Prevalence of antibiotic resistance and *blaIMP-1* gene among *Pseudomonas aeruginosa* strains isolated from burn and urinary tract infections in Isfahan, central Iran

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Abstract

Pseudomonas aeruginosa is one of the most important opportunistic pathogens responsible for various types of hospital infections. High prevalence of antibiotic resistance in *P. aeruginosa* strains of human clinical samples cause more severe diseases for a longer period of time. The current research was done in order to study the distribution of *blaIMP-1* gene among the imipenem-resistant *P. aeruginosa* strains isolated from burn and urinary tract infections of hospitalized patients. Two-hundred and forty-three *P. aeruginosa* isolates recovered from the cases of burn and urinary tract infections of inpatients and outpatients were analysis for antibiotic resistance pattern using the disk diffusion method. Then, imipenem-resistant isolates were further analyzed for distribution of *blaIMP-1* gene using the PCR. Of 243 *P. aeruginosa* isolates, 146 strains (60.08%) were taken from outpatients and 97 strains (39.91%) were taken from inpatients. *P. aeruginosa* isolates harbored the highest levels of resistance against streptomycin (100%), nalidixic acid (100%), aztreonam (100%), cotrimoxazole (95.47%), ciprofloxacin (88.47%), cefotaxime (84.36%) and gentamycin (83.95%). Inpatients had a relatively higher levels of antibiotic resistance. One-hundred and twenty-one out of 126 (96.03%) imipenem-resistant *P. aeruginosa* isolates harbored the *blaIMP-1* gene. Inpatients also had a relatively higher prevalence of *blaIMP-1* gene. High prevalence of *blaIMP-1* gene and also imipenem-resistant *P. aeruginosa* are important public health issue. Clinical laboratories should consider the detection of the *blaIMP-1* gene among the *P. aeruginosa* isolates of clinical samples.

Introduction

Superficial infections and especially burn infections (BIs) are important cause of emergency health care associated problems al-around the world. Superficial infections caused longer hospital stays, more expensive hospitalizations, and increased mortality.[1] The annual superficial infection care products market is projected to reach $15.5 billion by 2010.[1] Urinary tract infections (UTIs) are one of the most common bacterial infections diseases in human.[2-4] UTIs account for more than 8 million referrals to hospitals, 1.5 million hospitalizations, and 300,000 severe clinical syndromes in the United States annually.[2-4] UTIs is an important cause of mortality and morbidity al-around the world.[5,6]

Pseudomonas aeruginosa (*P. aeruginosa*) is a non-fermentative, aerobic, Gram-negative rod shape bacterium and is responsible for severe human clinical infections such as BIs, UTIs, pneumonia, wound, reproductive tract, respiratory tract and superficial and gastrointestinal infections, cystic fibrosis, ecthyma gangrenosum and black necrotic lesions.[7] It is also related to sever cases of hospital-acquired and healthcare associated infections globally.[7]

Resistant *P. aeruginosa* strains cause more severe clinical diseases which are mainly difficult to treatment with routine antibiotics.[8,9] Treatment of UTIs and BIs caused by this bacterium is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern.[8,9] However, a large proportion of uncontrolled antibiotic usage has subsidized to the development of resistance in *P. aeruginosa* strains.[8,9] *P. aeruginosa* strains exhibits the highest levels of resistance against fluoroquinolones, beta-lactams, penicilins, tetracyclines, carbapenems, aminoglycosides, macrolides and other types of antimicrobial agents. High levels of antibiotic resistance in the *P. aeruginosa* isolates of UTIs and BIs have been reported previously.[8,9] Recently, clinical isolates of the *P. aeruginosa* exhibited the high levels of resistance against imipenem antibiotic agent.[10] Documented data revealed that *blaIMP* gene is the most prevalent antibiotic resistance marker in the clinical isolates of imipenem-resistant *P. aeruginosa*.[10]

According to the high clinical standing of *P. aeruginosa* in hospital infections and also lack of microbiological and epidemiological investigations about the antibiotic resistance and also distribution of *blaIMP* gene, the current research was done to study the antibiotic resistance pattern of *P. aeruginosa* isolates of BI and UTIs and study the distribution of *blaIMP-1* gene among the imipenem-resistant strains.

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Materials and Methods

Ethical considerations

Ethical committees of the Alzahra and Kashani Hospitals were approved the general principles and framework of the current investigation. Written informed consent was obtained from all of the study patients or their parents. Personal information of all patients were remained secret.

