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Original article

Distribution of Integrons and Gene Cassettes among Metallo-β-Lactamase Producing *Pseudomonas aeruginosa* Clinical Isolates

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Abstract

Background: Integrons are considered as to play a significant role in the evolution and spread of antimicrobial resistance genes.
Materials and Methods: A total of 120 clinical isolates of *Pseudomonas aeruginosa* (collected from Zanjan hospitals between March 2015 and February 2016) were investigated for molecular characterization of MBLs and Class I and II integrons. Antimicrobial susceptibility testing was also performed based on the CLSI guidelines. The frequency of MBL producing isolates and the susceptibility to various antimicrobial agents were investigated.
Results: Based on the obtained results, *Bla*$_{IMP}$ was the most frequently detected metallo-β-lactamase. The frequency of *bla*$_{VIM}$, *bla*SPM, and *bla*$_{SIM}$, in MBL producing isolates was 17.1, 57.1, and 14.1%, respectively. No *bla*$_{GIM}$ harboring isolate was detected in our study. We detected two (5.7%) multidrug resistant *P. aeruginosa* strains isolated from the urine and sputum samples, which harbored *bla*$_{NDM-1}$. These isolates also contained *bla*$_{IMP}$ and *bla*$_{SPM}$. Class I integron was detected in 94.3% of the MBL positive isolates while 8.5% of the isolates contained Class II integrons. Of five different gene cassettes identified in Class I and II integrons, cassette encoding resistance to trimethoprim (*dfr*) was found to be predominant.
Conclusion: These results indicate that Class I integrons are widespread among the MBL producing *P. aeruginosa* isolates. Therefore, appropriate surveillance and control measures are essential to prevent the further spread of MBL and integron producing *P. aeruginosa* in hospitals.

Keywords: Antibiotic resistance, Integron, Metallo-β-lactamase, *Pseudomonas aeruginosa*

1. Background

The emergence of multidrug resistant (MDR) *Pseudomonas aeruginosa* has become a serious problem for healthcare settings in developing countries (1). The dissemination of antibiotic resistance genes by horizontal transfer has currently been thought to play a major role in development of MDR strains. Carbapenems are the most potent antimicrobial agents for the treatment of *P. aeruginosa* infections (2).However, carbapenem resistance have also been reported in some studies to be due to metallo-β-lactamase (MBL) production in *P. aeruginosa* (3), decreased outer membrane permeability via loss of the OprD (D_2) porins (4-5), over expression of an efflux pump system (6), and hyper production of the chromosomally encoded cephalosporinase AmpC (7-8). MBLs or Class B β-lactamases are zinc-dependent enzymes characterized by broad hydrolytic activity against β-lactams except for aztreonam (9). To date, several families of MBLs have been identified in *P. aeruginosa,* including IMP, VIM, GIM, SPM, SIM, AIM, KHM, NDM, and DIM (10).The most common and widespread acquired MBLs are the IMP and VIM types, showing a worldwide distribution (11). A novel MBL named NDM-1 (New Delhi metallo-β-lactamase) was identified from *Klebsiella pneumoniae* and *Escherichia coli* isolates recovered from a Swedish patient transferring from India. NDM-1 is distantly related to other MBLs, sharing only 32% amino acid identity with the most closely related enzymes VIM-1 and VIM-2. Sporadic cases of NDM-1 producing isolates have been reported from different countries, suggesting its widespread dissemination (12-14). Recently, the presence of NDM-1 in *K. pneumoniae* has been reported from Iran (15). The multidruge resistant nature of these strains limits their treatment options (15). The MBLs are usually encoded by genes on plasmids, associated with mobile genetic elements, mostly Class I integrons. Integrons have been identified as a primary source of resistance genes, playing an important role in antibiotic resistance dissemination within the microbial populations. They are able to capture one or more gene cassettes from the environment and incorporate them using site specific recombination. Gene cassettes confer resistance to a range of antimicrobial agents. There are more than nine classes of integrons, in which Class I integrons are mostly and commonly found in nosocomial and community environments, followed by ClassII (2, 16-17).

