J Med Bacteriol. *Vol. 1, No. 3, 4 (2012): pp. 25-36* *jmb.tums.ac.ir*

ISMB TUMS

Identification of Class-1 Integron and Various B-Lactamase Classes among Clinical Isolates of *Pseudomonas aeruginosa* at Children's Medical Center Hospital

Hossein Fazeli [1], *Hooman Sadighian* [1,2*], *Bahram Nasr Esfahani* [1], *Mohammad Reza Pourmand* [2,3]

[1] *Department of Microbiology, School of Medicine, Isfahan University of Medical sciences, Isfahan, IR Iran*
[2] *Urology Research Center, Sina Hospital, Tehran, IR Iran*
[3] *Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran*

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 10 Oct 2012
Revised: 11 Nov 2012
Accepted: 04 Dec 2012

Keywords:
InTI2 protein, *E. coli*
Integrons
Pediatrics
Pseudomonas aeruginosa
beta-Lactamases

ABSTRACT

Background: *Pseudomonas aeruginosa* is one of the most important opportunistic pathogens responsible for various types of infections. Children suffer significant morbidity and mortality due to nosocomial infections. The aim of this study was to investigate the presence of Class-1 integron, bla_{BEL}, bla_{PER}, bla_{KPC}, bla_{VIM}, bla_{IMP} and $bla_{OXA\text{-}group\text{-}1}$ genes among *P. aeruginosa* isolates at Children's Medical Center Hospital in Iran and to determine phenotypic evidence of ESBL and MBL production.

Methods: Antibiotic susceptibility tests were analyzed for 72 *P. aeruginosa* clinical isolates. Isolates were identified by using biochemical tests and confirmed by PCR assay for *oprL* gene. ESBL and MBL producer isolates were identified by phenotypic tests (double disc synergy tests). Detection of β-lactamase genes and class-1 integron were performed by PCR method.

Results: All of the isolates were susceptible to ceftazidime / clavulanate, meropenem, imipenem and ciprofloxacin. About 83.3% and 16.7% of isolates were resistant to ceftazidime and amikacin respectively. Approximately, 83.3% of isolates were considered as potential ESBL producers. None of the clinical isolates showed above β-lactamase genes. It seems that, the reason is the absence of class-1 integron in all of isolates. About 16.7% of strains were identified as multidrug resistant. Fortunately, all of the isolates were susceptible to meropenem and imipenem which are effective against ESBL producing strains.

Conclusion: The absences of class-1 integron decreases the probability of acquired β-lactamase especially MBL. Thus, absolute susceptibility to carbapenems and ciprofloxacin among *P. aeruginosa* isolates in pediatric hospital has important implications for empirical antimicrobial therapy. It seems that these properties help to decrease mortality of nosocomial infections within children.

- ***Please cite this paper as:*** Fazeli H, Sadighian H, Nasr Esfahani B, Pourmand MR. Identification of Class-1 Integron and Various β-lactamase Classes among Clinical Isolates of *Pseudomonas aeruginosa* at Children's Medical Center Hospital. *J Med Bacteriol.* 2012; **1** (3, 4): pp. 25-36.

***** *Corresponding Author:* Hooman Sadighian, PhD., Department of Microbiology, School of Medicine, Isfahan University of Medical sciences, Isfahan, IR Iran, *Tel:* +98 912 4091152, *E-mail:* sadighianh@yahoo.com

Introduction

Children suffer from significant morbidity and mortality due to nosocomial infections. The consequences of these infections include prolongation of hospitalization, transfer to intensive care units, antibiotic therapy, placement or replacement of invasive devices and surgical procedures (1, 2).

Pediatric wards and hospitals are particularly suited for the transmission of infection. Infants and toddlers constitute a large proportion of the patients admitted. They frequently harbor infectious organisms and may transfer pathogens, especially respiratory and gastrointestinal microorganisms (1, 3). Young children are also susceptible to many infections because they have not yet developed sufficient immunity. Behavioral characteristics of young children, such as incontinence, inadequate hygiene, frequent mouthing of hands and objects, drooling and direct contact between children during play, facilitate the spread of infection. Basic care requires frequent hands-on contact from health care personnel and parents. Multibed rooms, shared toys and playrooms, and visiting siblings contribute to the risk of transmission (1, 2, 4). Transmission rates increase with understaffing and overcrowding (5, 6).

