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Comparison of Biotyping and Antibiotyping of *Pseudomonas aeruginosa* Isolated from Patients with Burn Wound Infection and Nosocomial Pneumonia in Shiraz, Iran

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Abstract: The objective of present study was to compare and determine the prevalence of antibiotypes and biotypes of *Pseudomonas aeruginosa* isolated from patients with burn infection and nosocomial pneumonia in Shiraz, Iran. Thirty isolates from each group of patients were used. Antibiotyping (antibiotic sensitivity profiles) was performed by disk diffusion of Bauer-Kirby method using eleven antibiotics and biotyping (biochemical profiles) was done by standard biochemical procedures. High rate of multi-drug resistant isolates were observed by both groups of patients. *P. aeruginosa* isolated from burn infection were found more resistant (26.7%) to the all antibiotics used than those from nosocomial pneumonia (6.7%) $p \leq 0.04$. All *P. aeruginosa* (100%) isolates from burn infection were resistant to gentamycin, carbenicillin, cephtazidime and cephalothin. The lowest resistance rate was observed with meropenem. Antibiotic susceptibility profiles revealed 11 and 15 different antibiotypes among *P. aeruginosa* isolates from patients with burn infection and nosocomial pneumonia, respectively. The biochemical profiles consisting of 21 biochemical tests grouped *P. aeruginosa* into 8 different biotypes. Biotypes BVIII 15(50%) and BIII 11(36.7%) were the most prevalent isolates from burn infection and nosocomial pneumonia, respectively $p \leq 0.04$. Data obtained in this study revealed that different types of *Pseudomonas aeruginosa* are involved in burn wound infection and nosocomial pneumonia in this region.

Key words: Antibiotypes, biotypes, *Pseudomonas aeruginosa*, burn infection, nonsocomial pneumonia

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous, obligate aerobic, non fermentative, gram-negative bacillus which is widely distributed in humid environment. The ability of these bacteria to survive on inert material, minimum nutritional requirement, relative resistance of antimicrobial agents and antiseptics, contributes enormously to its survival and plays a major role as an effective opportunistic pathogen (Pollak, 2000). This organism rarely causes disease in normal healthy individuals and when introduced in the tissue of patients who are immunocompromised, mechanically ventilated, or patients undergoing antibiotic treatment, produce several virulence factors which consequently may lead to septicemia and death of the patients (Matar *et al*., 2002; Bang *et al*., 2002). *P. aeruginosa* has been incriminated in cases of meningitis, pneumonia, nosocomial, ocular and burn infections. Infections caused by *P. aeruginosa* are often difficult to treat, as the majority of isolates exhibit intrinsic resistance against many antimicrobial agents (Hancock, 1998). The increase of extended spectrum beta lactamase producing strains among the clinical isolates and exposure of these bacteria to various classes of antibiotics can result in the emergence of mutant strains that are highly resistant to multiple antibiotics (Ishii *et al*., 2005). Such Multi-Drug Resistant (MDR) strains can also present a major therapeutic problem for the patients. It is therefore necessary to carry out periodically, the susceptibility profile of this bacteria isolated from various infections to the routinely used antibiotics in order to modify the preventive and therapeutic strategies. For epidemiological investigation of infection caused by *P. aeruginosa*, various phenotypic and genotypic procedures such as biotyping, antibiogram, plasmid profile, serotyping, ribotyping, pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis have been used (Ndip *et al*., 2005; Freitas and Barth, 2004; Kinoshita *et al*., 1997; Fluge *et al*., 2001). Although DNA based techniques have been successfully applied, these procedures need expensive equipments and reagents which are not widely available in clinical laboratories of the developing countries, therefore phenotypic techniques such as biotyping and

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antibiotyping which are claimed to be 99% accurate and are relatively cheap could be applied (Kinoshita *et al*., 1997; Koneman *et al*., 1992).

In Iran, *P. aeruginosa* accounts for a significant proportion of burn wound and respiratory tract infections (Karimi-estabanati *et al*., 2002; Rastegar-lari and Aleghebardan, 2000). In our country, antibiotic consumption is high and sometimes without prescription which may lead to the development of MDR strains. The present study was carried out to investigate the antibiotic sensitivity profile and biotype of *P. aeruginosa* isolated from patients with nosocomial pneumonia and burn wound infections in medical centers of Shiraz University of Medical Sciences, Shiraz, southern Iran.