2. Objective

The aims of the present study were to investigate the pattern of antimicrobial resistance, the frequency of MBLs (*bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SPM}$, *bla*$_{SIM}$, *bla*$_{GIM}$ and *bla*$_{NDM-1}$) among the clinical isolates of *P. aeruginosa,* and finally the analysis of integrons in MBL producing isolates in Zanjan, Iran.

3. Materials and Methods

3.1. Bacterial isolates

During March 2015to February2016, one hundred and twenty *P. aeruginosa* clinical isolates were collected randomly from four major university hospitals in Zanjan, Iran. These strains were isolated from different clinical specimens such as urine, blood, sputum, and stool. The identification of the intended isolates was performed by routine biochemical tests. Confirmed isolates of *P. aeruginosa* were placed at –70°C in Tripticase Soy Broth (Merck, Germany) containing 20% (v/v) glycerol for further analysis.

3.2. Antimicrobial susceptibility testing and phenotypic characterization

Isolates susceptibility to the following antibiotics was examined using the disk diffusion method according to the

Clinical and Laboratory Standards Institute (CLSI, 2015) guidelines (18), including: Aztreonam (30µg), Amikacin (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefepime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Imipenem (10µg), PolymyxinB (300 unit), Piperacillin (100µg), (MAST, Merseyside, U.K). *P. aeruginosa* ATCC27853 was used as the control strain for susceptibility testing. All the strains resistant to imipenem were tested for the production of carbapenemases. MBL E-test strips (AB Biodisk, Solna, Sweden) using imipenem and imipenem-ethylenediamine-tetra acetic acid (EDTA) were used according to the protocol recommended by the manufacturer (18).

3.3. PCR amplification of metallo- β-lactamase genes

MBL producing isolates were tested for *bla* genes including bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{SIM}, bla_{GIM}, and bla_{NDM-1} using the primers listed in Table 1. Extraction of DNA was performed according to the protocol provided with the Qiagen Mini Amp kit. The PCR mixture with a final volume of 25 µL contained 2 µL template DNA, 0.2 mM of each deoxy nucleoside triphosphate, 10 pmol of each primer, 10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl, 1.5 U of Taq DNA polymerase. PCR was performed with the Gene Atlas 322 system (ASTEC, Japan). Amplification involved an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 50 s, annealing at 52°C for 40 s for bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{SIM}, bla_{GIM} and at 58°C for 60 s for bla_{NDM-1}, and an extension at 72°C for 1 min, with a final extension step at 72°C, for 8 min. The expected amplicons were ascertained by electrophoresis on a 1.5% agarose gel with appropriate molecular size markers (100bp DNA ladder; MBI Fermentas).

3.4. Integron characterization and sequencing of resistance-encoding gene cassettes

Metallo-β-lactamase producing isolates were tested for characterization of Class I and II integrons and their gene cassettes. The primer sequences used in this study are shown in Table 1 The PCR was performed in a reaction mixture with a total volume of 25 µL, containing 2 µL template DNA, 0.2 mM of each deoxynucleoside triphosphate, 10 pmol of each primer, 10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl, 1.5 U of Taq DNA polymerase. Amplification was done as follows: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min for *intI1* and *intI2*and at60°C for 1 min for *in* and *hep*, and an extension at 72°C for 2 min, followed by a final extension step at 72°C for 10 min. Amplified products were purified using QIAquick Gel Extraction Kit (Qiagen), and direct sequencing of internal variable regions (gene cassettes) of Class I and II integron was done using ABI 3730X capillary sequencer (Genfanavaran, Macrogen, Seoul, Korea). Nucleotide sequences were analyzed and compared using BLAST software (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov).

