Full Length Research Paper

Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* expressing *bla*GES and *bla*PER genes in two different hospitals

Omar B. Ahmed* and Atif H. Asghar

Department of Environmental and Health Research, The Custodian of the Two Holy Mosques Institute for Hajj and Umrah, Umm Al-Qura University, Makkah, Saudi Arabia.

Pseudomonas aeruginosa is intrinsically resistant to several antimicrobial drugs classes. Various extended-spectrum beta lactamases (ESBL) types have been found in *P. aeruginosa* such as Pseudomonas extended resistance (PER) and Guiana extended-spectrum (GES) enzymes. The study aimed to evaluate the susceptibility of the ESBL producing *P. aeruginosa* strains that express *bla*GES and *bla*PER genes to commonly used antibiotics. A total of 28 *P. aeruginosa* clinical isolates was identified as ESBL producers and subjected to polymerase chain reaction (PCR) technique for detection of *bla*GES, *bla*PER genes. Routine antimicrobial susceptibility was determined by the disc diffusion method. The highest resistance rate reached 71.4% for ciprofloxacin, while the lowest resistance rate (10.7%) was seen in imipenem followed by colistin (21.6%). *bla*GES gene was observed in 78.6% of the isolates, while *bla*PER appeared in 22.4%. It was concluded that imipenem and colistin showed good antipesuedomonal activity and *bla*GES was predominant gene among the ESBL producing *P. aeruginosa* in Makah hospitals. The results of the present study can help to prevent the mortality and morbidity associated with *Pseudomonas* infections in hospitals.

Key words: *P. aeruginosa*, extended-spectrum beta lactamases (ESBL), *bla*GES, *bla*PER, polymerase chain reaction (PCR).

INTRODUCTION

Pseudomonas aeruginosa is an obligate aerobic and can persist in both community and hospital settings due to its ability to survive on minimal nutritional requirements and to tolerate a variety of physical conditions (Lister and Wolter, 2009). The infections in hospitals mainly affect burn patients, patients in intensive care units, patients with urinary-tract infections and in catheterized and hospital-acquired pneumonia patients on respirators (Yetkin et al., 2006; Bodey et al., 1983).

The organism is intrinsically resistant to several antimicrobial drug classes and can rapidly develop resistance to other drugs during chemotherapy, making

*Corresponding author. E-mail: abuaglah1@hotmail.com.

Table 1. Primers used in the study.

Gene	Primer	Sequence (5′ to 3′)	Size	References
*Bla*GES	GES-1(F)	ATGCGCTTCATTCACGCAC	860	(Poirel et al., 2001)
	GES-1(R)	CTATTTGTCCGTGCTCAGG		
*Bla*PER	PER-1(F)	AATTTGGGCTTAGGGCAGAA	925	(Lee et al., 2005)
	PER-1(R)	ATGAATGTCATTATAAAAGC		

medical treatment difficult and ineffective. The development of beta-lactam resistance in *P. aeruginosa* can be caused by several mechanisms: (a) genetic mutations that lead to stable overexpression of AmpC, a chromosome-mediated cephalosporinase; (b) acquisition of transferable genes that code for a variety of beta-lactamases; (c) overproduction of efflux systems; and (d) reduced permeability (Livermore, 2002). Extended-spectrum beta lactamases (ESBL) are plasmid mediated enzymes that hydrolyze the oxyimino β monobactams (aztreonam) but have no effect on the cephamycins (cefoxitin and cefotitan) and the carbapenems (Imipenem) but they are inhibited by clavulanic acid and tazobactam (Livermore, 1995).

Various ESBL types found in *P. aeruginosa* are the SHV, TEM, CTX-M, PER, BEL-1, BES-1, SFO-1, TLA and IBC (Olowe and Adefioye, 2014) but many other types are also emerging. Examples of these types are Pseudomonas extended resistance (PER) and Guiana extended-spectrum (GES). PER-1 β-lactamase efficiently hydrolyzes penicillins and cephalosporins and is susceptible to clavulanic acid inhibition. PER is reported mostly in clinical isolates from Turkey, while GES is mostly in France, Greece and South Africa. PER-1 was identified first, in a *P. aeruginosa* isolate from 1991 recovered in France from a Turkish patient (Empel et al., 2007).

PER type was found to be the most common (or least rare) ESBL in *P. aeruginosa* in several countries such as Korea, Romania and Bulgaria (Libisch et al., 2008) and later on, has also been found in Belgium, Italy and Spain (Mirsalehian et al., 2010). GES-1 beta-lactamase was first detected in a *Klebsiella pneumoniae* isolate obtained in France in 1998 and subsequently detected in *P. aeruginosa* and other enterobacteriaceae from different geographical parts (Poirel et al., 2000; Garza-Ramos et al., 2015). The gene, *bla*GES-1, conferred an extended-spectrum cephalosporin resistance profile, including clavulanic acid (CA), tazobactam and imipenem (IPM). Generally, there are different GES variants, some are ESBL and some are carbapenemases and the integron genetic framework of GES is the essential factor that develops resistance to broad-spectrum β-lactam antibiotics and other dissimilar classes of antimicrobials (Tavajjohi et al., 2013; Weldhagen et al., 2003; Frase et al., 2011).

