# Molecular epidemiology of colistin-resistant *Pseudomonas aeruginosa* producing NDM-1 from hospitalized patients in Iran

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| ARTICLE INFO | ABSTRACT |
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| | ***Objective(s)***: Resistance to carbapenems is the principal reason for the continuing utilization of colistin as a last resort choice for treating the infections resulted from multidrug carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) isolates. The assessment of antimicrobial resistance pattern, the prevalence of carbapenem-resistance determinants, and molecular epidemiology of colistin-resistant isolates among CRPA strains were the aims of the present research.<br>***Materials and Methods:*** The current cross-sectional research was conducted on 269 CRPA isolates collected from various clinical samples from 2013 to 2016. After performing identification tests, disk diffusion as well as MIC methods were used for testing sensitivity to the antibiotics. Modified Hodge Test (MHT) was utilized to produce carbapenemase. PCR technique identified beta-lactamase classes A, B, and D genes.<br>***Results:*** In total, from 269 CRPA, five isolates (1.3%) were resistant to colistin. It was found that *blaNDM-1, blaIMP-1, blaVIM-2*, and *blaOXA-10* genes were present in 40%, 40%, 20%, and 100% of colistin-resistant isolates, respectively. DLST type 25-11 is a significant cluster of colistin-resistant *P. aeruginosa* isolates.<br>***Conclusion:*** The appearance of colistin-resistant isolates in CRPA carrying *bla*NDM-1 with multiple carbapenem-resistant genes shows the great problem in the treatment of *P. aeruginosa* infections. |

## Introduction

*Pseudomonas aeruginosa* is a highly opportunistic pathogen. Carbapenems are the antibiotics which are utilized for treating multidrug-resistant *P.aeruginosa* (MDRP) isolates. Carbapenem antibiotics used to be effective agents against MDRP when first presented. However, the growing prevalence of carbapenem-resistant *P. aeruginosa* (CRPA) has turned into a severe health problem recently (1-3). These strains lead to high mortality rates in patients infected by *P. aeruginosa* and there are also few effective drugs against them. Colistin is a key antimicrobial agent used to treat *P. aeruginosa* infections (1, 2). Resistance to carbapenems can be related to the production of carbapenemase enzymes such as serine carbapenemases (containing KPC and GES enzymes), metallo-β-lactamases (MBLs) such as IMP, VIM, and NDM enzymes, and oxacillinases (such as OXA enzymes) (4). $bla_{IMP}$ and $bla_{VIM}$ are the most frequently acquired MBLs. However, the recently emerged NDM-type (New Delhi metallo-β-lactamases) is becoming the most important carbapenemase (4, 5). Most $bla_{NDM-1}$ strains are resistant to a wide range of antibiotic groups, for example to aminoglycosides, sulfonamides, fluoroquinolones, and macrolides (4, 6). The clinically used polymyxin is effective against NDM-positive organisms (7). Polymyxins are antibiotics with a general structure containing a cyclic peptide and comprise five chemically different compounds (A–E). Polymyxins B and E (colistin) are used for curing Gram-negative bacterial pathogens (8). The great amount of activity that colistin has against many species of bacterial infections has been described in the recent literature. However, the use of colistin has been limited because it has serious neurotoxicity and nephrotoxicity (9). Colistin acts by connecting itself to lipopolysaccharide in the outer membrane of Gram-negative bacteria (10, 11). Although colistin resistance mechanisms have not been exactly understood, two principal functions of resistance to colistin in bacteria are adaptation and mutation (11). Various molecular typing methods have been utilized for analyzing the epidemiology of *P. aeruginosa*. Among these methods, pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are time-consuming and expensive and require specific technical abilities (12, 13). The recently described double-locus sequence typing (DLST) pattern permits us to gain a

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standardized and clear definition of types according to the partial sequencing of two extremely inconstant loci for typing *P. aeruginosa* isolates (12, 13). DLST has a remarkable discriminatory power and reproducibility and can detect high-risk endemic clones (13). Although reports of colistin-resistant cases are rare, its incidence is considered a serious menace. Hence, the present research has been designed to study the antimicrobial resistance pattern, the frequency of carbapenem-resistant determinants, and molecular epidemiology of colistin-resistant isolates among the CRPA collected from hospitalized patients in Iran by employing genotypic, typing, and phenotypic techniques.

