Investigation of the prevalence of genes conferring resistance to carbapenems in *Pseudomonas aeruginosa* isolates from burn patients

**Background and aim:** Currently, the rate of hospital-acquired infections due to drug-resistant *Pseudomonas aeruginosa* strains shows an increasing trend and remains one of the principal reasons for mortality in burn patients. This study aimed to investigate the prevalence of genes conferring resistance to carbapenems in *P. aeruginosa* isolates from burn patients.

**Methods:** A total of 50 *P. aeruginosa* isolates were tested for antibiotic susceptibility and presence of multidrug-resistant (MDR) and extensively drug resistant (XDR) isolates, using phenotypic tests. Screening for genes conferring resistance to carbapenems was investigated by multiplex PCR method.

**Results:** Susceptibility testing demonstrated the highest resistance against amikacin, ceftazidime (n=44/88% each), and gentamicin (84%), while colistin sulfate was the most effective antibiotic. The rate of MDR and XDR isolates was revealed as 50% and 40% respectively. We detected the following carbapenemase genes: *blaNDM* (32%), followed by *blaOXA-48* (18%), and *blaBIC-1* (14%). This study revealed a high antibiotic resistance in *P. aeruginosa* isolates with a total of 40% and 50% MDR and XDR isolates respectively, and 70% carbapenem resistance. The prevalence of carbapenem conferring genes tested among carbapenem-resistant isolates was demonstrated as 65.7%.

**Conclusion:** Due to the prevalence of *P. aeruginosa* strains carrying *blaOXA-48* and *blaNDM* genes in our hospital, more attention and implementation of effective control measures against nosocomial infection are recommended.

**Keywords:** *Pseudomonas aeruginosa*, carbapenems, encoding genes, antibiotics, drug susceptibility test

**Introduction**

Burn injuries is a common global public health problem, accounting for an estimated 180,000 deaths annually. *Pseudomonas aeruginosa* is an important pathogen causing a wide range of acute and chronic infections in burn patients. This microorganism is found in approximately 33% of all burn wounds and in 59% of extensive burns. Treatment and control of severe infections caused by *P. aeruginosa* are frequently complicated due to the limited susceptibility to antimicrobial agents and the emergence of antibiotic resistance during therapy. In 2011, the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention redefined conventionally-acquired antibiotic resistance profiles including multidrug-resistance, extensive drug resistance, and pan-drug resistance (PDR) for some bacterial species,
including *P. aeruginosa*. Literally, for *P. aeruginosa* specifically, multidrug-resistance means the isolate which is non-susceptible to at least one agent in ≥3 antimicrobial categories including aminoglycosides, carbapenems, and fluoroquinolones; extensive drug resistance means the isolate which is non-susceptible to at least one agent in all but two or fewer antimicrobial categories; PDR means resistant to all antibiotic classes available for empirical treatment. Carbapenems are the drugs used to treat multidrug-resistant (MDR) isolates, however, the increasing frequency of carbapenem-resistant *P. aeruginosa* has recently been mentioned in several studies. This resistance is mediated by carbapenem-hydrolyzing enzymes, including Ambler class A (eg, KPC), B (eg, IMP, VIM), and D (eg, OXA) beta-lactamas. Epidemiological studies showed that 88.3% of MDR *P. aeruginosa* isolates are resistant to carbapenems, aminoglycosides, and fluoroquinolones. This study was conducted to investigate the prevalence of genes conferring resistance to carbapenems in *P. aeruginosa* isolates from burn patients, using multiplex PCR.

### Materials and methods

The present study was conducted in Taleghani burn referral Hospital in Ahvaz, Iran, from March to August 2015. A total of 50 isolates of *P. aeruginosa* were collected from individual burn wound samples of admitted patients. The study was approved by the Institutional Review Board (IRB) and Ethics committee of the Islamic Azad University of Yasooj, after submission of the preliminary proposal, and necessary permission for sample collection was granted. Apart from this, as part of the teaching hospital’s policy, referred patients were requested to sign an informed consent in case their samples were to be used for research purposes apart from routine clinical investigation. We confirm that our study was conducted in accordance with the Declaration of Helsinki.

The isolates were identified as *P. aeruginosa* by standard culture and biochemical tests. Antimicrobial susceptibility testing (AST) was performed using the agar disk diffusion method (Kirby-Bauer) on Mueller-Hinton agar (EMD Millipore, Billerica, MA, USA) plates according to the Clinical and Laboratory Standards Institute (CLSI) guideline. The following antimicrobial discs were used: imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), piperacillin (100 μg), piperacillin-tazobactam (100/10 μg), colistin-sulfate (10 μg), and aztreonam (30 μg), (MAST Diagnostics, Merseyside, UK). *P. aeruginosa* ATCC 27853 was used for quality control.

