistance in *P. aeruginosa* against ampicillin (98.4%), ampicillin/sulbactum (85.3%), co-amoxiclave (83.8%), ofloxacin (68.4%), gentamicin (67.8%), tobramycin (44%), aztreonam (37%), piperacillin/tazobactum (35.1%), enoxacin (33%), ceftazidime (30.2%), meropenem (28%), imipenem (26%) and amikacin (24%) (Khan *et al*., 2008).

It is apparent from the present study that antibiotic resistant strains are prevailing in the environment which may be hazardous to the health as transmission of such drug resistant strains may be responsible for spreading some fatal

diseases particularly in immuno-compromised persons. Thus, the increasing problem of drug resistance requires regular monitoring and evolution of new antimicrobial drugs.

Table 1. Criteria for the interpretation of antibiotic resistance/susceptibility.

Antibiotics	Disc code	Potency (µg)	Inhibition zone diameter in mm		
			Resistant	Intermediate	Susceptible
Meropenem	MEM	10	≤13	14-15	≥16
Trimethoprim-sulfamethoxazole	SXT	1.25/23.75	≤10	11-15	≥16
Pip/tazobactum	TZP	100/10	≤17	---	≥18
Amikacin	AK	30	≤14	15-16	≥17
Ciprofloxacin	CIP	5	≤15	16-20	≥20
Gentamicin	CN	10	≤12	13-14	≥15
Polymyxin B	PB	300 units	≤11	---	≥12
cefoperazone	SCF	75	≤15	16-20	≥21
Aztreonam	ATZ	30	≤15	16-21	≥22
Azithromycin	ATM	15	≤13	14-17	≥18
Ampicillin	AMP	10	≤13	14-16	≥18
Amoxicillin/clavulanic acid	AMC	20/10	≤13	14-17	≥18
Ceftazidime	CAZ	30	≤14	15-17	≥18

Table 2. Antibiotic resistant patterns of Pseudomonas aeruginosa isolated from different sources.

Samples	No. of isolates	Percentage (number) of isolates resistant to												
		MEM	SXT	TZP	AK	CIP	CN	PB	SCF	CAZ	ATZ	ATM	AMP	AMC
Pus	254	8.7 (22)	6.3 (16)	10.2 (26)	25.2 (64)	36.2 (92)	43.3 (110)	1.6 (04)	7.1 (18)	2.8 (70)	0 (0)	1.8 (46)	0 (0)	0 (0)
Water	184	12.0 (22)	10.9 (20)	15.2 (28)	3.3 (06)	6.5 (12)	4.3 (08)	3.3 (06)	0 (0)	46.7 (86)	0 (0)	0 (0)	0 (0)	0 (0)
Blood	28	35.7 (10)	0 (0)	0 (0)	57.1 (16)	28.6 (08)	50.0 (14)	35.7 (10)	28.6 (08)	28.6 (8)	0 (0)	14.3 (04)	42.9 (12)	21.4 (06)
Sputum	14	42.9 (06)	14.3 (02)	42.9 (06)	28.6 (04)	28.6 (04)	42.9 (06)	0 (0)	42.9 (06)	50.0 (07)	0 (0)	0 (0)	0 (0)	0 (0)
Urine	30	13.3 (04)	20.0 (06)	33.3 (10)	13.3 (04)	33.3 (10)	26.7 (08)	0 (0)	13.3 (04)	36.7 (11)	6.7 (02)	26.7 (08)	6.7 (02)	13.3 (04)
Ear swab	30	26.7 (08)	0 (0)	13.3 (04)	20.0 (06)	46.7 (14)	66.7 (20)	20.0 (06)	13.3 (04)	53.3 (16)	6.7 (02)	0 (0)	0 (0)	0 (0)
Throat swab	04	0 (0)	0 (0)	0 (0)	0 (0)	50.0 (02)	50.0 (02)	0 (0)	0 (0)	75.0 (03)	0 (0)	50.0 (02)	0 (0)	0 (0)
Fluids	40	30.0 (12)	15.0 (06)	35.0 (14)	35.0 (14)	75.0 (30)	75.0 (30)	0 (0)	30.0 (12)	77.5 (31)	5.0 (02)	25.0 (10)	0 (0)	5.0 (02)
Total	584	14.4 (84)	8.6 (50)	15.1 (88)	19.5 (114)	29.5 (172)	33.9 (198)	4.5 (26)	8.9 (52)	39.7 (232)	1.0 (06)	12.0 (70)	2.4 (14)	2.1 (12)

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