## Materials and Methods

### Bacterial isolation and identification

This cross-sectional work was conducted in three main hospitals in Ahvaz, Tehran, and Isfahan, Iran within a three-years period from October 2013 to July 2016.

This research was approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Iran and conformed to the declaration of Helsinki. Overall 236 non-duplicate carbapenem-resistant *P. aeruginosa* isolates were collected from different medical specimens. By using standard microbiological techniques and also the genotypic method (the presence of the *gyrB* gene) (15), the bacteria were detected. The confirmed isolates were kept at the temperature of -80 °C in Trypticase Soy Broth comprising 15% glycerol.

### Testing antimicrobial sensitivity

The antibiotic sensitivity of all the isolates was tested by employing the Kirby-Bauer's technique as suggested by the CLSI (16). The twelve standard antibiotic disks used include: IPM; imipenem (10 μg), MEM; meropenem (10 μg), ETP; ertapenem (10 μg), CTX; cefotaxime (30 μg), CT; ceftazidime (30 μg), FEP; cefepime (30 μg), GEN; gentamicin (10 μg), AN; amikacin (30 μg), TZP; piperacillin/tazobactam (100/10 μg), CIP; ciprofloxacin (5 μg), CST; colistin (10 μg), and ATM; aztreonam (30 μg) (Mast Group Ltd, UK). Isolates with resistance against a minimum of three groups of antibacterial agents were considered as MDR (17). The MHT was carried out to detect the carbapenemase-generating strains as recommended by CLSI (16). To detect ESBL-producing isolates, the combined disk technique by disks of ceftazidime (30 mg) with (10 mg) and without clavulanic acid (Mast Group Ltd, UK) was used. A growth in the area diameter of ≥5 mm around ceftazidime disc with and without clavulanic acid was assumed to be a positive result for ESBL production (18).

### E-test

The E-test (imipenem 0.002-32μg/ml and colistin 0.064-1024 μg/ml) (Liofilchem, Roseto degli Abruzzi, Italy) was conducted based on the guidelines of the manufacturer. The tests were considered positive for imipenem and colistin when the ratio was ≥ 8 μg/ml (16). The E-test method was used to specify the minimum inhibitory concentrations (MICs) of imipenem and colistin (for colistin, MIC was detected only in resistant isolates by using Kirby-Bauer's technique).

### PCR amplification of carbapenem resistant genes

A DNA extraction set (Sinaclon, Iran) was employed for DNA extraction from the colistin-resistant isolates

**Table 1.** Primers used in this study

| Primers | Sequence (5′–3′) | Product size (bp) | Reference |
|---|---|---|---|
| ms172 | GGATTCTCTCGCACGAGGT TACGTGACCTGACGTTGGTG | 400 | 12 |
| ms217 | TTCTGGCTGTCGCGACTGAT GAACAGCGTCTTTTCCTCGC | 350 | 12 |
| *bla* NDM-1 | GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC | 621 | 19 |
| *bla* IMP | GGAATAGAGTGGCTTAATTC GGTTTAACAAAACAACCACC | 233 | 31 |
| *bla* VIM | GTTTGGTCGCATATCGCAAC AATGCGCAGCACCAGGATAG | 382 | 32 |
| *bla* SPM | AAAATCTGGGTACGCAAACG ACATTATCCGCTGGAACAGG | 271 | 19 |
| *bla* KPC | CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG | 798 | 31 |
| *bla* OXA-10 | ATTATCGGCCTAGAAACTGG CTTACTTCGCCAACTTCTCTG | 170 | 33 |