4. Results

4.1. Frequency of MBL producing isolates

A total of 120 clinical *P. aeruginosa* isolates were collected from the clinical specimens of four hospitals in Zanjan. A majority of the isolates were recovered from urine specimens (56 isolates, 46.7%), followed by blood (33 isolates, 27.5%), sputum (21 isolates, 17.5%), and stool (10 isolates, 8.3%). Among which, 35 isolates (29.2%) were MBL positive.

4.2. *Susceptibility to antimicrobial agents*

Antimicrobial susceptibility of *P. aeruginosa* isolates are presented in Tables 2The highest resistance rate among the isolates was shown against to Cefotaxime (43.3%), followed by Piperacillin (41.6%), Aztreonam and Gentamicin (37.5%). Only 68.3% of the isolates were imipenem susceptible whilst Polymixin B showed the highest activity against all the isolates (95.8% of the isolates were susceptible). A total of 50 (41.6%) isolates of *P. aeruginosa* were multidrug resistant (MDR). Moreover, all of the MBL producing isolates (35 isolates) were MDR. The most prevalent MDR pattern was resistance to β-lactams, Gentamicin, and Ciprofloxacin.

Table 1. Primer Sequences for Detection of metallo-β-lactamase, integrons and gene cassettes genes in *Pseudomonas aeruginosa*.

Target gene	Primer sequence (5' → 3')	Ampliconsize (bp)	Reference
bla_{IMP-F}	GGAATAGAGTGGCTTACATCTC	188	29
bla_{IMP-R}	CCAAACCACTACGTTATCT		
bla_{SPM-F}	AAAATCTGGGTACGCAAACG	271	29
bla_{SPM-R}	ACATTATCCGCTGGAACAGG		
bla_{SIM-F}	TACAAGGGATTCGGCATCG	570	29
bla_{SIM-R}	TAATGGCCTGTTCCCATGTG		
bla_{VIM-F}	GATGGTGTTTGGTCGCATA	390	29
bla_{VIM-R}	CGAATGCGCAGCACCAG		
bla_{GIM-F}	TCGACACACCTTGGTCTGAA	477	29
bla_{GIM-R}	AACTTCCAACTTTGCCATGC		
bla_{NDM1-F}	CTTCCAACGGTTTGATCGTC	263	15
bla_{NDM1-R}	ATTGGCATAAGTCGCAATCC		
Int1-F	CAGTGGACATAAGCCTGTTC	160	17
Int1-R	CCCGAGGCATAGACTGTA		
Int2-F	CACGGATATGCGACAAAAAGGT	788	17
Int2-R	GATGACAACGAGTGACGAAATG		
in-F (5'CS)	GGCATCCAAGCAGCAAGC	Variable	30
in-R (3'CS)	AAGCAGACTTGACCTGAT		
hep-F	CGGGATCCCGGACGGCATGCACGATTTGT	Variable	30
hep-R	GATGCCATCGCAAGTACGAG		

Table 2. Antimicrobial susceptibility of *P. aeruginosa* clinical isolates collected from Zanjan hospitls.

Intermediate [n (%)]	Susceptible [n (%)]	Resistant [n (%)]	Antimicrobial agent
22(18.3)	53(44.2)	45(37.5)	Aztreonam
8(6.6)	86(71.7)	26(21.7)	Amikacin
10(8.3)	58(48.4)	52(43.3)	Cefotaxime
5(4.1)	80(66.7)	35(29.2)	Ceftazidime
7(5.8)	84(70)	29(24.2)	Cefepime
14(11.3)	77(64.2)	39(32.5)	Ciprofloxacin
2(1.1)	73(60.4)	45(37.5)	Gentamicin
3(2.5)	82(68.3)	35(29.2)	Imipenem
2(1.6)	68(56.6)	50(41.6)	Piperacillin
2(1.7)	115(95.8)	3(2.5)	PolymixinB