MATERIALS AND METHODS

Isolations of *P. aeruginosa* from patients: Wound swabs obtained from patients with burn wound infection who were admitted in Ghotb-e-din hospital, the burn center of Shiraz University of Medical Sciences and sputum samples from patients with nosocomial pneumonia admitted in ICU in our university hospital were used for isolation of *P. aeruginosa*. The samples were cultured on blood agar, cetrimide and Mac-Conkey agar (Merk, KGaA, 64271 Darstadt, Germany) plates and incubated at 37°C for 24-48 h. *P. aeruginosa* were then identified on the basis of colony morphology, mucoidy, zone of hemolysis on blood agar, pigmentation on cetrimide agar and positive oxidase and citrate tests (Forbes *et al*., 1998). Only those specimens which revealed pure or heavy growth of *P. aeruginosa* were included in this study. Thirty isolates of *P. aeruginosa* from each group of patients were collected and then subjected to biotyping using the API20E (Biomerieux SA) kit according to the manufacturer recommendations. Any repeat isolate from the same patient was excluded.

Antibiotic susceptibility testing: *P. aeruginosa* isolates were subjected to disk antibiotic susceptibility testing using the standard method of Bauer-Kirby. Briefly a 1: 100 dilution from the bacterial suspension with turbidity equivalent to #0.5 McFarland were prepared by using Muller Hinton broth (Merk, KgaA, 64271 Darstadt, Germany). A sterile cotton swab was dipped into standardized bacterial suspension and spread on a Muller Hinton agar. Antibiotic disks with the following drug contents, amikacin (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), gentamycin (10 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), meropenem (10 μg), imipenem (10 μg), tetracycline (30 μg), tobramycin (10 μg), carbenicillin (100 μg) were placed on the plates. The plates were incubated at 37°C for 24 h after which zones of inhibition were measured and evaluated as recommended by NCCLS (2000).

Statistical analysis: The significant difference of the antibiotic resistances patterns between *P. aeruginosa* isolates from the two groups of patients were determined by the Chi-square and fisher exact tests. SPSS, version 11.5 was used to perform statistical analysis.

RESULTS

Table 1 represent the results of antibiotic sensitivity tests carried out on *P. aeruginosa* isolated from patients with nosocomial pneumonia and burn wound infection. The resistance rate of *P. aeruginosa* isolated from patients with burn wound infection varied from 36.7-100%. All (100%) *P. aeruginosa* isolated from these patients were resistant to cephalothin, gentamicin, carbenicillin and ceftazidime while the lowest resistance was observed with meropenem. The resistance rate of *P. aeruginosa* isolated from the sputum of patients with nosocomial pneumonia varied from 23.3-96.7% in which the lowest resistance was seen with meropenem and ciprofloxacin, i.e., 23.3%. Overall, among 60 isolates of *P. aeruginosa*, in this study, 42 (70%) of isolates were sensitive to meropenem followed by 37 (61.7%) to imipenem and only 1 (1.7%) of the isolates was sensitive to cephalothin. Table 2 shows the antimicrobial (antibiotypes) resistance patterns of *P. aeruginosa* isolated from patients with

Table 1: Antibiotic sensitivity tests on *P. aeruginosa* strains isolated from clinical specimens

Drugs	Wound (n = 30) No. resistance (%)	Sputum (n = 30) No. resistance (%)	p-value	Total (n = 60) resistance (%)
Amikacin	28 (93.3)	10 (33.3)	0.0001*	38 (63.3)
Cephalothin	30 (100.0)	29 (96.7)	1.00	59 (98.3)
Chloramphenicol	29 (96.7)	27 (90.0)	0.61	56 (93.3)
Gentamycin	30 (100.0)	11 (36.7)	0.0001*	41 (68.3)
Tetracycline	29 (96.7)	29 (96.7)	1.00	58 (96.7)
Tobramycin	27 (90.0)	12 (40.0)	0.0002*	39 (65.0)
Carbenicillin	30 (100.0)	26 (86.7)	0.11	56 (93.3)
Cephtazidime	30 (100.0)	27 (90.0)	0.24	57 (95.0)
Ciprofluxacin	25 (83.3)	7 (23.3)	0.00002*	32 (53.3)
Meropenem	11 (36.7)	7 (23.3)	0.40	18 (30.0)
Imipenem	14 (46.7)	9 (30.0)	0.29	23 (38.3)