based on the guidelines of the manufacturer. PCR amplification for the detection of $bla_{NDM}$, $bla_{IMP}$, $bla_{VIM}$, $bla_{KPC}$, $bla_{GES}$, $bla_{SPM}$, and $bla_{OXA-10}$ was done using specific primers as described previously (Table 1) (19). In this study, a pentaplex PCR assay was used in a thermal cycler (Eppendorf AG, Germany), with an initial denaturation of 4 min at the temperature of 94 °C followed by 30 cycles of a denaturation of 60 sec at the temperature of 94 °C, annealing 56 °C for $bla_{OXA-10}$, 59 °C for $bla_{SPM}$, and 55 °C pentaplex PCR and extension of 60 sec at 72 °C, with a single final extension of 7 min at 72 °C (19). Bioneer Company (Bioneer, Daejeon, South Korea) accomplished the sequencing of the amplicons. BLAST in NCBI was employed to analyze the nucleotide sequences.

### Double-locus sequence typing method

On the DLST website, the full procedure for DLST technique of *P. aeruginosa* is accessible. In brief, nucleotide extracts were utilized for PCR production of both ms172 and ms217 loci by employing particular primers (Table 1). The typical gel electrophoresis was performed, and gels marked with ethidium bromide (Sinaclon, Iran) were surveyed below UV light for the existence of one detectable perfect band for each PCR. The sizes of DNA sequences varied among strains. Bioneer Corporation (Bioneer, Daejeon, South Korea) purified and sequenced the PCR products. For allele assignment, the sequences were submitted to www. dlst.org. Two numbers were granted to each strain and represented its DLST type. In case no result for allele assignment was obtained, it was considered null (13, 20).

### Statistical analysis

SPSS™ software, version 19 (IBM Corp, USA) was employed for the statistical analysis.

## Results

In total, 236 isolates of carbapenem-resistant *P. aeruginosa* were collected from 369 patents. The outcomes of the antibiotic sensitivity pattern demonstrated that 90.7% and 90.7% of the isolates had resistance against imipenem and meropenem, respectively. Table 2 presents the complete outcomes of the antibiotic resistance pattern for all CRPA isolates. Most CRPA isolates were collected from urine samples [28 isolates (21%)], followed by trachea samples (19 isolates (16.3%)), and wound samples [18 isolates

**Table 2.** Antimicrobial sensitivity of carbapenem-resistant *Pseudomonas aeruginosa (CRPA)* isolates

| Antimicrobial agent | The number of CRPA isolates | Number of sensitive isolates (%) | Number of intermediate isolates (%) | Number of resistant isolates (%) |
|---|---|---|---|---|
| imipenem | 236 | 12 (5.1) | 10 (4.2) | 214 (90.7) |
| meropenem | 236 | 11 (4.7) | 11 (4.7) | 214 (90.7) |
| ertapenem | 236 | 18 (7.6) | 10 (4.2) | 208 (88.2) |
| piperacillin-tazobactam | 236 | 47 (19.9) | 56 (23.7) | 133 (56.4) |
| cefepime | 236 | 27 (11.5) | 18 (7.6) | 191 (80.9) |
| amikacin | 236 | 67 (28.4) | 15 (6.4) | 154 (65.2) |
| colistin | 236 | 231 (97.9) | 0 | 5 (2.1%) |
| ciprofloxacin | 236 | 25 (10.6) | 11 (4.7) | 200 (84.7) |
| gentamicin | 236 | 35 (14.8) | 0 | 201 (85.2) |
| ceftazidime | 236 | 44 (18.6) | 11 (4.7) | 181 (76.7) |
| cefotaxime | 236 | 3 (1.3) | 22 (9.3) | 211 (89.4) |
| aztreonam | 236 | 47 (19.9) | 86 (36.4) | 103 (43.7) |