Pseudomonas aeruginosa, an aerobic Gram-negative organism and an opportunistic pathogen that is commonly discovered in soil, water, and plants, rarely causes illness in healthy people. However, *P. aeruginosa* sepsis often occurs in patients with burns, malignancies or immunodeficiency or in preterm infants. Most of these infections are nosocomially acquired (1). *P. aeruginosa* is a virulent organism that is susceptible to a limited number of antimicrobial agents including antipseudomonal penicillins and cephalosporins, carbapenems, fluoroquinolones and ciprofloxacin (7-10). The intrinsic resistance of *P. aeruginosa* to various antibiotics is generally due to its low outer membrane permeability, production of inducible chromosomal cephalosporinases (AmpC), and multidrug efflux pumps (8, 11). Despite recent improvements in therapy, *P. aeruginosa* bacteremia still remains fatal in children (12). In a large multi-center study of all age groups, *P. aeruginosa* bloodstream infection was associated with crude mortality rates of 39% in all patients and 48% in intensive care unit patients (13).

Thus, drugs effective against *P. aeruginosa* infections are limited to aminoglycosides (e.g., gentamicin, amikacin), fluoroquinolones (ciprofloxacin remains the most active), selected β-lactams (e.g., ceftazidime, carbapenems), and one β-lactam / β-lactamase inhibitor combination (piperacillin / tazobactam) (8, 14, 15).

P. aeruginosa, like other Gram-negative pathogens, is known to acquire resistance by producing various β-lactamases (such as VEB, OXA, PER, BEL, KPC, VIM and IMP), which are mostly plasmid-mediated β-lactamases. Among plasmid-mediated β-lactamases, extended-spectrum β-lactamases (ESBL) are generally known to hydrolyze cephamycins and/or carbapenems which cannot be easily hydrolyzed by traditional β-lactamases (8, 16). VEB-type ESBLs were the predominant ESBL reported in *P. aeruginosa* (8). The production of AmpC variants with improved activity against oxyiminocephalosporins (e.g., ceftazidime),

cefepime, and carbapenems (including imipenem), first described in the Enterobacteriacae and referred to as extended-spectrum AmpC (17), have been reported in clinical isolates of *P. aeruginosa* (18, 19).

OXA enzymes (molecular class D β-lactamases) (20) are mostly narrow-spectrum β-lactamases that confer resistance to aminopenicillins and carboxypenicillins and narrow-spectrum cephalosporins (21). Although several OXA-type enzymes are ESBLs (22).

PER-1-type β-lactamase is an ESBL that hydrolyzes various β-lactams except for carbapenems, oxacillin and cephamycins (23, 24). BEL-1 (25) is not frequently observed in *P. aeruginosa*, but it was reported in ESBL positive *P. aeruginosa* in a Belgium study (26, 27).

KPC is a class a β-lactamase with activity against carbapenems. Only KPC-2 and KPC-5 have been reported in *P. aeruginosa*. Interestingly, KPC-2 is more active against carbapenems than is KPC-5 while the latter shows better activity against ceftazidime (8, 28). Class B MBLs are by far the major determinants of β-lactamase mediated resistance to carbapenems and the major cause of high level resistance to these agents, VIM and IMP β-lactamases (molecular class B) are carbapenemases (35). The predominance of VIM vs. IMP in *P. aeruginosa* appears to be geographical, with IMP-type MBLs predominating in Asia where it was first discovered and VIM-type enzymes predominating in Europe though both enzymes are now disseminated globally, with VIM-2 in particular well established on five continents (30, 31).

OXA group-1 β-lactameses especially OXA-10 has the most frequency among all OXA-type β-lactameses in Iran (32, 33, 34).

In Iran, the prevalence of VIM β-lactamases and IMP β-lactamases were 11-23% and 0-5% respectively (35-38).

Prevalence of PER-1 type extended-spectrum β-Lactamase in clinical strains of *Pseudomonas aeruginosa* isolated from Iran is 17 to 27.5% (39, 40).

According to many surveys in Iran, the most prevalent ESBL genes were $bla_{\text{OXA-10}}$and bla_{VEB}genes. The prevalence of *veb* gene is about 31% (32, 33, 40).

BEL β-Lactamases have never been studied in Iran. This study for the first time surveys these genes.

Integrons and transposons have been repeatedly detected in multi-drug resistant *P. aeruginosa* isolates (41, 42). Integrons are genetic elements encoding the components of a site specific recombination system that recognizes and captures mobile gene cassettes, mostly resistance determinants (43). Such elements may be located within transposons, which in turn actively contribute to the dissemination of resistance determinants for aminoglycosides and β-lactams among Gram-negative species (44). Moreover, it has been demonstrated that integrons and transposons are associated with the spread of resistance to third-generation cephalosporins when they encode extended-spectrum β-lactamases (ESBLs) such as VEB-1, GES-1 or TEM-21 (42).