*Statistically significant

Table 2: Antimicrobial resistance (Antibiotypes) patterns of *P. aeruginosa* strains isolated from patients with nosocomial pneumonia

Antibiotypes[a]												No. of strains showing pattern (%)
A1	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}	MER^{R}	IMI^{R}	2 (6.70)
A2	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}			1 (3.33)
A3	CF^{R}	C^{R}	TET^{R}	CB^{R}	CAZ^{R}							7 (23.30)
A4	AN^{R}	CF^{R}	C^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}					5 (16.70)
A5	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}					3 (10.00)
A6	CF^{R}	C^{R}	TET^{R}	CB^{R}	CAZ^{R}	IMI^{R}						2 (6.70)
A7	CF^{R}	GM^{R}	TET^{R}	CIP^{R}								2 (6.70)
A8	AN^{R}	CF^{R}	C^{R}	TET^{R}	CAZ^{R}	CIP^{R}						1 (3.33)
A9	AN^{R}	CF^{R}	C^{R}	TET^{R}	CB^{R}	CAZ^{R}	CIP^{R}	MER^{R}	IMI^{R}			1 (3.33)
A10	CF^{R}	C^{R}	GM^{R}	TET^{R}	CB^{R}	CAZ^{R}	CIP^{R}	MER^{R}	IMI^{R}			1 (3.33)
A11	CF^{R}	C^{R}	TET^{R}	GM^{R}	CAZ^{R}	MER^{R}						1 (3.33)
A12	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	CB^{R}	CAZ^{R}	MER^{R}	IMI^{R}			1 (3.33)
A13	CF^{R}	C^{R}	TET^{R}	CB^{R}	CAZ^{R}	MER^{R}	IMI^{R}					1 (3.33)
A14	CF^{R}	C^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}						1 (3.33)
A15	TOB^{R}	IMI^{R}										1 (3.33)
Total												30 (100.00)

[a]Abbreviations: AN, Amikacin; CF, Cephalothin; C, Chloramphenicol; GM, Gentamycin; TET, Tetracycline; TOB, Tobramycin; CB, Carbenicillin; CAZ, Ceftazidime; CIP, Ciprofloxacin; MER, Meropenem; IMI, Imipenem; R, Resistance

Table 3: Antimicrobial resistance (Antibiotypes) patterns of *P. aeruginosa* strains isolated from patients with burn wound infections

Antibiotypes[a]												No. of strains showing pattern (%)
A1	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}	MER^{R}	IMI^{R}	8 (26.7)
A2	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}			11 (36.7)
A3	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}	IMI^{R}		3 (10.0)
A4	CF^{R}	C^{R}	GM^{R}	TET^{R}	CB^{R}	CAZ^{R}						1 (3.3)
A5	AN^{R}	CF^{R}	GM^{R}	TET^{R}	CB^{R}	CAZ^{R}	CIP^{R}					1 (3.3)
A6	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	CB^{R}	CAZ^{R}	IMI^{R}				1 (3.3)
A7	AN^{R}	CF^{R}	C^{R}	GM^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}				1 (3.3)
A8	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}				1 (3.3)
A9	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	IMI^{R}				1 (3.3)
A10	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	IMI^{R}			1 (3.3)
A11	AN^{R}	CF^{R}	C^{R}	GM^{R}	TET^{R}	TOB^{R}	CB^{R}	CAZ^{R}	CIP^{R}	ME^{R}		1 (3.3)
Total												30 (100.0)

[a]Abbreviations: AN, amikacin; CF, cephalothin; C, chloramphenicol; GM, gentamycin; TET, tetracycline; TOB, tobramycin; CB, carbenicillin; CAZ, ceftazidime; CIP, ciprofloxacin; MER, meropenem; IMI, imipenem; R, resistance

Table 4: Biotypes of *P. aeruginosa* strains isolated from sputum and wound of patients with nosocomial pneumonia and burn wound infections