The study aimed to evaluate the susceptibility of the ESBL producing *P. aeruginosa* strains that express *bla*GES and *bla*PER genes to the commonly used antibiotics.

MATERIALS AND METHODS

A total of 28 out of 108 non-duplicated *P. aeruginosa* clinical isolates, obtained from admitted patients of various body sites, sputum specimen (n=12), urine specimen (n=10) tracheal aspirates (n= 4) and wound swabs (n=2) were identified as ESBL producer by double disc synergy methods in two main tertiary care hospitals in Makkah, Hera General Hospital (HGH) and King Abdulaziz Hospital (KAH), during the period of September 2014 to August 2015. The isolates were tested for their antimicrobial susceptibility then to determine the prevalence of *bla*GES and *bla*PER genes.

The following antibiotics were used to indicate ESBL production (Poirel et al., 2001): cefpodoxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg) and aztreonam (30 µg). In each plate, four discs were placed at inter-disc distances of 25 or 30 nm away from an amoxicillin/clavulanic acid disc (20/10 µg) according to the Clinical and Laboratory Standards Institutes (CLSI) criteria (CLSI, 2012). Any distortion (keyhole) or increase in the zone towards the disc of amoxicillin-clavulanate was considered as positive for the ESBL production.
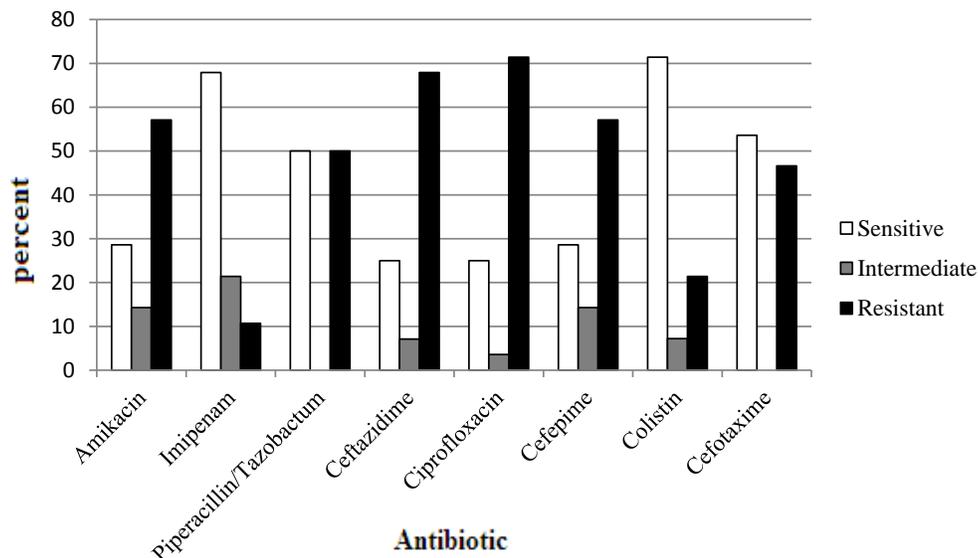
Routine antimicrobial susceptibility was determined by the disk diffusion method according to the guidelines of the CLSI (CLSI 2012). The isolates were tested against ceftazidime (30 µg), cefotaxime (30 µg), ciprofloxacin (10 µg), amikacin (30 µg), cefepime (30 µg), Piperacillin/Tazobactum (100/10 µg), Imipenam (10 µg) and colistin (10 µg).

Single colony from each ESBL-producing isolate was transferred into 100 µL of sterile distilled water and the bacterial DNA was extracted by using boiling method including microwave pre-heating according to Ahmed et al. (2014). All ESBLs producers isolates were subjected to polymerase chain reaction (PCR) technique for detection of *bla* GES and *bla* PER genes. The primers used in this study (Table 1) were obtained from IDT Integrated DNA technologies (IDT, Belgium). Amplification of DNA was performed using Master cycler Personal Thermal Cycler (Eppendorhoff, Germany). PCR was carried in 50 µl PCR reaction volumes containing 4 µl of template DNA, 1 µl (100 pmol) of each primer and a 25 µl of Taq PCR Master Mix (promega company). The conditions of the reaction were as follows: pre-denaturation at 94°C for 4 min, followed by 35 amplification cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1.5 min, with a final extension step of 72°C for 5 min. Amplified PCR products were detected in 1% agarose gel electrophoresis for 35 min at 90 V using 5 x TBE running buffer (4.84 g/L Tris, 0.37 g/L EDTA, pH 8). Gels were stained with ethidium bromide (2 g/ml) and DNA bands were viewed under UVP BioDoct-IT digital imaging system.