4.3. Molecular characterization of MBL genes

All the MBL producing isolates were subjected to PCR experiments to detect metallo- β-lactamase genes, including bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{SIM}, bla_{GIM}, and bla_{NDM-1}. bla_{IMP} was the most frequently isolated metallo- β-lactamase, which was detected in 80% (28/35) of MBL producing *P. aeruginosa* isolates. The frequency of bla_{VIM}, bla_{SPM}, and bla_{SIM} among the MBL producing isolates was 17.1% (6 isolates), 57.1% (20 isolates), and 14.1% (5 isolates), respectively. No bla_{GIM} harboring isolate was detected in our study. Two (5.7%) multidrug resistant strains of *P. aeruginosa* isolated from the urine and sputum samples harbored bla_{NDM-1}. These isolates contained bla_{IMP} and bla_{SPM} simultaneously.

4.4. Analysis of integrons

The presence of integrons was confirmed in 33 (94.3%) cases of MBL producing isolates, of which 33 (94.3%) and 3 (8.5%) cases were identified as Class I (*intI1*) and Class II (*intI2*) integrons, respectively. Three (8.5%) isolates harboread both *intI1* and *intI2*; moreover, *intI1* was more frequent in comparison with *intI2* ($P<.001$). All of the MBL positive isolates harboring *intI1* or *intI2* were MDR. We amplified cassette regions of Class I and II integrons by primers 5'CS/3'CS and *hep*F/*hep*R, respectively. Four different amplicons were identified in Class I integrons with the following size, including 480 bp (14 isolates), 707 bp (2 isolates), 750 bp (7 isolates), and 990 bp (3 isolates). No product was obtained for seven (21.2%) of the *intI1* positive isolates. Six strains (3.8%) had two amplicons. A 1400 bp amplicon was obtained from a single isolate of *P. aeruginosa* harboring *intI2*. The sequence analysis of integron cassettes indicated the presence of dihydrofolate reductase Type A17 (*dfrA17*), dihydrofolate reductase Type I (*dfrA7*), aminoglycoside-2'-adenylyltransferase (*aadB*), aminoglycoside 3'-adenyltransferase (*aadA1*), and dihydrofolatereductase 1- streptothricin acetyltransferase 2 (*dhfr1-sat2*) resistance gene cassettes among the isolates, corresponding to 480, 750, 707, 990, and 1400 bp PCR products, respectively.

5. Discussion

Antimicrobial resistance and the spread of metallo-β-lactamases among *P. aeruginosa* isolates has become a major public health problem in developing countries (19). The treatment of infections associated with multidrug resistant *P. aeruginosa* has been further complicated in Asian countries such as Japan, Taiwan, India, and Iran (2). Furthermore, the increase in carbapenem-resistant Enterobacteriaceae and non-fermenting Gram-negative bacilli is a major concern worldwide (15). In our study, 41.6% of the *P. aeruginosa* isolates were resistant to at least three different classes of antimicrobial agents and determined as multidrug resistant. Only 68.3% of the isolates were imipenem susceptible whilst Polymixin B (95.8%) showed the highest activity against all the isolates, followed by Amikacin (71.7%) and Cefepime (70%). On average, resistance to third and fourth -generation of cephalosporins was 32.2%: 43.3% to cefotaxime, 29.2% to ceftazidime, and 24.2% to cefepime.

During the last decade, several metallo-β-lactamases have been identified in *P. aeruginosa*, beginning with IMP-1 and its derivatives, which are widespread in Japan and China (20). Previous investigations indicated that IMP and VIM types of MBLs are also widespread in Asian countries such as Japan, Korea, China, Taiwan, and Iran (21-23). According to the results, 29.1% (35 isolates) of the *P. aeruginosa* isolates were MBL positive. Similar to a study conducted in China, bla_{IMP} was the most commonly detected MBL in *P. aeruginosa* isolates (21). The frequency of bla_{VIM}, bla_{SPM}, and bla_{SIM} among the MBL producing isolates was 17.1, 57.1, and 14.1%, respectively. No bla_{GIM} harboring isolate was detected in our study. The frequency of bla_{IMP} was significantly higher than bla_{VIM} ($P<.001$). In contrast, Saderi et al. (2010) (25) and Shahcheraghi et al. (2010) (21) indicated higher frequency of bla_{VIM} in comparison with bla_{IMP} (20-24).

