Characterization of *Pseudomonas aeruginosa* Strains Isolated from Burned Patients Hospitalized in a Major Burn Center in Tehran, Iran

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Abstract- *Pseudomonas aeruginosa* is an important life-threatening nosocomial pathogen and plays a prominent role in serious infections in burned patients. The current study was undertaken to characterize *P. aeruginosa* strains isolated from burned patients in Tehran, Iran. The study was conducted in a major burn center in Tehran, Iran in 2007. A total of seventy specimens obtained from different clinical origin with positive culture results for *P. aeruginosa* were included in the study. Antimicrobial susceptibility test was performed according to the standard CLSI guideline. The relationship between the strains was also determined using antimicrobial drug resistance pattern analysis and plasmid profiling. All strains were multi drug resistant. The percentage of resistance to tested antibiotics was: imipenem 97.5%, amikacin 90%, piperacillin 87.5%, ceftizoxime 72.7%, gentamicin 67.5%, ciprofloxacin 65%, ceftriaxone 60%, and ceftazidime 57.5%. Thirteen resistant phenotypes were recognized, R3 (TET, IPM, AMK, CIP, PIP, GM, CAZ, CRO, CT) was the predominant resistance pattern seen in 27.5% of isolates. Results obtained from E-test showed that 100% of *P. aeruginosa* strains were resistant to cefoxitin, 97% to cefotetan, 93% to ticarcillin, 89% to ticarcillin/clav, 76% to gentamicin and imipenem, 63% to piperacillin, 49% to tetracycline, and 20% to meropenem. Nine different plasmid profiles were observed among the strains. The current study showed an increase rate of resistance for some antibiotics tested among *P. aeruginosa* strains isolated from burned patients in Tehran. A combination of antibiotic susceptibility testing and profile plasmid analysis, which are relatively cheap and available methods, showed to be useful to characterize the clinical strains of *P. aeruginosa* isolated from burned patients in Iran.

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Keywords: *Pseudomonas aeruginosa*; Antimicrobial susceptibility; Plasmid profiles

Introduction

Burn injury as one of the most common and devastating forms of trauma, is a major public health problem worldwide (1). The risk of infections in burns is well known. Available current techniques of burn wound care have significantly decreased the incidence of infections in patients with burn wounds (2), however severely burned patients may still develop life-threatening infections. *Pseudomonas aeruginosa* as an important life-threatening nosocomial pathogen plays a prominent role in serious infections in burn patients. The condition for patients infected by this bacterium is particularly problematic since the organism is intrinsically resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs (3). *P. aeruginosa* isolates are resistant to many commonly used antibiotics (4). Some studies carried out in Iran have also indicated that infections caused by multi drug resistant (MDR) *P. aeruginosa* are widespread among Iranian hospitals (5-6). Many simple molecular methods such as plasmid profile have been used for epidemiological investigation of infections caused by multi drug resistant *P. aeruginosa* (5-6).

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The current research was undertaken to assess the new situation of antimicrobial resistance and to study the plasmid profiles of *P. aeruginosa* strains isolated from burn patients hospitalized in a main burn center in Iran in 2007.

Material and Methods

The study was conducted from March through November 2007 at Motahari Hospital, a major center for admission of burned patients in Tehran, Iran. A total of 70 specimens obtained from different clinical origins with positive culture results for *P. aeruginosa* were included in the study. Cultures of the burn wounds were performed using swabs on the admission and all clinically indicated cultures such as blood, tissue, and urine were also evaluated. They were plated primarily onto blood agar and incubated at 37 ºC for 24–48 h. Suspect isolates were presumptively identified by colony morphology, growth at 44 ºC, pigment formation, positive oxidase test, glucose fermentation, hydrolysis of arginine, nitrate production and growth on acetamide agar (7,8).

Antimicrobial susceptibility test was performed according to the standard CLSI guideline (9) by disk diffusion using 9 antibiotic disks (MAST Group LTD, Merseyside, UK): ceftriaxone (CRO, 30 µg); ceftizoxime (CT, 30 µg); ceftazidime (CAZ, 30 µg); amikacin (AMK, 30 µg); tetracycline (TET, 30 µg); ciprofloxacin (CIP, 5 µg); gentamicin (GM, 10 µg); piperacillin (PIP, 100 µg) and imipenem (IPM, 10 µg). Standard strain of *P. aeroginosa* ATCC 27853 was used as a quality control strain. E-test method was used for determination of MIC using cefoxitin, cefotetan, ticarcillin, ticarcillin/clav, gentamicin, imipenem, piperacillin, tetracycline, and meropenem strips.

Plasmids were extracted as previously described (10-12). Extracted plasmids were then separated on a 0.8% agarose gel in tris-borate-EDTA buffer (TBE×1) (pH 8.2) by electrophoresis at 45V for 4–5 h, stained with ethidium bromide and visualized under UV illumination. The strains were grouped depending on the size and number of the plasmid DNA bands.