The present study reviews the prevalence of different groups of β-lactamase genes (bla_{BEL}, bla_{PER},bla_{KPC}, bla_{VIM}, bla_{IMP} and $bla_{\text{OXA-group-1}}$) and the existence of class-1 integron in clinical isolates of *P. aeruginosa* as a principle pathogen in nosocomial infections at Children's Medical Center Hospital in Tehran, Iran.

Materials and Methods

Sample collection and clinical data

During a 3 month period, from November 2011 to January 2012, 72 clinical isolate (*P. aeruginosa*) collected from pediatric patients younger than 18 years old who had one positive sample at Children's Medical Center Hospital (the major pediatric hospital in Tehran, Iran).

These samples included thirteen blood specimen, thirty five urine specimens, five wound specimens, twelve tracheal specimens, two throat specimens, three CSF specimen and other biological fluids (two specimens). Over the study period, all the isolates were identified by using microscopic observation and biochemical tests, and then strains were confirmed by PCR for *opr*L gene, which is specific for *P. aeruginosa*. Isolates were analyzed for susceptibility to anti-*Pseudomonas* antibiotics.

To avoid duplicates only one isolate was selected from each patient, unless isolates showed different resistance profiles.

Antimicrobial Susceptibility tests and detection of ESBLs and MBLs by phenotypic methods

Antibiotic susceptibility of *P. aeruginosa* isolates was determined by the disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, UK). Susceptibility was defined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (45). The following β-lactam antibiotics were used: piperacillin 100 µg, piperacillin / Tazobactam 100/10 µg, meropenem 10 µg, imipenem 10 µg, ceftazidime 30 µg, cefepime 30 µg, ceftazidime/clavulanic acid 30/10 µg, amikacin 30 µg, ciprofloxacin 5µg and aztreonam 30 µg discs (MAST, Bootle, Merseyside, UK). *P. aeruginosa* ATCC PAO1 was used as reference strains in susceptibility test.

To detect possible ESBL production, double disc synergy test (DDST) was performed with discs containing ceftazidime (30 mg) alone and in the presence of clavulanate (10 mg). Ceftazidime resistant isolates were suspected to be ESBL producers when a positive double disc synergy test was observed (increase in ceftazidime inhibition zone of > 5mm in the presence of clavulanic acid as compared with when tested alone) (CLSI guidelines) (45).

To screen for Metallo-β-lactamase producing strains, initial screening tests and phenotypic confirmatory tests were performed. For this reason, determination of resistance to meropenem (45) and then phenotypic detection of MBL in all the isolates was performed by double-disc synergy test with discs containing imipenem and EDTA (46).

PCR amplification for detection of P. areruginosa, β-lactamesegenes and class-1 integron

The presence of class 1 integron was tested by PCR using primers specific for the integron integrase genes (*intI*1) (51).

The total DNA from *P. aeruginosa* isolates was extracted by DNA extraction kit (DNeasy blood and tissue, QIAGEN). PCR was carried out in a 50µl volume by using 25 µl Emerald Amp MAX HS PCR Master Mix (Takara Shuzo, Shiga, Japan), 50ng of genomic DNA of the test strain and 0.2 µM of each primer.

PCR with the following cycling parameters was performed: initial denaturation at 94°C for 240 s; 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s and extension at

72°C for 60s; and a final extension at 72°C for 300s. Published primers (25, 47-51) or sets of primers which were designed in this study were used *(Table 1)*.

Table 1. Oligonucleotides used as primers for PCR amplification of class-1 integron, β lactamase and oprL genes