This is the first report of NDM-1 producing *P. aeruginosa* isolates in Iran. Sporadic cases of NDM-1 producing isolates have been reported from different countries, suggesting its widespread dissemination (12-14). NDM-1 positive *P. aeruginosa* isolates are capable to destroy carbapenem antibiotics, which are the most potent antimicrobial agents for the treatment of *P. aeruginosa* infections. Therefore, the spread of the pathogenic microorganisms carrying NDM-1 gene now has become potentially a major global health threat (25). According to the results, two *P. aeruginosa* isolates harboring NDM-1 were resistant to all of the tested antibiotics, including third-generation cephalosporins, imipenem, ciprofloxacin, amikacin, gentamicin, aztreonam, piperacillin, and polymixin B. Although aztreonam is not hydrolyzed by MBLs, azteronam resistance observed in our study could be due to the presence of other beta-lactamase genes. In this study, NDM-1 positive isolates also harbored bla_{IMP} and bla_{SPM}. A majority of the patients with NDM-1-positive bacteria usually have a travel background to India or Pakistan (15), but in the present study, the patients had traveled to Iraq before hospitalization. The ability of NDM-1 to spread among Enterobacteriaceae and Pseudomonaceae implies the possibility of numerous new NDM-1 cases to be detected in the near future.

Integrons have been identified as a primary source of resistance genes, and are suspected to serve as reservoirs of antimicrobial resistance genes within microbial populations (26). The present study characterized Class I and II integrons in 91.4 and 8.5% of the MBL producing *P. aeruginosa* isolates. Class II integrons are most frequently associated with members of the family Enterobacteriaceae (2). Previous studies have shown the association between betalactamases and integrons and plasmids of bacteria responsible for nosocomial outbreaks (27). We found a relatively high occurrence of Class I integrons within the MBL producing isolates. Several other reports have indicated the elevated occurrence of Class I integrons among *P. aeruginosa* in comparison to Class 2 (2, 28-29).Also, two NDM-1 positive isolates harbored Class I integrons. According to the results, Class I integrons with identical cassette array were detected in most of the isolates, suggesting that these isolates may have the same mechanisms for resistance acquisition. Gene cassettes encoding resistance to trimethoprim (*dfr*) were found to be predominant in the Class I integrons (63.6% of the isolates harboring *intI1*). Also, two NDM-1 positive isolates harbored *dfrA17* gene cassette. The *aad* cassettes conferring resistance to aminoglycosides were also detected in 15.1% of the isolates harboring *intI1*. *dhfr1-sat2*gene cassette detected in one isolate of Class II integrons may reflect co-transfer of resistance genes due to the genetic linkage between *dhfr* and *sat* cassettes. Our results are also consistent with the previous reports worldwide on the predominance of *dfrA* and *aad* gene cassettes among Enterobacteriaceae (19, 30).

6. Conclusion

According to the results, integrons are continuing to threaten the usefulness of antibiotics as therapeutic agents, especially in *P. aeruginosa* infections. Therefore, appropriate surveillance and control measures are essential to prevent the further spread of MBL and integron producing *P. aeruginosa* in hospitals. Further studies should be carried out for a better understanding of the impact of integrons on the dissemination of antimicrobial resistance in clinical practice.

Conflict of interests

Authors have no conflict of interest to declare.

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Authors' Contribution

Dr Zeighami and Dr Haghi designed the study and wrote the manuscript, Keramati and Hemati performed the experiments and analyzed data.