(15.5%)]. The number of ESBLs in CRPA carrying MBL was 13 (11.2%) of which one was a colistin-resistant isolate. Five CRPA isolates resistance against colistin were collected from urine, blood, and trachea specimens from ICU and gynecology wards. Nevertheless, two of these isolates were carrying $bla_{NDM-1}$ gene and three of them had other MBL genes. All colistin-resistant isolates were carrying $bla_{OXA-10}$ gene. However, $bla_{KPC}$, $bla_{GES}$, and $bla_{SPM}$ genes could not be identified in the colistin-resistant isolates. Interestingly, one of the colistin-resistant isolates had resistance against all kinds of antibiotics. Colistin-resistant isolates demonstrated an immense rate of resistance (100%) to ertapenem, ceftazidime, and ciprofloxacin antibiotics. 80% of colistin-resistant isolates were also MDR. By employing the E-test, it was found that Eieyhty percent of colistin-resistant isolates were resistant to imipenem (MIC ≥ 8 mg/ml). The sources of colistin-resistant isolates, MBLs genes, antibiogram patterns, specimens, and wards have been given in Table 3. In the current research, the DLST method was used in five colistin-resistant *P. aeruginosa* isolates obtained from various hospital wards during a
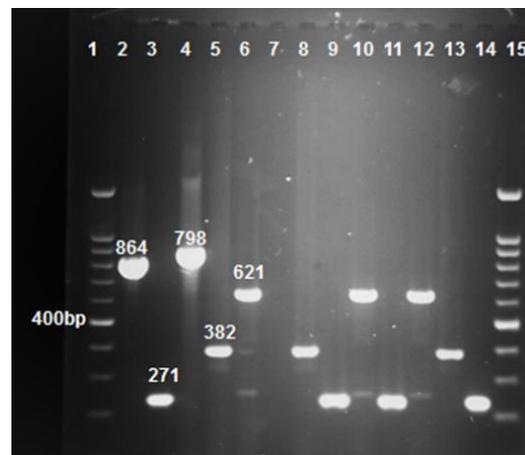


**Figure 1.** Gel electrophoresis of multiplex PCR products following amplification with specific primers. Line 1 and 15 ladder, line 2, 3, 4, 5, and 6 positive control *bla* KPC, *bla* IMP, *bla* GES, *bla* VIM and *bla* NDM (864, 271, 798, 382, and 621 bp, respectively), line 7 deionized water as control negative, line 8-14 samples. All positive controls were provided by the Pasteur Institute, Iran

**Table 3.** The presence of non-sensitive isolates to colistin carrying NDM-1 and other carbapenemase genes in carbapenem-resistant *Pseudomonas aeruginosa (*CRPA) isolates

| City/ Hospital | Gender | Sample/ ward | carbapenemase genes | | | | | | | [a]DLST type | [b]MHT | [c]MIC (mg/ml) CST | [d]MIC (mg/ml) IPM | [e]ESBL | [f]MDR | Pattern antibiogram | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | NDM-1 | VIM | IMP | KPC | GES | SPM | OXA-10 | | | | | | | IMP | MEM | ETP | TZP | CEP | AN | CST | CIP | GEN | CAZ | CTX | AZT |
| Isfahan/ General | M | Urine/ ICU | + | - | + | - | - | - | + | 25-11 | + | 8 | ≥32 | + | + | R | R | R | I | R | R | R | R | R | R | R | R |
| Ahvaz/ General | M | Urine/ Urology | - | - | + | - | - | - | + | 25-11 | + | 32 | ≥32 | - | + | R | R | R | R | I | R | R | R | R | R | R | R |
| Ahvaz/ General | M | Blood/ ICU | + | + | - | - | - | - | + | 25-11 | + | 16 | ≥32 | - | + | R | R | R | R | I | R | R | R | R | R | R | R |
| Tehran/ General | F | Trachea/ Women | - | - | - | - | - | - | + | 5-91 | + | 8 | ≥32 | - | + | R | R | R | R | R | R | R | R | R | R | R | R |
| Ahvaz/ General | F | Trachea/ Women | - | - | - | - | - | - | + | null | + | 32 | 4 | - | - | I | I | R | S | R | S | R | R | S | R | S | S |

Resistance rate of non-sensitive *Pseudomonas aeruginosa* isoleates to colistin against tested antibiotics imipenem (IPM), meropenem (MEM), ertapenem (ETP), piperacillin-tazobactam (TZP), cefepime (CFP), amikacin (AN), colistin (CST), ciprofloxacin (CIP), gentamicin (GEN), ceftazidime (CAZ), cefixime (CTX), aztreonam (AZT).
[a] Double-locus sequence typing, [b] modified hodge test, , [c] minimum inhibitory concentration of colistin, [d] minimum inhibitory concentration of imipenem, [e] extended-spectrum β-lactamases, [f] multi-drug resistant

period of 3 years. The majority cluster included three patients (60%) infected or colonized by the similar genotype DLST 25-11.