Name	Target	Sequence (5`>>>3`)	Annealing temperature (°C)	Amplicon Size (bp)	Reference
OXA1-F	$bla_{OXA\text{-}group\text{-}1}$	TCAACAAATCGCCAGAGAAG	61	276	47
OXA1-R		TCCCACACCAGAAAAACCAG			
PER-F	bla_{PER}	CCTGACGATCTGGAACCTT	56	641	25
PER-R		CCGTCCATCAGGCAACA			
KPC-F	bla_{KPC}	AGTTCTGCTGTCTTGTCTC	55	798	This study
KPC-R		CTGTGCTTGTCATCCTTG			
BEL-F	bla_{BEL}	TGCTGTTCCTTGTCATTC	55	782	This study
BEL-R		TAATAACGCCCTTTCCTC			
VIM-F	bla_{VIM}	GTCCGTGATGGTGATGAGT	58	437	48
VIM-R		ATTCAGCCAGATCAGCATC			
IMP-F	bla_{IMP}	CATGGTTTGGTGGTTCTTGT	56	448	49
IMP-R		ATAATTTGGCGGACTTTGGC			
AmpC-F	bla_{AmpC}	GCTCCACCAACGGCTTC	58	124	This study
AmpC-R		CTGAGGATGGCGTAGGC			
oprL-F	oprL	ATGGAAATGCTGAAATTCGGC	57	504	50
oprL-R		CTTCTTCAGCTCGACGCGACG			
IntI1-F	Intl-1	CCTCCCGCACGATGATC	63	280	51
IntI1-R		TCCACGCATCGTCAGGC			

Results

Seventy-two patients (38 females and 34 males) were included in this study. Samples were comprised various specimens such as blood, urine, wound, tracheal, throat, CSF and other biological fluids. Of these, 60 (83.3%) isolates were resistance to ceftazidime (inhibition zone diameter of antibiotic disc ≤ 14 mm) and resistance to piperacillin (inhibition zone diameter of antibiotic disc ≤ 17 mm) and resistance to cefepime (inhibition zone diameter of antibiotic disc ≤ 14 mm). The ceftazidime resistant strains were subjects for double disc synergy tests (DDST).

DDST using ceftazidime alone and with clavulanic acid, allowed the detection of 60 potential ESBL producers among 72 isolates (observation of the positive synergy test).

The presence of genes for various ESBLs was investigated using PCR. All of the isolates were evaluated for the presence of ESBL-encoding genes (bla_{PER}, bla_{BEL} and bla_{KPC}) by PCR. Also all the isolates were evaluated for $bla_{OXA\text{-}group\text{-}1}$ and bla_{AmpC} and examined for the presence of class-1 integron (*intl*-1). As a result, all of the isolates had *ampC* gene and none of them had bla_{PER}, bla_{BEL} and bla_{KPC}, $bla_{OXA\text{-}group\text{-}1}$ and class-1 integron.

Whole clinical isolates (100%) were susceptible to meropenem and imipenem, and phenotypic tests for MBLs were performed for all isolates. None of them were positive and they were not suspected for MBL. In order to confirm it, PCR for two MBL genes (bla_{VIM}, bla_{IMP}) was performed for all isolates. Results showed that there were not any MBL genes in these isolates. Furthermore all isolates were susceptible to ciprofloxacin, 60 (83.3%) isolates were susceptible to amikacin and 57 (79.2%) isolates were susceptible to aztreonam. We found that most of the *P. aeruginosa* strains isolated from Children's

Medical Center Hospital, had high level of resistance to ceftazidime (The inhibition zone diameter of ceftazidime disc for most of the isolates was zero) *(Table 2)*. Of all isolates, 16.7% of clinical isolates were identified as multidrug resistant (MDR) which showed resistance to piperacillin, ceftazidime, cefepime, aztreonam and amikacin.

Table 2. Antimicrobial susceptibility of 72 P. aeruginosa by disc diffusion method

Antibiotics	Con. (µg)	Sensitive No. (%)	Resistance No. (%)
Piperacillin	100	12 (16.7)	60 (83.3)
Piperacillin/tazobactam	100/10	66 (91.6)	6 (8.4)
Ceftazidime	30	12 (16.7)	60 (83.3)
Ceftazidime/clavulanate	30/10	72 (100)	0 (0)
Cefepime	30	12 (16.7)	60 (83.3)
Imipenem	10	72 (100)	0 (0)
Meropenem	10	72 (100)	0 (0)
Aztreonam	30	57 (79.2)	15 (20.8)
Ciprofloxacin	5	72 (100)	0 (0)
Amikacin	30	60 (83.3)	12 (16.7)

Discussion

This study is the first report on β-lactamase genes among *P. aeruginosa* clinical isolates in pediatric hospital in Iran and the ESBL screening results demonstrated a high frequency of ESBLs in *P. aeruginosa* isolates examined.