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References

1. Ullah W, Qasim M, Rahman H, Bari F, Khan S, Rehman ZU, et al. Multidrug resistant *Pseudomonas aeruginosa*: Pathogen burden and associated antibiogram in a tertiary care hospital of Pakistan. Microb Pathog. 2016; 97:209-12.
2. Khosravi Y, Tee Tay S, Vadivelu J. Analysis of integrons and associated gene cassettes of metallo- β -lactamase-positive *Pseudomonas aeruginosa* in Malaysia. J Med Microiol. 2011; 60:988–994.
3. Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a β-lactamase gene, blaGIM-1, encoding a new subclass of metallo-β-lactamase. Antimicrob Agents Chemother. 2004; 48(12):4654–4661.
4. Tsakris A, PoulouA, Kristo I, PittarasT, SpanakisN, PournarasS, et al. Large dissemination of VIM-2–metallo-β-lactamase–producing *Pseudomonas aeruginosa*strains causing health care-associated community-onset infections. J Clin Microbiol. 2009; 47:3524–3529.
5. Giakkoupi P, Petrikkos G, Tzouvelekis LS, Tsonas S, Legakis NJ, Vatopoulos AC. Spread of integron-associated VIM-type metallo-β-lactamase genes among imipenem-nonsusceptible *Pseudomonas aeruginosa*strains in Greek hospitals. J Clin Microbiol. 2003; 41(2):822–825.
6. Hernando-Amado S, BlancoP,Alcalde-Rico M, Corona F,Reales-CalderónJ, Sánchez MB, et al. Multidrug efflux pumps as main players in intrinsic and acquired resistance to antimicrobials. Drug Resist Updat. 2016; 28: 13–27
7. Juan C, Moya´B, Pe´rez JL, Oliver A. Stepwise up regulation of the *Pseudomonas aeruginosa* chromosomal cephalosporinase conferring high-level beta-lactam resistance involves three AmpD homologues. Antimicrob Agents Chemother. 2006; 50(5):1780–1787.
8. Livermore DM, Woodford N. Carbapenemases: a problem in waiting? Curr Opin Microbiol. 2000; 3(5):489–495.
9. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev.2007; 20(3):440–458.
10. Hammami S, Boutiba-Ben Boubaker I, Ghozzi R, SaidaniM, Amine S, Redjeb S. Nosocomial outbreak of imipenem-resistant *Pseudomonas aeruginosa* producing VIM-2 metallo-β-lactamase in a kidney transplantation unit. Diagn Pathol. 2011; 6:106.
11. Koratzanis E, Souli M, Galani I, Chryssouli Z, Armaganidis A, Giamarellou H. Epidemiology and molecular characterisation of metallo-ß-lactamase-producing Enterobacteriaceae in a university hospital intensive care unit in Greece. Int J Antimicrob Agents. 2011; 38(5): 390– 397.
12. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β- lactamases: a last frontier for β-lactams? Lancet Infect Dis.2011; 11(5): 381-393.
13. Tota M, Coque TM, Ruiz-Garbajosa P, Pintado V, Cobo J, Sader HS, et al. Complex clonal and plasmid epidemiology in the first outbreak of Enterobacteriaceae infection involving VIM-1 metallo-beta-lactamase in Spain towards endemicity? Clin infect Dis. 2007; 45(9): 1171-1178.
14. Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA. New delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada. Emerg Infect Dis.2011; 17(1):103–106.
15. Shahcheraghi F, Nobari S, RahmatiGhezelgeh F, Nasiri S, Owlia P, Nikbin VS, et al. First report of new delhi metallo-beta-lactamase-1-producing *Klebsiella pneumoniae* in Iran. Microb Drug Resist. 2013; 19(1):30-6.
16. Xu Z, Li L, Shirtliff ME, Alam MJ, Yamasaki S, Shi L. Occurrence and characteristics of Class 1 and 2 integrons in *Pseudomonas aeruginosa* isolates from patients in southern China. J Clin Microbiol. 2009; 47(1): 230–234.