DNA was extracted from colonies of *P. aeruginosa* isolates by the simple boiling method as described elsewhere, and the concentration of extracted DNA was determined at 260 nm, using Nanodrop instrument (ThermoFisher Scientific, Waltham, MA, USA). Multiplex PCR was performed using previously described oligonucleotide primers to detect *blaOXA-48*, *blaNDM*, *blaKPC*, and *blaBIC* beta-lactamase genes, as shown in Table 1. PCR mixture was prepared in a final volume of 25 μL comprising 10× PCR buffer, 1.5 mmol of MgCl2, 0.2 mmol of each dNTPs, 5 U/μL of Taq DNA polymerase, 1 μmol of each primer, 8 μL of distilled water, and 8 ng of template DNA. The amplification was carried out in a thermocycler (Eppendorf, Germany) with the following cycling conditions: initiation denaturation at 95°C for 10 minutes, and 36 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 40 seconds, extension at 72°C for 50 seconds, and a final extension at 72°C for 5 minutes. DNA fragments were analyzed by electrophoresis on a 2% agarose gel containing 0.5 μg/mL ethidium bromide. The bands were visualized under UV light using a gel documentation system (Protein Simple, USA). The PCR products were sent to Bioneer Corporation, Daejeon, South Korea, for sequencing.

### Table 1 Sequence of primers used in the present study

| Primer | Sequence (5’→3’) | Gene | Product size (base pair) |
|--------|-----------------|------|-------------------------|
| KPC F  | CGTCGATGTTGGTGCTGGTTG | *blaKPC* | 798 |
| R      | CTTGATGTTGGTGCTGGTTG |  | |
| OXA-48 F| GCGTGTTAGGATGAACAC | *blaOXA-48* | 438 |
| R      | CATCAAGTTACAACCCAACGC |  | |
| NDM-1 F| GTTTGGCGATCGTTGTTTTC | *blaNDM* | 621 |
| R      | CGGAATGGCTCATACGATC |  | |
| BIC-1 F| TAGATGCTGCTTTAAGGGCC | *blaBIC* | 537 |
| R      | TCATTGGCCGGTTCGACAC |  | |

*Notes:* F, forward primer; R, reverse primer.
Results and discussion
In the present study, 50 confirmed *P. aeruginosa* isolates were selected for further investigation. The isolates were recovered from burn wound infections of 22 (44%) male and 28 (56%) female patients with a mean age of 38.3 years. According to AST, high antibiotic resistance against most of the tested antibiotics was demonstrated in this study (40% and 50% MDR and extensively drug resistant [XDR] isolates respectively), except for four isolates (8%) that were susceptible to all applied antibiotics. The antibiotic susceptibility profile of *P. aeruginosa* isolates is presented in Figure 1. The highest resistance was seen against amikacin and ceftazidime (n=44/88% each) and gentamicin (84%), and the most effective antibiotic against the isolates was colistin sulfate (100%). Twenty-five isolates (50%) were resistant to at least three different classes of antibiotics according to previously described criteria, and were considered as MDR isolates. Moreover, according to the same criteria, 20 isolates (40%) were resistant to at least six different classes of antibiotics and were considered as XDR isolates. In Figure 2 the rate of antimicrobial susceptibility of MDR and XDR *P. aeruginosa* isolates to eleven antimicrobial agents is presented. The significantly high rate of 70% resistance to carbapenems (imipenem and meropenem) among the isolates may be related to frequent use of carbapenems as drug of choice for the treatment of MDR *P. aeruginosa* in our burn hospital for all patients, which facilitates increasing carbapenem-resistant isolates, as stated by other investigators. In fact, the resistance to carbapenems in the region of study started long ago, as shown in a report by Khosravi et al. During the past 10 years the rate of resistance to both imipenem and meropenem has increased from 41% in 2008 to 70% currently. This increase in carbapenem resistance in the same burn center, which is also true for other categories of antibiotics, could be due to several reasons, including the use of different antibiotic regimes, antibiotic overuse, presence of different persistent strains in the hospital, the quality of hygiene, and duration of hospital stays for patients with antibiotic-resistant infections. The high resistance (up to 90%) to carbapenems among burn patients was also reported in a recent study from Iran. Moreover, according to recent reports, MDR *P. aeruginosa* strains and resistance to carbapenems are a matter of concern worldwide as well. Even though carbapenems are still being used in Iran and some other countries as the last antibiotic of choice for the treatment of MDR *P. aeruginosa*, the acquisition of new resistance determinants such as extensive drug resistance remains a therapeutic challenge as effective antimicrobial therapy is severely limited. In the present study, 40% of *P. aeruginosa* isolates exhibited an XDR phenotype. This high resistance rate is very worrying. Unfortunately, according to statistics published in previous studies, we are witnessing an increasing rate in Iran, and other parts of the world.