Bacterial strains

From March 2013 to June 2014, a total of 243 *P. aeruginosa* isolates were referred to the Microbiology Research Center of the Islamic Azad University of Shahrekord, Iran. Isolates were recovered from impa-

tient and outpatients of the Kashani and Alzahra Hospitals, Isfahan, Iran. *P. aeruginosa* strains were isolated from the cases of BIs (n=170) and UTIs (n=73). All of the *P. aeruginosa* isolates were further identified according to the Gram staining, oxidase test, citrate utilization test, motility test, urease production test, gelatinase liquefaction, catalase test, triple sugar iron (TSI) agar test, oxidative-fermentative test, alkaline protease production, nitrate reduction test, indole test, haemolysin production and lecithinase production.

Antibiotic susceptibility test

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique. The Mueller-Hinton agar (Merck, Germany) medium was used for this purpose. Antibiotic resistance of *P. aeruginosa* strains against 22 commonly used antibiotics in the cases of UTIs was determined using the instruction of Clinical and Laboratory Standards Institute guidelines.[11] Susceptibility of *P. aeruginosa* strains were tested against gentamycin (10 µg/disk), imipenem (30 u/disk), cefotaxime (30 µg/disk), ciprofloxacin (5 µg/disk), cotrimoxazole (30 µg/disk), ceftazidime (30 µg/disk), chloramphenicol (30 µg/disk), streptomycin (10 µg/disk), nalidixic acid (30 µg/disk) and aztreonam (30 µg/disk) antibiotic agents (Oxoid, UK). All of the inoculated plates were aerobically incubated at 37°C for 18-24 h in an aerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2012).[11] In all reactions, the *P. aeruginosa* (ATCC 27853) was used as quality control organisms.

DNA extraction

Total genomic DNA was extracted from the bacterial colonies. A single colony was inoculated on 5 mL of brain heart infusion broth and incubated over night at 37ºC. Then 1.5 mL of a saturated culture was harvested with centrifugation for 5 min. at 14,000 rpm. The cell pellet was resuspended and lysed in 200µl of lysis buffer (40 mM Tris-acetate pH 7.8, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) by vigorous pipetting. To remove most proteins and cell debris, 66 µL of 5M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10 min. at 4ºC. After transferring the clear supernatant into a new Eppendorf tube, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugation at 14,000 rpm for 5 min, the supernatant is then removed to another eppendorf tube and double volume of 100% ethanol was added. The tubes were inverted 5 to 6 times gently, then centrifuged at 10,000rpm for 5minutes. The supernatant was discarded and 1 mL of ethanol (70%) was added to the pellet, and tubes centrifuged at 10,000 rpm for 5 minutes. Finally, the supernatant discarded and the pellet was dried for 10 min at room temperature, the pellet was resuspended by 100 µL H2O. The stock was kept at −20ºC until use.

PCR amplification of *blaIMP-1* gene

Imipenem-resistant *P. aeruginosa* strains were subjected to PCR amplification of the *blaIMP-1* gene. The PCR mixture contained 200 µM of each dNTP (Fermentas, Germany), PCR buffer (10 mM Tris/HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), DMSO at a final concentration of 4 %, 12.5 pmol of each primer (*blaIMP-1*; F: 5′-ACCGCAGCAGAGTCTTTGCC-3′ and *blaIMP-1*; R: 5′-ACAACCAGTTTTGC-CTTACC-3′ (587 bp),[12] 1 U Taq DNA polymerase (Fermentas, Germany) and 25 ng DNA template. The DNA was amplified in a programmable thermal cycler (Eppendorf, Mastercycler® 5330, Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) PCR device using the following protocol: 95ºC for 5 min, 30 cycles of 94ºC for 30 s, 55ºC for 30 s, 72ºC for 30 s, and 72ºC for 5 min. *P. aeruginosa ATCC* 27853 were used as positive controls and PCR grade water Fermentas, Germany) was used as a negative control in all PCR reactions. Ten microliters of PCR products were resolved on a 1% agarose gel containing 0.5 mg/ml of ethidium bromide in Tris-borate-EDTA buffer at 90 V for 30 min, also using suitable molecular weight markers (100 bp, Fermentas, Germany). The products were examined under ultraviolet illumination.

Statistical analysis

The results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using SPSS/21.0 software (SPSS Inc., Chicago, IL) for significant relationship between pattern of antibiotic resistance and also distribution of the *blaIMP-1* gene of *P. aeruginosa* isolates. The chi-square test and Fisher's exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at a $P<0.05$.