	Biochemical tests																						
Biotypes	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	N	(%)
BI	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	6	1.0
BII	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	5	8.3
BIII	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	15	25.0
BIV	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	4	6.7
BV	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+	+	7	11.7
BVI	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	1	1.7
BVII	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	3	5.0
BVIII	-	+	-	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	19	31.7

A, ortho-nitrophenyl-galactoside; B, adenine dihydrolase; C, lysine decarboxylse; D, ornithine decarboxylase; E, citrate utilization; F, hydrogen sulphide production; G, urease; H, tryptophane deaminase; I, indol production; J, acetoin production (Voges Proskauer); K, gelatinase; L, glucose; M, mannitol; N, inositol; O, sorbitol; P, rhamnose; Q, sucrose; R, melibiose; S, amygdaline; T, arabinose; U, cytochrome oxidase; N, number of strains; %, % abundance +, positive; -, Negative

Table 5: Specimen sources and occurrence of biotypes of *P. aeruginosa* isolates

	Biotypes (%)								
Specimens	BI	BII	BIII	BIV	BV	BVI	BVII	BVIII	Total
Sputum	5 (16.7)	2 (6.7)	11 (36.7)	2 (6.7)	4 (13.3)	1 (3.3)	1 (3.3)	4 (13.3)	30
Burn wound	1 (3.3)	3 (10.0)	4 (13.3)	2 (6.7)	3 (10.0)	0 (0.0)	2 (6.7)	15 (50.0)	30
Total	6 (10.0)	5 (8.3)	15 (25.0)	4 (6.7)	7 (11.7)	1 (1.7)	3 (5.0)	19 (31.7)	60

nosocomial pneumonia. The predominant resistance pattern, cephalothin, chloramphenicol, tetracycline, carbenecillin and ceftazidime (CF^{R}, C^{R}, TET^{R}, CB^{R}, CAZ^{R}, anibiotype A3) were observed in 23.3% of the isolates and only 2 (6.7%) strains were resistant to the eleven antibiotics used, i.e., antibiotype A1. The least resistance pattern was exhibited by 1 (3.3%) isolate showing resistance corresponding to patterns A2 and A8-A15 antibiotypes.

Table 3 shows the antimicrobial resistance patterns of 30 *P. aeruginosa* isolates from burn wound infections. Eight (26.7%), 11(36.7%) and 3(10%) of isolates were resistant to 11, 9 and 10 antibiotics used in this study, respectively. The least resistance pattern was exhibited by

one isolate (3.3%) showing resistance corresponding to patterns A4-A11 antibiotypes.

Table 4 shows the results of biotyping of *P. aeruginosa* strains isolated from both groups of patients using 21 different biochemical tests and enzymes assay. Eight different biotypes, BI-VIII, were identified. Table 5 represents distribution of *P. aeruginosa* biotypes isolated from these two groups of patients. The most prevalent biotypes among patients with nosocomial pneumonia and burn wound infection were BIII 11(36.7%) and BVIII 15 (50%), respectively. Biotype BVI, was not detected among patients with burn wound infection.