Figure 1 Antibiotic resistance pattern of the 50 clinical *P. aeruginosa* isolates.

Abbreviations: AK, amikacin; GM, gentamicin; IMP, imipenem; MEM, meropenem; CAZ, ceftazidime; CIP, ciprofloxacin; CPM, cefepime; PTZ, piperacillin-tazobactam; CL, colistin; PIP, piperacillin; ATM, aztreonam.
Although the most common genes conferring resistance to carbapenems are \textit{bla IMP} and \textit{bla VIM}, these genes were studied in our region previously,\textsuperscript{13,18} so this work was designed to investigate other non-studied genes conferring resistance to carbapenems in the region. PCR analysis showed the presence of genes conferring resistance to carbapenems in 26 (52\%) isolates, of which, in 22 isolates (84.6\%) only one gene was detected. \textit{blaOXA-48} gene was detected in nine (18\%) isolates, seven (14\%) isolates carried \textit{blaBIC-1} gene, and 14 (28\%) isolates carried \textit{blaNDM-1} gene which was the most prevalent gene in the present study. \textit{blaKPC-2} gene was not detected in any of the isolates. Of the 25 MDR isolates, five, three, and five were positive for \textit{blaNDM-1}, \textit{blaBIC-1}, and \textit{blaOXA-48} genes respectively, while the distribution of carbapenemases genes in XDR isolates was nine, three, and four, for \textit{blaNDM-1}, \textit{blaBIC-1}, and \textit{blaOXA-48} genes respectively. To the best of our knowledge, this is the first report of detection of \textit{blaNDM-1} gene from \textit{P. aeruginosa} in the region of study. Even in a similar recent study from Iran, no incidence of \textit{blaNDM-1} positive \textit{P. aeruginosa} was reported.\textsuperscript{19} NDM-1, an Ambler class B metallo-\textbeta-lactamase, renders the bacteria resistant to almost all \textbeta-lactam antibiotics, aminoglycosides, and fluoroquinolones.\textsuperscript{20} While \textit{KPC}-producing organisms have been described quite often in Iran, we did not find any \textit{blaKPC-2} positive isolates in this region.\textsuperscript{15,21}

In our study, nine isolates (18\%) harbored \textit{blaOXA-48}, seven of them were resistant to all antibiotics except for colistin, and two isolates were susceptible to carbapenems as well. Despite the fact that \textit{blaOXA-48} gene is mainly reported in \textit{Enterobacteriaceae} members, there have been recent reports of isolation of this gene in \textit{P. aeruginosa} strains as well.\textsuperscript{22,23} Among carbapenem-resistant isolates (n=35), 23 isolates harbored any of the tested resistance genes (65.7\%). The emergence of carbapenemases, that have been acquired via mobile elements such as transposons or plasmids, can lead to a high degree of antibiotic resistance in clinical pathogens which can be transmitted among patients in a hospital setting.\textsuperscript{24} In the current study, 12 out of 35 carbapenem-resistant \textit{P. aeruginosa} isolates lacked carbapenemase genes (Table 2). It is critical to note that carbapenemases are not the only mechanisms of acquired resistance to carbapenems,\textsuperscript{25} and various resistance mechanisms have been involved in antibiotic resistance in nosocomial pathogens. This study, due to its qualitative description design, had a few limitations. Because of limited time, the current study focused on the main problem faced by the burn center, ie, antibiotic ineffectiveness. Detailed research work is needed in future on the mechanisms of antibiotics, and mainly carbapenem resistance, for a better understanding of the nature of antibiotic resistance in our setting.

**Conclusion**

The current study revealed the highest antibiotic resistance in \textit{P. aeruginosa} isolates we have ever seen in our region, which is very alarming for our public health sector. The rate of resistance had increased to 84\%–88\% for some of the antibiotics used, with 50\% multidrug resistance and 40\% extensive drug resistance.
Table 2 The distribution of encoding genes among Pseudomonas aeruginosa isolates