17. Zeighami H, Haghi F, Masumian N, Hemati F, Samei A, Naderi G. Distribution of integrons and gene cassettes among uropathogenic and diarrheagenic *Escherichia coli*isolates in Iran. Microb Drug Resist. 2015; 21 (4):435-40.
18. Clinical and Laboratory Standards Institute; 2015. Performance standards for antimicrobial susceptibility testing; 21th informational supplement. CLSI document M100-S25.
19. Yan H, Li L, Zong M, Alam MJ, Shinoda S. Occurrence and characteristics of Class 1 and 2 integrons in clinical bacterial isolates from patients in south China. J Health Sci.2010; 56(4): 442-450.
20. Shahcheraghi F, NikbinVS, Feizabadi MM. Identification and genetic characterization of metallo-beta-lactamase-producing strains of *Pseudomonas aeruginosa* in Tehran, Iran. New Microbiol. 2010; 33(3): 243-248.
21. Dong F, Xu XW, Song WQ, LU P, Yu SJ, Yang YH, et al. Characterization of multidrug-resistant and metallo-beta-lactamase-producing *Pseudomonas aeruginosa* isolates from a pediatric clinic in China. Chin Med J. 2008; 121:1611-1616.
22. Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-β-lactamase- producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. Jpn J Infect Dis.2011; 64(1): 69-71.
23. Franco MR, Caiaffa-Filho HH, Burattini MN, Rossi F. Metallo-beta-lactamases among imipenem-resistant *Pseudomonas aeruginosa* in a Brazilian university hospital. Clinics 2010; 65(9):825-829.
24. Saderi H, Lotfalipour H, Owlia P, Salimi H. Detection of metallo-β-lactamase producing *Pseudomonas aeruginosa* isolated from burn patients in Tehran, Iran. Lab Med. 2010; 41(10):609-12.
25. Bhattacharya D, Thamizhmani R, Bhattacharya H, SudharamaSayi D, Muruganandam N, Roy S, et al. Emergence of new delhi metallo-b-lactamase 1 (NDM-1) producing and multidrug resistant uropathogens causing urinary tract infections in Andaman Islands, India. Microb Drug Resist.2013; 19(6):457-462
26. Dıaz-Mejıa JJ, Amabile-Cuevas CF, Rosas I, Souza V. An analysis of the evolutionary relationships of integron integrases, with emphasis on the prevalence of Class 1 integrons in *Escherichia coli* isolates from clinical and environmental origins. Microbiology. 2008; 154(Pt 1):94–102.

27. Machado E, Canton R, Baquero F, Galan JC, Rollan A, Peixe L, et al.Integron content of extended-spectrum-ß-lactamase-producing *Escherichia coli* strains over 12 years in a single hospital in Madrid, Spain. Antimicrob Agents Chemother. 2005; 49:1823–1829
28. SekiguchiJI, Asagi T, Miyoshi-Akiyama T, Kasai A, Mizuguchi Y, AraakeM, et al. Outbreaks of multidrug-resistant *Pseudomonas aeruginosa* in community hospitals in Japan. J Clin Microbiol. 2007; 45:979–989.
29. Cheng X, Wang P, Wang Y, Zhang H, Tao C, Yang W, et al. Identification and distribution of the clinical isolates of imipenem-resistant *Pseudomonas aeruginosa* carrying metallo-beta-lactamase and/or Class 1 integron genes. J Huazhong Univ Sci Technolog Med Sci. 2008; 28(3):235-8.
30. Phongpaichit S, Tunyapanit W, Pruekprasert P. Antimicrobial resistance, Class 1 integrons and extended spectrum betalactamases in *E. coli* clinical isolates from patients in south Thailand. JHS. 2011; 57(3): 281-288.

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