Results

Among all 243 isolates of *P. aeruginosa*, 146 strains (60.08%) were taken from outpatients, while 97 strains (39.91%) were taken from inpatients. Table 1 represents the results of the disk diffusion method for the *P. aeruginosa* strains isolated from human clinical samples. *P. aeruginosa* strains of our investigation harbored the highest levels of resistance against streptomycin (100%), nalidixic acid (100%), aztreonam (100%), cotrimoxazole (95.47%), ciprofloxacin (88.47%), cefotaxime (84.36%) and gentamycin (83.95%). *P. aeruginosa* strains of samples taken from outpatients had the highest levels of resistance against

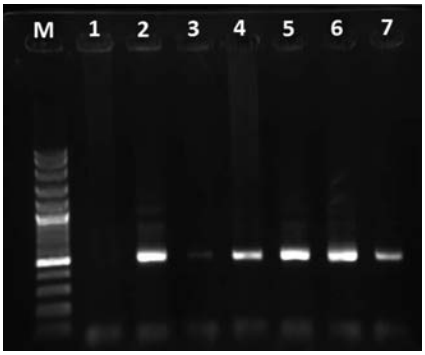

Figure 1. Results of the gel electrophoresis for amplification of the *blaIMP-1* gene among studied *P. aeruginosa* isolates. M: 100 bp ladder (Fermentas, Germany), 1: Negative control, 2: Positive control and 3-7: Positive samples for the *blaIMP-1* gene (587 bp).

Table 1. Antibiotic resistance pattern of the *P. aeruginosa* strains isolated from human clinical infections.

Clinical infections (N. *P. aeruginosa*)	Antibiotic resistance pattern (%)									
	G10	Imp	Cef	Cip	Cot	Cfz	C30	S10	Nlx	Az
Outpatients (146)	114 (78.08)	74 (50.68)	115 (78.77)	124 (84.93)	137 (93.83)	105 (71.92)	107 (73.29)	146 (100)	146 (100)	146 (100)
Inpatients (97)	90 (92.78)	52 (53.61)	90 (92.78)	91 (93.81)	95 (97.94)	83 (85.57)	85 (87.63)	97 (100)	97 (100)	97 (100)
Total (243)	204 (83.95)	126 (51.85)	205 (84.36)	215 (88.47)	232 (95.47)	188 (77.36)	192 (79.01)	243 (100)	243 (100)	243 (100)

*G10: gentamycin (10 µg/disk), Imp: imipenem (30 u/disk), Cef: cefotaxime (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Cot: cotrimoxazole (30 µg/disk), Cfz: ceftazidime (30 µg/disk), C30: chloramphenicol (30 µg/disk), S10: streptomycin (10 µg/disk), Nlx: nalidixic acid (30 µg/disk), Az: aztreonam (30 µg/disk).

streptomycin (100%), nalidixic acid (100%), aztreonam (100%), cotrimoxazole (93.83%) and ciprofloxacin (84.93%), while those of inpatients had the highest against streptomycin (100%), nalidixic acid (100%), aztreonam (100%), cotrimoxazole (97.94%), ciprofloxacin (93.81%), cefotaxime (92.78%) and gentamycin (92.78%). There were no statistically significant differences for the prevalence of antibiotic resistance between types of samples. Totally, 51.85% of *P. aeruginosa* strains had resistance against imipenem.

Table 2 represents the distribution of *blaIMP-1* gene among the imipenem resistant *P. aeruginosa* strains isolated from human clinical infections. Prevalence of the *blaIMP-1* gene among the imipenem-resistant *P. aeruginosa* strains recovered from outpatients and inpatients were 95.94% and 96.15%, respectively (Figure 1).

Discussion

The present investigation focused on the antibiotic resistance pattern of the *P. aeruginosa* strains of BIs and UTIs. As it showed, *P. aeruginosa* had significant impact in the occurrence of BIs and UTIs. High impact of *P. aeruginosa* in hospital infections has been reported from other sites of the world including Brazil,[13] Italy,[14] Germany,[15] United States[16] and South Africa.[17] We also found that *P. aeruginosa* strains harbored the high levels of resistance against commonly used antibiotic and especially streptomycin, nalidixic acid, aztreonam, cotrimoxazole, ciprofloxacin, cefotaxime and gentamycin. Of studies which were conducted in this field,[18-23] all have shown a high distribution of antibiotic resistance against ampicillin, gentamycin, cotrimoxazole, ciprofloxacin, cefotaxime and amikacin. Presence of considerable levels of resistance against imipenem have been reported previously from Iran,[19,20] Turkey,[24] India[25] and Indonesia.[26] Onguru *et al.* (2008)[27] reported that the *P. aeruginosa* strains of various clinical sources were resistant to imipenem (44.1%) which was entirely high. They showed that imipenem resistant strains were also resistant to amikacin (70%), gentamycin (85%), tobramycin (87%), cefepime (81%), piperacillin (61%) and ciprofloxacin (77%). Our results revealed that 51.85% of *P. aeruginosa* strains were resistant to imipenem. Indiscriminate, unauthorized and illegal prescription on antibiotic agents and especially imipenem are the main factors causing high prevalence of resistance of *P. aeruginosa* strains.