Results

The distribution of *P. aeruginosa* among the clinical samples was as follows: burn wound swab 72.3%, tissue biopsy 15.4%, urine 7.7%, sputum 3%, and blood 1.5%.

All isolates were resistant to tetracycline. The percentage of resistance to other antibiotics for these isolates was as follows: imipenem 97.5%, amikacin 90%, piperacillin 87.5%, ceftizoxime 72.7%, gentamicin 67.5%, ciprofloxacin 65%, ceftriaxone 60%, and ceftazidime 57.5%. The predominant resistance pattern, R3 (TET, IPM, AMK, CIP, PIP, GM, CAZ, CRO, CT) was observed in 27.5% of isolates. The least resistance patterns were exhibited by 2.5% of isolates showing resistance corresponding to patterns R2, R5, R8, R9 and R13 (Table 1).

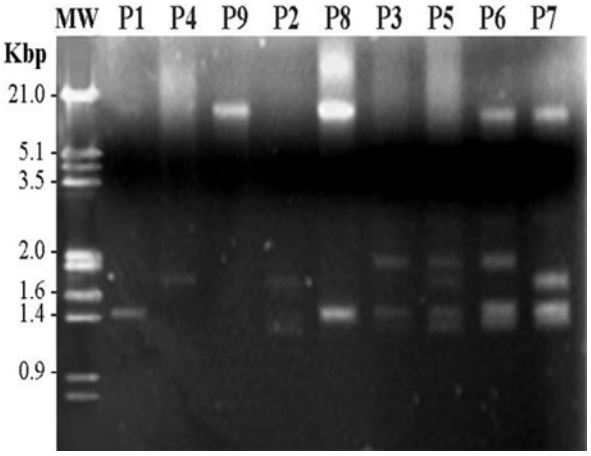

Figure 1. Plasmid profiles of *P. aeruginosa* isolates. Lanes P1-P9 are representative plasmid profiles of the strains. Lane M is DNA marker (molecular ladder).

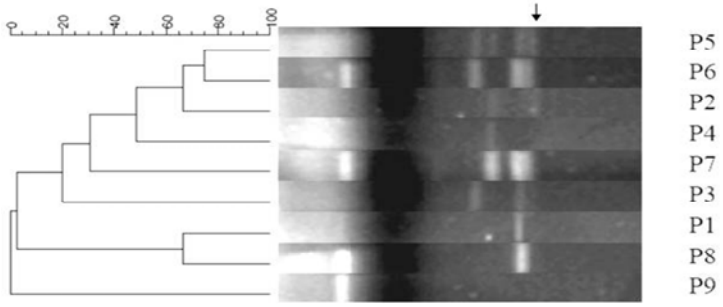

Figure 2. A UPGMA dendrogram showing distribution of plasmid profiles of *Pseudomonas aeruginosa* isolates. Lanes P1-P9 are representative plasmid profiles of the strains. The arrow indicates the DNA band evident in most isolates. UPGMA=Un-weighted pair group method with arithmetic averages.

Table 1. Plasmid profiles and antimicrobial resistance patterns of *P. aeruginosa*

Plasmid pattern	Size (kbp) of plasmids	% of isolates	Resistance Pattern (%)	Resistance phenotype*
P1	1.4	12.5	R1 (7.5)	TET, IPM, AMK, PIP, CRO
			R2 (2.5)	TET, IPM, AMK, PIP, GM, CAZ, CT
			R3 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CRO, CT
P2	1.3, 1.7	30	R4 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CT
			R5 (2.5)	TET, IPM, AMK, CIP, PIP, CT
			R6 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CT
			R1 (2.5)	TET, IPM, AMK, PIP, CRO
			R7 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ
			R9 (2.5)	TET, IPM, AMK, PIP, GM, CAZ, CRO
			R3 (15)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CRO, CT
P3	1.4, 1.9	22.5	R10 (5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CRO
			R11 (10)	TET, IPM, CT
			R6 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CT
			R7 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ
			R8 (2.5)	TET, AMK,CIP, PIP, GM, CRO, CT
P4	1.9	7.5	R12 (5)	TET, IPM, AMK, PIP, CRO, CT
			R13 (2.5)	TET, IPM, AMK,CIP, GM, CAZ, CT
P5	1.3, 1.4, 1.7, 1.9	2.5	R3 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CRO, CT
P6	1.3, 1.4, 1.9, 20	2.5	R12 (2.5)	TET, IPM, AMK, PIP, CRO, CT
P7	1.3, 1.4, 1.7, 20	15	R3 (7.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CRO, CT
			R12 (2.5)	TET, IPM, AMK, PIP, CRO, CT
			R10 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CRO
			R6 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ, CT
P8	1.4, 1.20	2.5	R7 (2.5)	TET, IPM, AMK,CIP, PIP, GM, CAZ
P9	20	5	R4 (5)	TET, IPM, AMK,CIP, PIP, GM, CT

* CRO: ceftriaxone; CT: ceftizoxime; CAZ: ceftazidime; AMK: amikacin; TET: tetracycline; CIP: ciprofloxacin; GM: gentamicin; PIP: piperacillin; IPM: imipenem

Results obtained from E-test showed that 100% of *P. aeruginosa* strains were resistant to cefoxitin, 97% to cefotetan, 93% to ticarcillin, 89% to ticarcillin/clav, 76% to gentamicin and imipenem, 63% to piperacillin, 49% to tetracycline, and 20% to meropenem.