| Number | Susceptibility patterns | MDR | XDR | bla genes |
|--------|-------------------------|-----|-----|-----------|
|        | A  | B  | C  | C  | D  | E  | F  | G  | H  |           |
|        | GM | AK | IMP | MEM | CAZ | CPM | CIP | PTZ | PIP | ATM | CL |
| 1      | R  | R  | R  | R  | R  | R  | R  | S  | R  | S  | S   | –  | +  | BIC |
| 2      | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | –  | +  | NDM |
| 3      | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | –  | +  | –  |
| 4      | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | +  | –  | NDM |
| 5      | R  | R  | R  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | –  |
| 6      | R  | R  | R  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | –  |
| 7      | R  | R  | R  | R  | R  | R  | S  | R  | S  | S  | S   | –  | +  | NDM |
| 8      | R  | R  | R  | R  | R  | R  | S  | R  | S  | S  | +  | –  | –  | NDM |
| 9      | S  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | –  | +  | –  |
| 10     | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | +  | –  | –  |
| 11     | S  | S  | S  | S  | S  | S  | S  | S  | S  | S  | S   | –  | –  | –  |
| 12     | R  | R  | R  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | –  |
| 13     | R  | R  | R  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | NDM |
| 14     | R  | R  | R  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | –  |
| 15     | R  | R  | S  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | –  |
| 16     | R  | R  | R  | R  | R  | R  | S  | S  | S  | S  | +  | –  | –  |
| 17     | S  | S  | R  | R  | S  | S  | S  | S  | S  | S  | S   | –  | –  | BIC |
| 18     | S  | S  | S  | S  | S  | S  | S  | S  | S  | S  | S   | –  | –  | –  |
| 19     | R  | R  | R  | R  | R  | R  | S  | R  | S  | S  | +  | –  | NDM |
| 20     | R  | R  | R  | R  | R  | R  | S  | R  | S  | S  | +  | –  | NDM/BIC |
| 21     | R  | R  | R  | R  | S  | R  | S  | R  | S  | R  | S   | +  | –  | –  |
| 22     | R  | R  | S  | R  | R  | S  | R  | S  | R  | S  | S   | +  | –  | –  |
| 23     | S  | R  | S  | S  | R  | S  | S  | R  | R  | S  | S   | –  | –  | –  |
| 24     | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | +  | –  | –  | OXA |
| 25     | R  | R  | R  | R  | R  | R  | S  | S  | S  | S  | S   | +  | –  | –  |
| 26     | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | +  | –  | OXA |
| 27     | S  | S  | R  | R  | R  | S  | R  | R  | S  | R  | S   | +  | –  | OXA |
| 28     | R  | R  | R  | R  | S  | R  | S  | R  | S  | R  | S   | +  | –  | OXA |
| 29     | R  | R  | R  | R  | R  | R  | S  | R  | R  | R  | S   | +  | –  | OXA |
| 30     | R  | R  | S  | S  | S  | R  | R  | S  | S  | R  | S   | +  | –  | OXA/BIC |
| 31     | R  | R  | R  | R  | R  | R  | S  | R  | R  | R  | S   | +  | –  | NDM |
| 32     | S  | S  | S  | S  | S  | S  | S  | S  | S  | S  | S   | –  | –  | –  |
| 33     | R  | R  | S  | S  | S  | R  | S  | R  | S  | R  | S   | +  | –  | –  |
| 34     | R  | R  | R  | R  | R  | R  | S  | R  | S  | R  | S   | +  | –  | NDM |
| 35     | R  | R  | S  | S  | R  | S  | S  | S  | S  | S  | S   | +  | –  | –  |
| 36     | R  | R  | R  | R  | R  | R  | S  | R  | S  | S  | –  | –  | NDM |
| 37     | R  | R  | R  | R  | R  | R  | S  | R  | S  | S  | –  | –  | BIC |
| 38     | R  | R  | S  | S  | R  | R  | S  | R  | S  | R  | S   | +  | –  | –  | BIC |
| 39     | R  | R  | R  | R  | R  | R  | S  | R  | R  | S  | S   | +  | –  | NDM/BIC |
| 40     | R  | R  | R  | R  | R  | R  | R  | S  | S  | S  | –   | +  | –  | –  |
| 41     | R  | R  | R  | R  | R  | R  | S  | S  | R  | S  | S   | +  | –  | OXA |
| 42     | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | +  | –  | NDM/OXA |
| 43     | R  | R  | S  | S  | R  | R  | S  | R  | S  | R  | S   | +  | –  | –  |
| 44     | R  | R  | R  | R  | R  | R  | R  | R  | S  | S  | S   | –  | +  | –  |
| 45     | R  | R  | R  | R  | R  | R  | S  | S  | S  | R  | S   | +  | –  | NDM |
| 46     | R  | R  | S  | S  | R  | R  | R  | R  | S  | S  | S   | +  | –  | OXA |

(Continued)
resistance, compared to other previous studies from Iran. To clarify other resistance mechanisms apart from carbapenemases, further investigations are needed. Moreover, due to the prevalence of *P. aeruginosa* strains carrying *blaOXA-48*, *blaKPC*, and *blaNDM* genes in our hospital, greater attention and implementation of effective control measures against nosocomial infection are recommended.

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**Disclosure**

The authors report no conflicts of interest in this work.

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