According to our data, 83.3% of *P. aeruginosa* isolates from major pediatric hospital in Tehran, Iran, were ceftazidime resistant. The results of investigation by Pourakbari *et al* at Children's Medical Center Hospital, among the Gram-negative isolates, *P. aeruginosa* were the most frequently isolated species (52). Previous research at Children Hospital in Tabriz, Iran showed 50% ceftazidime resistant (CAZ_{res}) isolates (53). In another study in Tehran, Iran among burned patients with infection by *P. aeruginosa*, the rate of resistance to ceftazidime was 91.8% (54). Therefore, the rate of *P. aeruginosa* CAZ_{res} strains in Children Hospital was less than general hospital (usually admitted adults). But in our study, the prevalence of *P. aeruginosa* CAZ_{res} isolates (83.3%) obtained from children at Children's Medical Center Hospital was really high and it seems unusual findings.

The results of current study, confirmed the potent of ESBL in 83.3% of the isolates based on DDST.

In present study, all clinical isolates (100%) were susceptible to meropenem and imipenem. But in another study among burned patients in Iran, 32.1% were resistant to imipenem (54) and as discussed by Ranjbar *et al*, 97.5% of isolates found among burned patients in Tehran, Iran, were imipenem resistant (55). In our study 100% and 82.6% of isolates were susceptible to ciprofloxacin and amikacin, respectively. Similar to our results, in a survey at Children Hospital in Tabriz, Iran the rate of resistant isolates to ciprofloxacin was low (53). While in another study in Iran among burned patients with *P. aeruginosa* infection, 77.2% and 47.2% of isolates were resistant to ciprofloxacin and amikacin, respectively (54, 55).

Investigation for four types of β-lactamases (class D and class A ESBLs) including $bla_{\text{OXA group-1}}$, bla_{PER}, bla_{BEL} and bla_{KPC} (car-

bapenemase) by PCR method, showed that none of them existed in our clinical isolates.

It is the first time that BEL and KPC is investigated in Iran and, fortunately, none of them detected. According to previous study in Iran, OXA-10 was more frequently detected than other classes of β-lactamase in *P. aeruginosa*, especially in adults (32, 33). Therefore, we searched for OXA group-1 including OXA-10, in *P. aeruginosa* isolates obtained from children, but there was not any β-lactamase gene from this group among the isolates.

PER type enzymes were reported in different studies in Iran. All of them were detected in *P. aeruginosa* isolates from adults, but among our samples from children, PER was not detected. Carbapenems (e.g., meropenem, imipenem) are an important class of anti-pseudomonas β-lactam owing to their stability to most β-lactamases and are of particular use in treating infections associated with ESBL- and AmpC-producers (8). In our study, the clinical isolates of *P. aeruginosa* in pediatric hospital, showed no resistance to imipenem and meropenem and MBL phenotypic tests were negative. It suggested that none of the isolates have MBL which was confirmed by PCR for bla_{VIM} and blaIMP. But as discussed by Owlia, Ranjbar and Shahcheraghi, notable number of imipenem resistant isolates of *P. aeruginosa* (mostly obtained from adults) have been detected in burned patients in Iran (32, 38, 54).

According to our investigation on antibiotic susceptibility in *E. coli and Klebsiella pneumonia* isolates from children in a pediatric hospital (data not published yet), the susceptibility of these isolates was higher than the susceptibility of same isolates from adults in other hospitals (samples obtained from adults) (56).

Our findings demonstrated the growing problem of extended-spectrum β-lactams resistance such as CAZ_{res} in pediatric *P. aeruginosa* isolates. Also, multi-drug resistant (resistance to piperacillin, ceftazidime, cefepime, aztreonam and amikacin) *P. aeruginosa* isolates were detected at Children's Medical Center Hospital. In contrast to wide spread of β-lactamase genes and other resistance mechanisms in *P. aeruginosa* strains isolated from adult patients, all of the studied isolates were susceptible to carbapenems (meropenem, imipenem) and ciprofloxacin. It seems that, the absence of class-1 integron (*intl-1*) prevents the isolates from acquiring β-lactamase genes that cause resistance to carbapenems. Also, the absence of class-1 integron, probably, helps to decrease horizontal gene transfer among bacteria that prevents creation of resistant strains.

Conclusion

In conclusion, our findings showed that absolute susceptibility to carbapenems and ciprofloxacin and high rate of aminoglycoside susceptible strains among *P. aeruginosa* clinical isolates in pediatric hospital have important implications for empirical antimicrobial therapy. Thus, it helps to decrease the mortality rate of nosocomial infections by ESBL producing *P. aeruginosa* within children.