All isolates harbored at least a single plasmid band. The electrophoretic analysis of plasmid DNAs showed the existence of 1 to 4 DNA bands ranging from 1.3 to larger than of 20 kbp in the strains tested (Figures 1 and 2). The DNA band of 1.4 kbp was evident in 57.5% of the strains. Based on size and number of DNA bands, plasmid analysis of *P. aeruginosa* isolates resulted in 9 different profiles labeled P1-P9. P2 (30%) was the dominant type followed by P3 (22.5%) (Table 1, Figure 1).

Discussion

We carried out this study in the year 2007 to determine antibiotic susceptibility and plasmid profiles of *P. aeruginosa* strains isolated from burned patients hospitalized at the Motahari Burn Center in Tehran, Iran. All strains showed multiple drug resistance. As shown in table 1, more than 90% of the isolates were resistant to imipenem, amikacin and tetracycline.

When compared to previous studies carried out in Iran, our finding indicated that the rate of resistance against some antibiotics tested has increased in recent years. Previously, resistance to imipenem has been reported 32.9%, 25%, 2.9 and 5.6% in our country in the years 2003 (13), 2005 (14), 2006 (6) and 2007 (15) respectively.

More than 87% of our isolates were found to be resistant to piperacillin while previous resistance rate for this antibiotic was reported 33.7% and 84% by Nikbin *et al.* (6) and Japoni *et al.* (13) respectively. Similarly, lower resistance rates against tetracycline and ceftriaxone were reported among *P. aeruginosa* strains isolated in Iran in the years 2002 (5) and 2006 (6). On the other hand, the resistance rate was relatively constant

for amikacin and ciprofloxacin when compared to previous reports in Iran (5,13,16).

Interestingly our results showed that the percentage of resistance to ceftizoxime (72.7%) and gentamicin (67.5%) was decreased in comparison with previous reports published from our country (5,6,16).

Here we also used antimicrobial drug resistance pattern analysis as an epidemiological marker for our isolates. This method is least expensive and could be considered as a preliminary screening tool in assessing the strain relatedness. This simple method has been widely applied in epidemiologic studies of *P. aeruginosa* through the years (5,17,18). Using this method, 13 resistant phenotypes were recognized suggesting this method may represent a discriminative approach for differentiation of *P. aeruginosa*.

Some previous studies have used plasmid profiles to characterize the isolates of *P. aeruginos* (5,6,19-25). This method is cheap and quick, requiring one hour approximately of hands-on time, and 24 hours to be completed. The results obtained from electrophoretic analysis of plasmid DNAs showed the presence of plasmid bands in all strains tested. The sizes of the plasmids among *P. aeruginosa* isolates ranged from 1.3 to 20 kb. Nine different plasmid profiles were observed among the strains, P2 was predominant (30%) followed by P3 (22.5%). In a recent study carried out in our country, Nikbin *et al*. identified 15 plasmid patterns among 31 strains of *P. aeruginosa* recovered from clinical and environmental specimens in Tehran, in the year 2006. Those patterns contained 1-7 plasmids which ranged in size from 1.7 to 100 kb (6).

Poh *et al*. found the plasmid profiling was to be a useful method adjunct to serotyping for the epidemiological typing of *P. aeruginosa* (25). This statement was also noted by Wu in a study carried out on 120 clinical strains of *P. aeruginosa* isolated in Nanjing City (22).

Walia and colleagues demonstrated that the combination of plasmid DNA profile or serotyping with electrophoretic patterns of soluble proteins can be of value in the epidemiologic fingerprinting of clinical isolates of *P. aeruginosa* (23).

In another study carried out by Plesiat and colleagues, however this technique appeared to be less appropriate as an epidemiological tool for this organism than other techniques since only 13.9% of the strains tested harbored plasmids and the majority of these plasmids were antibiotic resistant (19).

In this study we found any obvious correlation between plasmid profiles and antibiotic resistance patterns among *P. aeruginosa* strains. No correlation was also observed between the antibiotic resistance patterns and the kind of specimens in which the isolates were isolated. The results obtained from plasmid profiling indicated that 70% of the strains isolated from burn wounds have shown plasmid pattern 2 however there was any obvious correlation between other profiles and specimens.

The current study showed an increase rate of resistance for some antibiotics tested among *P. aeruginosa* strains isolated from burned patients in Tehran that merits immediate attention. We recommend Iranian health care practitioners and policy makers to address this problem by implementing an appropriate use of antibiotics. A combination of antibiotic susceptibility testing and profile plasmid analysis, which are relatively cheap and available methods in our country, could be useful for epidemiological studies particularly in research laboratories in the developing world with limited resources.

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