Shahini *et al.* (2012)[28] reported that *P. aeruginosa* strains of Iranian cases of infections had the highest levels of resistance amoxicillin (100%), cefepime (52%), trimethoprim (100%), imipenem (60%), tetracycline (100%), clavunic acid (69.2 %), ampicillin (100%), carbenicillin (90%), gentamycin (50%), ticarcillin (100%), ceftazidime (80%), and ciprofloxacin (40 %). Fazeli and Momtaz (2014)[29] reported more similar results with our findings. They showed that *P. aeruginosa* strains of clinical infections harbored the highest levels of resistance against penicillin (100%), tetracycline (90.19%), streptomycin (64.70%) and erythromycin (43.13%). Shiny *et al.* (2013)[30] reported that *P. aeruginosa* strains of pus and urine samples had the highest levels of resistance against imipenem (100%) and cefotaxime (93.75%). Akingbade *et al.* (2012)[31] reported that *P. aeruginosa* isolates of infectious samples harbored considerable levels of resistance against tetracycline (70.9%), amoxicillin (92.7%), erythromycin (72.7%), ampicillin (90%), cotrimoxazole (77.3%), cloxacillin (88.2%), ofloxacin (690%) and streptomycin (65.5%). Availability of antibiotic agents, cost of them and idea of medical practitioners for prescription of antibiotics are the main factors caused differences in the prevalence of antibiotic resistance in various studies.

The most actual antibiotics that can be used against *P. aeruginosa* infections are beta-lactam antibiotics in which imipenem as a carbapenem is considered as the most suitable antibiotic. However, carbapenem resistance occurs because of decrease in antibiotics absorption due to lack of an outer membrane porin, exclusion from the cell by efflux pump, decrease in outer membrane permeability and production of metallo-beta-lactamases (MBL). The *IMP* type is the most clinically significant carbapenemases which encoded by *blaIMP* gene and harden all treatments done using the imipenem.[31] Our results also showed that about 96.03% of imipenem-resistant *P. aeruginosa* strains harbored the *blaIMP-1* gene which was considerable. Peymani *et al*. (2015)[32] revealed that 107 (35.66%) *P. aeruginosa* isolates were non-susceptible to imipenem and/or meropenem and among them, 56 (52.3%) isolates were MBL producer. Twenty-four isolates of 56 (42.85%) MBL producer strains harbored MBL-encoding genes. Prevalence of *blaIMP-1* gene was 25% which also was lower than our findings. Moosavian *et al*. (2015)[33] reported that of 236 examined *P. aeruginosa* isolates, 122 isolates (51.40%) were resistant to imipenem. They showed that 67 strains (55%) of imipenem-resistant *P. aeruginosa* isolates harbored *blaIMP-1* gene which was lower than our findings. Abiri *et al*. (2015)[34] reported that among the 225 *P. aeruginosa* isolates, 33.7% (76/225) and 18.1% (41/225) were resistant to imipenem and meropenem, respectively. They showed that of the 76 imipenem-resistant *P. aeruginosa* strains, 45 (59.2%) were positive for MBLs and 34 (75%) strains carried the *blaIMP-1* gene which was similar to our findings. Tarashi *et al*. (2016)[35] indicated that among 278 imipenem-resistant *P. aeruginosa* strains, 178 (64.02%) were MBL producers. The *blaIMP-1* gene was detected in 16.8% of *P. aeruginosa* isolates. As far as we know, the present study reported the highest prevalence of *blaIMP-1* gene among the imipenem-resistant *P. aeruginosa* strains of human clinical samples. Differences in types of samples is the main factor for various prevalence rate of *blaIMP-1* gene reported in different studies.

Conclusions

In conclusion, we identified a large number of *P. aeruginosa* strains in the BIs and UTIs samples taken from inpatients and outpatients. Resistance against streptomycin, nalidixic acid, aztreonam, cotrimoxazole, ciprofloxacin, cefotaxime and gentamycin was considerable in the *P. aeruginosa* strains of clinical samples. Moderate prevalence of resistance against imipenem was supported by the high prevalence of *blaIMP* gene. High prevalence of this gene poses an important public health threat regarding the occurrence of resistance against imipenem in the clinical isolates of *P. aeruginosa*. It seems that prescription of imipenem is not effective for treatment of the cases of *P. aeruginosa* infection.

Table 2. Prevalence of *blaIMP-1* gene among the imipenem resistant *P. aeruginosa* strains isolated from human clinical infections.

Samples (No. imipenem-resistant isolates)	Distribution of *blaIMP*-1 gene (%)
Outpatients (74)	71 (95.94)
Inpatients (52)	50 (96.15)
Total (126)	121 (96.03)

However, additional investigations should perform to found other epidemiological aspects of the MBL producing *P. aeruginosa*, detection of *blaIMP-1* gene recommended to be important in all clinical microbiology laboratories.

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