## Discussion

In the present research, the presence of $bla_{NDM-1}$ in colistin-resistant isolates in university teaching hospitals of Iran has been reported. To date, there has only been one report about the prevalence of $bla_{NDM-1}$ in patients (21) and as far as we know, the present work represents the first report about the occurrence of colistin-resistant *P. aeruginosa* isolates co-existing with $bla_{NDM-1}$ or other carbapenemase genes in Iran. Although reports are rare, its incidence is important because *P. aeruginosa* is an organism with a potent colonization ability in the hospital (22). As far as we know, this is the first study of colistin-resistant isolates with DLST type 25-11 in CRPA clinical isolates co-harboring $bla_{NDM-1}$ identified in Iran and the second report on the detection of colistin-resistant isolates with $bla_{NDM-1}$, the first one being reported by Mataseje *et al.* from North America (23). In other studies, conducted earlier in the northwest of Iran, Goli (11) and Saderi and Owlia (24) reported 4.8% and 9.1% resistance of isolates against colistin, respectively. These rates are slightly higher than the results obtained in our study.

However, reports from other countries explained that resistance to colistin varies from 0% (25) to 31.7% (26). This discrepancy can be due to the misuse of drugs, dissimilar policies of hospitals for controlling the infection, sanitation, and topographical distribution.

Another notable aspect in our results was the emergence and dissemination of colistin-resistant strains in three cities of Iran (Ahvaz, Tehran, and Isfahan) which showed the importance of prescribing antibiotics and optimizing effective infection control policies in our healthcare settings. Our research has also shown that besides colistin which acts as the antibiotic of choice for treating infections caused by CRPA isolates, amikacin is a very effective antibiotic as well. In line with our results, Liu *et al.* noted a very large amount of sensitivity to amikacin (91%) among clinical *P. aeruginosa* isolates (27). However, in contrast to our findings, Goli *et al.* revealed that piperacillin/tazobactam had the highest amount of activity against MDR strains of *P. aeruginosa* (11). Data from the previous studies showed that one of the colistin-resistant strains had resistance against all kinds of antibiotics, collected from trachea and carrying $bla_{OXA-10}$. Furthermore, molecular typing of the isolates using DLST revealed a distinct pattern with 5-91 DLST type. A study from an Iranian burn hospital showed that the mortality rates of patients diseased by MBL-producing *P. aeruginosa* were higher than those infected by non-MBL-producing strains (28). In DLST method, the main cluster comprised 3 patients (60%) infected or colonized by the genotype DLST 25-11 which is important in MLST technique and Cholley *et al.* (13) considered it to be equal to ST-244. DLST is a novel and promising technique and can be utilized for future endemic superintendence of *P. aeruginosa* isolates. This typing pattern was founded on the fractional sequencing of two extremely inconstant loci and permitted us to gain a clear and constant classification of the varieties. DLST is an excellent technique for endemic studies of *P. aeruginosa* strains (12, 13, 20). The great stability, typability, and discriminatory power of DLST considerably decrease analysis prices and working time. All previous DLST published studies (12, 13, 20, 29, 30) only considered the genotyping of *P. aeruginosa* strains gathered from various samples. However, an absolutely experimental study with regard to colistin-resistant *P. aeruginosa* isolates or isolates presenting carbapenem-resistant genes is still unaccessible.

## Conclusion

The high frequency of MDR and colistin-resistant *P. aeruginosa* carrying $bla_{NDM-1}$ indicated the importance of routine surveillance over infection control since this antibiotic is the last line of curing infections resulted from MDR *P. aeruginosa*. Moreover, performing MIC sensitivity testing and combined drug therapy is recommended. Molecular typing of the isolates suggested that DLST type 25-11 was a dominant clone and DLST 5-91 was a high-risk clone with resistance to all used antibiotics. Therefore, further studies for accurate and specific use of this antibacterial agent which can help control the dissemination of colistin-resistant strains are needed.