Acknowledgments

We acknowledge Dr. Kyungwon Lee from department of laboratory medicine, Yonsei

University College of Medicine Seoul, Korea for kindly providing *intl-1 and* bla_{IMP}-positive *P. aeruginosa* isolate as a gift. We would like to thank Dr. Shahcheraghi from department of bacteriology, Pasteur institute of Iran, who gave us plasmids which contain bla_{VIM} and bla_{PER} genes. The authors wish to thank Dr. Nikmanesh and Ms. Naseri for assistance with sample collection.

Conflict of Interest

None declared conflicts of interest.

References

1. Pier GB, Ramphal R, 2005. *Pseudomonas aeruginosa.* In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious diseases*, 6th ed. Churchill Livingstone, USA, Philadelphia, pp. 2587-615.
2. Gaynes R, Edwards JR. National nosocomial infections surveillance system: overview of nosocomial infections caused by Gram negative bacilli. *Clin Infec Dis* 2005; **41** (6): 848.
3. Jarvis WR, Robles B. Nosocomial infections in pediatric patients. *Adv Pediatr Infec Dis* 1996; **12**: 243-95.
4. Harris JS. Pediatric nosocomial infections: Children are not little adults. *Infec Control Hosp Epidemiol* 1997; **18** (1): 739-42.
5. Lambert ML, Suetens C, Savey A, Palomar M, Hiesmayr M, Morales I, *et al*. Clinical outcomes of healthcare-associated infections and antimicrobial resistance in patients admitted to European intensive-care units: a cohort study. *Lancet Infect Dis* 2011; **11** (1) 30-38.
6. Archibald LK, Manning ML, Bell LM, *et al*. Patient density, nurse to patient ratio and nosocomial infection risk in a pediatric cardiac intensive care unit. *Pediatr Infec Dis J* 1997; **16** (11): 1045-8.
7. Alvarez-Ortega C, Wiegand I, Olivares J, *et al*. The intrinsic resistance of *Pseudomonas aeruginosa* to β-lact ams. *Virulence* 2011; **2** (2): 144-6.
8. Poole K, Frank D, Tolmasky M. *Pseudomonas aeruginosa*: resistance to the max. *Front Microbiol* 2011; **2** (65): 1-13.
9. Navon-Venezia S, Ben-Ami R, Carmeli Y. Update on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections in the healthcare setting. *Curr Opin Infec Dis* 2005; **18** (4): 306-13.
10. Cao B, Wang H, Sun H, *et al*. Risk factors and clinical outcomes of nosocomial multidrug resistant *Pseudomonas aeruginosa* infections. *J Hosp Infec* 2004; **57** (2): 112118.
11. Li XZ, Livermore DM, Nikaido H. Role of efflux pumps in intrinsic resistance of *Pseudomonas aeruginosa*: active efflux as a contributing factor to b-lactam resistance. *Antimicrob Age nts Chemother* 1994; **38** (8): 1742-52.
12. Lucignano B, Ranno S, Liesenfeld O, *et al*. Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. *J Clin Microbiol* 2011; **49** (6): 2252-8.