DISCUSSION

In the present investigation the antibiotypes and biotypes of *P. aeruginosa* isolated from patients with burn infection and nosocomial pneumonia were identified and compared. All (100%) of the *P. aeruginosa* isolated from patients with burn wound infection were resistant to cephalothin, gentamycin, carbenicillin and cephtazidime which are in agreement with the findings of other investigators (Karimi-Estahbanati *et al*., 2002; Komolafe *et al*., 2003; Corona-Nakamura *et al*., 2001). However, lower resistance rate to these antibiotics were reported from other regions (Singh *et al*., 2003; Farjadian *et al*., 1996). In this study, *P. aeruginosa* isolated from nosocomial pneumonia in ICU revealed 36.7-96.7% resistance to the four above mentioned antibiotics. Using amikacin, gentamycin, tobramycin and ciprofloxacin, highly significant differences were observed between the resistance of *P. aeruginosa* isolates from the two groups of patients ($p<0.0001$). These findings indicate the lower antibacterial resistance rate among the isolates from patients with nosocomial pneumonia. A similar conclusion was also obtained in a study from Italy (Segatore *et al*., 1999). In this study, *P. aeruginosa* isolates from both groups of patients showed the lowest resistance to meropenem (burn infection, 36.7% and nosocomial pneumonia, 23.3%) and imipenem (burn infection, 46.7% and nosocomial pneumonia 30%). Moreover, Bonfiglio *et al*. (1998) have found meropenem four times more active than imipenen against these bacteria. Goossens (2003) reported that 29.1 and 44.9% of MDR *P. aeruginosa* were resistant to meropenem and imipenem respectively which are in close accordance with our findings. Although imipenem resistant strains have been reported more frequently than strains resistant to meropenem (Blandino *et al*., 2004; Turner *et al*., 1999) however, in this study no significant differences between the two antibiotics were observed. It is noteworthy that meropenem is expensive and was recently introduced to our country. This might be an explanation for the lowest resistance rate of *P. aeruginosa* to this antibiotic. During the last decade, the resistance pattern of these bacteria has changed drastically in our medical centers. In 1996, all (100%) of the *P. aeruginosa* isolates from nosocomial infections were sensitive to ceftazidime and ciprofloxacin (Farjadian *et al*., 1996), while in this study, 100 and 83.3% of the isolates from burn infection have been found to be resistant to these antibiotics, respectively. However, we observed significant differences between the resistance rates of isolates from the two groups of patients to ciprofloxacin ($p\leq0.00002$). *P. aeruginosa* recovered from burn infection were highly resistant to the 11 antibiotics used as, 26.7% were resistant to all, 80% to 9 or more and 100% of the isolates to 6 or more antibiotics. On the other hand, *P. aeruginosa* isolates from the sputum of patients with nosocomial pneumonia showed less resistance as only 2 (6.7%) of the isolates were resistant to all antibiotics used ($p\leq0.04$). There was a tendency for isolates derived from burn infection to demonstrate more antimicrobial resistance than those from nosocomial pneumonia. This might be due to the involvement of different types of *P. aeruginosa* in these infections. Considering the antibiotypes, in this study 11 and 15 different antimicrobial resistance patterns were identified among patients with burn infection and nosocomial pneumonia, respectively. Only two resistance patterns A1 and A2 which comprised 40% of all isolates were similar in both groups of patients. Considering these two antibiotypes, significant differences were observed between the isolates from the two groups of patients. The biotyping scheme applied in this study made use of biochemical tests in the API20E kit which is believed to be 99% accurate (Koneman *et al*., 1992; Ndip *et al*., 2005; Freitas and Barth, 2004; Balows *et al*., 1991; Wu *et al*., 2004). Based on these phenotypic procedures, eight biotypes were identified. Among patients with burn infection, 15 (50%) of the *P. aeruginosa* isolates were biotype BVIII while the prevalence of this biotype was 4 (13.3%) among patients with nosocomial pneumonia ($p\leq0.003$). BIII biotype was the most prevalent 11 (36.7%) among nosocomial pneumonia while 4 (13.3%) of this biotype was identified among patients with burn infection ($p\leq0.04$). These findings are in agreement with the data obtained by Ndip *et al*. (2005) who have reported the higher prevalence of BIII and BVIII biotypes in wound and sputum of patients with nosocomial infections, respectively. Although phenotypic procedures such as biochemical and antimicrobial tests have some limitations for detection of various *P. aeruginosa* isolates relationships, however, it seems useful to employ the two procedures in combination to produce a composite biochemical-antimicrobial profile to establish their relations. Data presented in this study indicate that high levels of MDR *P. aeruginosa* with different resistance pattern were isolated from patients with burn infection and nosocomial pneumonia. *P. aeruginosa* isolates from burn infection were significantly more resistant to

antibiotics than the isolates from nosocomial pneumonia in ICU. Overall, our findings revealed that meropenem followed by imipenem showed higher level of activity against clinical isolates of these bacteria than other antibiotics tested. The phenotypic procedures used in this study indicate that certain types of *P. aeruginosa* might be involved in burn wound infection and nosocomial pneumonia. Although newer antibiotics are needed to overcome the MDR isolates, our results provide a guideline for prescription of the most effective antibacterial agents to treat patients with nosocomial infections in our setting.

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