## Acknowledgment

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## References

1. Vaez H, Moghim S, Nasr Esfahani B, Ghasemian Safaei H. Clonal relatedness among imipenem-resistant *Pseudomonas aeruginosa* isolated from ICU-hospitalized patients. Crit Care Res Pract 2015; 2015:1-5 .

2. Mikucionyte G, Zamorano L, Vitkauskiene A, López-Causapé C, Juan C, Mulet X, *et al.* Nosocomial dissemination of VIM-2-producing ST235 *Pseudomonas aeruginosa* in Lithuania. Eur J Clin Microbiol Infect Dis 2016; 35:195-200.

3. Leylabadlo HE, Asgharzadeh M, Aghazadeh M. Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. Iran J Microbiol 2015; 7: 226-246.

4. Merie Queenan A, Bush K. carbapenemases: the versatile B-lactamases. Clin Microbiol Rev 2007; 20: 440-458.

5. Jovčić B, Lepšanović Z, Begović J, Filipić B, Kojić M. Two copies of blaNDM-1 gene are present in NDM-1 producing *Pseudomonas aeruginosa* isolates from Serbia. Antonie van Leeuwenhoek 2014; 105: 613-618.

6. Halaji M, Rezaei A, Zalipoor M, Faghri J. Investigation of class I, II, and III Integrons among *Acinetobacter Baumannii* isolates from hospitalized patients in Isfahan, Iran. Oman Med J 2018; 33: 37-42.

7. Wei W-J, Yang H-F, Ye Y, Li J-B. New Delhi metallo-β-lactamase-mediated carbapenem resistance: origin, diagnosis, treatment and public health concern. Chin Med J 2015; 128: 1969-1976.

8. Lee J-Y, Na IY, Park YK, Ko KS. Genomic variations between colistin-susceptible and-resistant *Pseudomonas aeruginosa* clinical isolates and their effects on colistin resistance. J Antimicrob Chemother 2014; 69: 1248-1256.

9. Falagas ME, Fragoulis KN, Kasiakou SK, Sermaidis GJ, Michalopoulos A. Nephrotoxicity of intravenous colistin: a prospective evaluation. Int J Antimicrob Agents 2005; 26: 504-507.

10. Dhariwal A, Tullu M. Colistin: Re-emergence of the 'forgotten' antimicrobial agent. J Postgrad Med 2013; 59: 208-215.

11. Goli HR, Nahaei MR, Rezaee MA, Hasani A, Kafil HS, Aghazadeh M. Emergence of colistin resistant *Pseudomonas aeruginosa* at Tabriz hospitals, Iran. Iran J Microbiol 2016; 8: 62-69.

12. Basset P, Blanc D. Fast and simple epidemiological typing of *Pseudomonas aeruginosa* using the double-locus sequence typing (DLST) method. Eur J Clin Microbiol Infect Dis 2014; 33: 927-932.

13. Cholley P, Stojanov M, Hocquet D, Thouverez M, Bertrand X, Blanc DS. Comparison of double-locus sequence typing (DLST) and multilocus sequence typing (MLST) for the investigation of *Pseudomonas aeruginosa* populations. Diagn Microbiol Infect Dis 2015; 82: 274-277.

14. Luce E. Koneman's color atlas and textbook of diagnostic microbiology. Plast Reconstr Surg 2010; 125: 414-415.

15. Lavenir R, Jocktane D, Laurent F, Nazaret S, Cournoyer B. Improved reliability of *Pseudomonas aeruginosa* PCR detection by the use of the species-specific ecfX gene target. J Microbiol Methods 2007; 70: 20-29.

16. Patel J, Cockerill F, Alder J, Bradford P, Eliopoulos G, Hardy D, *et al*. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. CLSI standards for antimicrobial susceptibility testing 2014; 34: 1-226.

17. Amjad A, Mirza I, Abbasi S, Farwa U, Malik N, Zia F. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol 2011; 3: 189-193.