13. Wisplinghoff H, Bischoff T, Tallent SM, *et al*. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clinical Infectious Diseases* 2004; **39** (3): 309-317.
14. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). *Diagn Microbiol Infec Dis* 2004; **50** (1): 59-69.
15. Rodriguez-Martinez JM, Poirel L, Nordmann P. Treatment and control of severe infections caused by mul ti-resistant *Pseudomonas aer-uginosa*. *Clin Microbiol Infec* 2005; **11** (4): 17.
16. Strateva T, Yordanov D. *Pseudomonas aeruginosa* – a phenomenon of bacterial resistance. *J Med Microbiol* 2009; **58** (9): 1133-48.
17. Nordmann P, Mammeri H. Extended-spectrum cephalosporinases: structure, detection and epidemiology. *Future Microbio* 2007; **2** (3): 297.
18. Rodriguez-Martinez JM, Poirel L, Nordmann P. Extended spectrum cephalosporinases in *Pseudomonas aeruginosa*. *Antimicrob Agents Che mother* 2009; **53** (5): 1766-71.
19. Rodriguez-Martinez JM, Poirel L, Nordmann P. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aerugin-osa*. *Antimicrob Agents Chemother* 2009; **53** (11): 4783-8.
20. Bush K, George AJ. Updated functional classification of β-Lactamases. Antimicrob Agents Chemother 2010; **54** (3): 969-76.
21. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β-lactamases. *Antimicrob Age nts Chemother* 2010; **54** (1): 24.
22. Carrer A, Poirel L, Yilmaz M, *et al*. Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob Age nts and Chemother* 2010; **54** (3): 1369-73.
23. Nordmann P, Ronco E, Naas T, *et al*. Characterization of a novel extended-spectrum b-lactamase from *Pseudomonas aeruginosa*. Ant-imicrob Age nts Chemother 1993; **37** (5): 962-9.
24. Nordmann P, Ronco E, Naas T. Sequence analysis of PER-1 exte ndedspectrum b-lactamase from *Pseudomo nas aeruginosa* and comparison with class Aβ-lactamases. *Antimicrob Age nts Chemother* 1994; **38** (1): 104-14.
25. Aktaş Z, Poirel L, Salcioğlu M, *et al*. PER-1- and OXA-10-like betalactamases in ceftazidime-resistant *Pseudomonas aeruginosa* isolates from intensive care unit patients in Istanbul, Turkey. *Clin Microbiol Infec* 2005; **1** (3): 193-8.
26. Glupczynski Y, Bogaerts P, Deplano A, *et al*. Detection and characterization of class an extended-spectrum b-lactamase-producing *Pseudomonas ae ruginosa* isolates in Belgian hospitals. *J Antimicrob Chemother* 2010; **65** (5): 866-71.
27. Bogaerts P, DeplanoA ,Glupczynski Y. Emergence and Dissemination of BEL-1-Producing *Pseudomonas aeruginosa* isolates in Belgium. *Antimi*

crobal Agents and Chemother 2007; **51** (4): 1584-5.
28. Poirel L, Nordmann P, Lagrutta E, *et al*. Emergence of KPC- producing *Pseudomonas aeruginosa* in the United States. *Antimicrob Agents Chemo ther* 2010; **54** (7): 3072-6.
29. Walsh TR, Toleman MA, Poirel L, *et al*. Metallo-β- lactamases: the quiet before the storm. *Clin Microbiol Rev* 2005; **18** (2): 306-25.
30. Gupta V. Metallo beta lactamases in *Pseudomonas aeruginosa* and *Acin etobacter* species. *Expert Opin Inve stig Drugs* 2008; **17** (2): 131-43**.**
31. Walsh TR. Emerging carbapenemases: a global perspective. *Int J Ant imicrob Agents* 2010; (36): S8-S14.
32. Mirsalehian A, Feizabadi M, Nakhjavani FA, *et al*. Detection of VE B-1, OXA-10 and PER-1 genotypes in extended-spectrum beta-lactamase-prod ucing *Pseudomonas ae ruginosa* strai ns isolated from burn patients. *Burns* 2010; **36** (1): 70-4.
33. Jabalameli F, Mirsalehian A, Sotoudeh N. Multiple-locus variable number of tandem repeats (VNTR) fingerprinting (MLVF) and antibacterial resistance profiles of extended spectrum beta lactamase (ESBL) producing *Pse udomonas aeruginosa* am ong burnt patients in Tehran. *Burns* 2011; **37** (7): 1202-07.
34. Kalantar E, Taherzadeh S, Ghadimi T, *et al*. *Pseudomonas aeruginosa* an emerging pathogen among burn patie nts in Kurdistan Province, Iran. *Southeast Asian J Trop Med Public Health* 2012; **43** (3): 712-7.
35. Khosravi AD, Mihani F. Detection of metallo-β-lactamase producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn Microbiol Infec Dis* 2008; **60** (1): 125-8.
36. Rezaei YH, Behzadian NG, Peerayeh SN. Prevalence and detection of metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* strains from clinical isolates in Iran. *Ann Microbiol* 2007; **57** (2): 293-5.
37. Yousefi S, Farajnia S, Nahaeib MR, *et al*. Detection of metallo-β lactamase–encoding genes among clinical isolates of *Pseudomonas aeruginosa* in northwest of Iran. *Diagn Microbiol Infect Dis* 2010; **68** (3): 322-5.
38. Shahcheraghi F, Nikbin VS, Feizabadi MM. Identification and genetic characterization of metallobetalact am ase-producing strains of *Pseudomonas aeruginosa* in Tehran, Iran. *New Microbiolo* 2010; **33** (3): 243-8.
39. Akhi MT, Khalili Y, Ghottaslou R, *et al*. Prevalence of PER-1-type Extended-Spectrum Beta-Lactamaes in Clinical Strains of *Pseudomonas aeruginosa* Isolated from Tabriz, Iran. *Iranian J Basic Med Sci* 2012; **15** (1): 678-682.
40. Shahcheraghi F, Nikbin VS, Feizabadi MM. Prevalence of ESBLs genes among multidrug resistant isolates of *Pseudomonas aeruginosa* isolated fro m patients in Tehran. *Microb Drug Re sis* 2009; **15** (1): 37.
41. Riccio ML, Pallecchi L, Docquier JD. Clonal relatedness and conserved integron structures in epidemiological-