18. Qu T-t, Zhang J-l, Wang J, Tao J, Yu Y-s, Chen Y-g, *et al*. Evaluation of phenotypic tests for detection of Metallo-β-lactamase-producing *Pseudomonas aeruginosa* strains in China. J Clin Microbiol 2009; 47: 1136-1142.

19. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011; 70:119-123.

20. Pappa O, Beloukas A, Vantarakis A, Mavridou A, Kefala A-M, Galanis A. Molecular characterization and phylogenetic analysis of *Pseudomonas aeruginosa* isolates recovered from Greek aquatic habitats implementing the Double-Locus Sequence Typing Scheme. Microb Ecol 2017; 74: 78-88.

21. Shokri D, Khorasgani MR, Fatemi SM, Soleimani-Delfan A. Resistotyping, phenotyping and genotyping of New Delhi metallo-β-lactamase (NDM) among Gram-negative bacilli from Iranian patients. J Med Microbiol 2017; 66: 402-411.

22. Shanthi M, Sekar U, Kamalanathan A, Sekar B. Detection of New Delhi metallo beta lactamase-1 (NDM-1) carbapenemase in *Pseudomonas aeruginosa* in a single centre in southern India. Indian J Med Res 2014; 140: 546-550.

23. Mataseje L, Peirano G, Church D, Conly J, Mulvey M, Pitout J. Colistin-nonsusceptible *Pseudomonas aeruginosa* sequence type 654 with blaNDM-1 arrives in North America. Antimicrob Agents Chemother 2016; 60: 1794-800.

24. Saderi H, Owlia P. Detection of multidrug resistant (MDR) and extremely drug resistant (XDR) *P. aeruginosa* isolated from patients in Tehran, Iran. Iran J Pathol 2015; 10: 265-271.

25. Maroui I, Barguigua A, Aboulkacem A, Ouarrak K, Sbiti M, Louzi H, *et al*. First report of VIM-2 metallo-β-lactamases producing *Pseudomonas aeruginosa* isolates in Morocco. J Infect Cemother 2016; 22: 127-132.

26. Gill MM, Usman J, Kaleem F, Hassan A, Khalid A, Anjum R, *et al*. Frequency and antibiogram of multi-drug resistant *Pseudomonas aeruginosa*. J Coll Physicians Surg Pak 2011; 21: 531-534.

27. Bahar MA, Jamali S, Samadikuchaksaraei A. Imipenem-resistant *Pseudomonas aeruginosa* strains carry metallo-β-lactamase gene $bla_{VIM}$ in a level I Iranian burn hospital. Burns 2010; 36: 826-830.

28. Liu P-Y, Weng L-L, Tseng S-Y, Huang C-C, Cheng C-C, Mao Y-C, *et al*. Colistin resistance of *Pseudomonas aeruginosa* isolated from snakes in Taiwan. Can J Infect Dis Med Microbiol 2017; 2017:1-5.

29. Tissot F, Blanc D, Basset P, Zanetti G, Berger M, Que Y-A, *et al*. New genotyping method discovers sustained nosocomial *Pseudomonas aeruginosa* outbreak in an intensive care burn unit. J Hosp Infect 2016; 94: 2-7.

30. Blanc D, Magalhaes BG, Abdelbary M, Prod'hom G, Greub G, Wasserfallen J, *et al*. Hand soap contamination by *Pseudomonas aeruginosa* in a tertiary care hospital: no evidence of impact on patients. J Hosp Infect 2016; 93: 63-67.

31. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis 2011; 17: 1791-1798.

32. Fallah F, Borhan RS, Hashemi A. Detection of *bla* (IMP) and *bla* (VIM) metallo-β-lactamases genes among *Pseudomonas aeruginosa* strains. International . Int J Burns Trauma 2013; 3: 122-124.

33. Golshani Z, Sharifzadeh A. Prevalence of blaOxa10 type beta-lactamase gene in carbapenemase producing *Pseudomonas aeruginosa* strains isolated from oatients in Isfahan. Jundishapur J Microbiol 2013; 6:1-4 .