ly unrelated *Pseudomonas aeruginosa* strains producing the VIM-1 Metallo-β-Lactamase from different Italian hos pitals. *Antimic-rob Agents Chemot her* 2005; **49** (1): 104-10.

42. Stalder T, Barraud O, Casellas M, *et al*. Integron involvement in environmental spread of antibiotic resistance. *Front Microbiol* 2012; **3**: 119-23.
43. Recchia GD, Hall RM. Gene cassettes: a new class of mobile element. *Microbiol* 1995; **141** (Pt12): 3015-27.
44. Partridge SR, Collis CM, Hall RM. Class 1 integron containing a new gene cassette, aadA10, associated with Tn1404 from R151. *Antimic-rob Age nts Chemother* 2002; **46** (8): 2400-8.
45. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. Twentieth Informational Supplement M100-S12.Wayne, PA: CLSI, 2010.
46. Lee K, Lim YS, Yong D, *et al*. Evaluation of the Hodge test and the imipenem-EDTA double-disc synergy test for differentiating metallo-β-la ctamase-producing isolates of *Pseudomonas* spp. and *Acinetobac-ter* spp. *J Clin Microbiol* 2003; **41** (10): 4623-9.
47. Bert F, Branger C, Lambert-Zec hovsky N. Identification of PSE and OXA β-lactamase genes in *Pseudomo nas aeruginosa* using PCR–restriction fragment length polymorphism. *J Antimicrob Che-mother* 2002; **50** (1): 11-8.
48. Bogaerts P, Huang T, Rodriguez-Villalobos H, *et al*. Nosocomial infections caused by multidrug-resistant *Pse udomonas putida* isolates producing VIM-2 and VIM-4 metalloblactamases. *J of Antimic-rob Chemo ther* 2008; **61** (3): 749-51.
49. Jeong SH, Lee K, Chong Y, *et al*. Characterization of a new integron containing VIM-2, a metallo-β-lact amase gene cassette, in a clinical isolate of *Enterobacter cloacae*. *J Antimicrob Chemother* 2003; **51** (2): 397-400.
50. Deschaght P, Baere TD, Simaey LV, *et al*. Comparison of the sensitivity of culture, PCR and quantitative real-time PCR for the detection of *Pseudomonas aeruginosa* in sputum of cystic fibrosis patients. *BMC Microbiol* 2009; **9**: 244.
51. Bass L, Liebert CA, Lee MD, *et al*. Incidence and Characterization of Integrons, Genetic Elements Mediating Multiple-Drug Resistance, in Avian *Escherichia coli*. *Antimicrob Agents Chemother* 1999; **43** (12): 2925-9.
52. Pourakbari1 B, Sadr A, Ashtiani MT, *et al*. Five-year evaluation of the antimicrobial susceptibility patterns of bacteria causing bloodstream infections in Iran. *J Infec Dev Ctries* 2005; **6** (2): 120-5.
53. Ghorashi Z, Nezami N, Ghotaslou R, *et al*. Pattern of *Pseudomonas aeruginosa*drug resistance in Tabriz Children Hospital. *Pak J Biol Sci* 2010; **13** (8): 400-4.
54. Owlia P, Bahar MA, Sadri, *et al*. Antibiotic resistance patterns of isolated *pseudomonas aeruginosa* strains from infections of burned patients. *J Med Council IRI* 2007; **25** (1): 26-33.

55. Ranjbar R, Owlia P, Saderi H, *et al*. Characterization of *Pseudomonas aeruginosa* Strains Isolated from Burned Patients Hospitalized in a Major Burn Center in Tehran, Iran. *Acta Med Iran* 2011; **49** (10): 675-9.

56. Eftekhar F, Rastegar M, Golalipoor M, *et al*. Detection of Extended Spectrum β-Lactamases in Urinary Isolates of *Klebsiella pneumoniae* in Relation to Bla_{SHV}, Bla_{TEM} and Bla_{CTX-M} Gene Carriage. *Iran J Public Health* 2012; **41** (3): 127-32.