The results outcomes were analyzed and assessed using Statistical Package for Social Sciences"IBM Corp. Released 2012.

Table 2. Samples, distribution.

Specimen source		No.	%
Hospital	HGH	17	60.7
	KAH	11	39.3
	Total	28	100
Gender	Males	18	64.3
	Females	10	35.7
	Total	28	100
Age	Less 20	3	10.7
	20-40	7	25
	41-60	8	28.6
	Above 60	10	35.7
	Total	28	100
Ward	MW	14	50
	FW	7	25
	ICU	4	14.3
	SW	3	10.7
	Total	28	100

Figure 1. Antimicrobial susceptibility of different types of antibiotics of ESBL producing *P. aeruginosa*.

IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp".

RESULTS

The distribution of *P. aeruginosa* isolates is shown in Table 2. Antibiotic susceptibility rates among ESBL *P. aeruginosa* isolates were seen as maximum in colistin (71.4%) followed imipenem (67.9%), cefotaxime (53.6%) and piperacillin/tazobactum (50%), while the highest resistance rates was seen in ciprofloxacin (71.4%) followed by ceftazidime (67.9%), cefepime (57.1%) and amikacin (57.1%) (Figure 1). The results of PCR of the *bla*PER and *bla*GES genes are shown in Figures 2 and 3, respectively. The *bla*GES (860 bp) was the most frequent
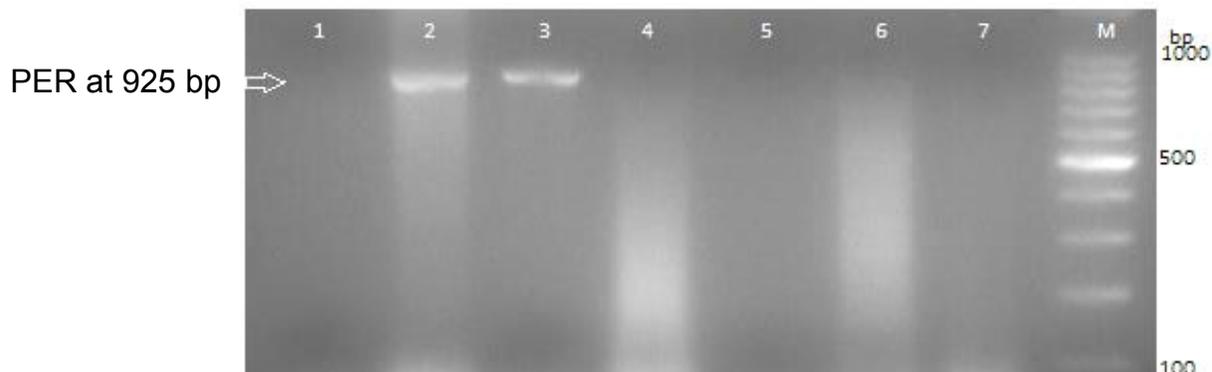
Figure 2. The ESBL genes after PCR on 2% agarose gel electrophoresis. Lane 1: Control (negative). Lane 2; control (positive) *bla*PER, Lanes 3; positive *bla*PER, Lanes 4 to 7: negative *bla*PER, Lane M: 100-bp DNA ladder.
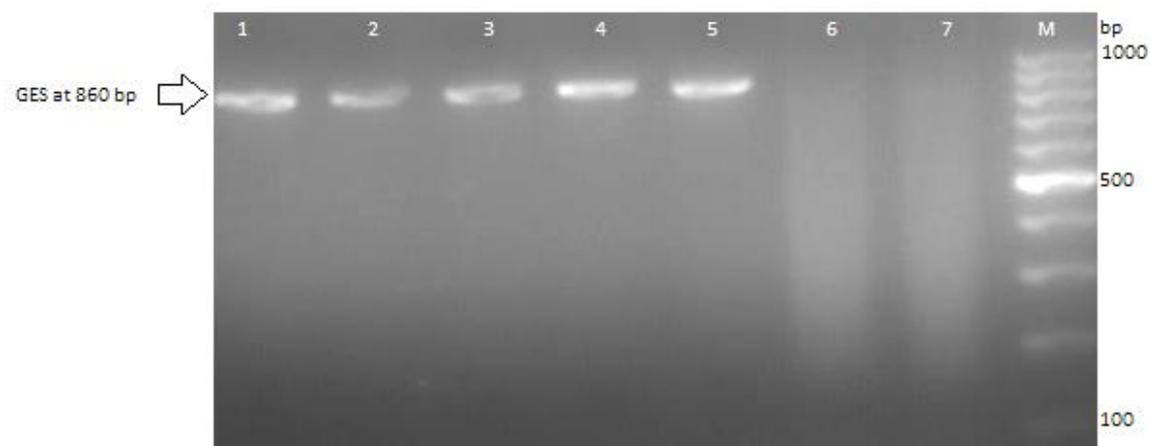

Figure 3. The ESBL genes after PCR on 2% agarose gel electrophoresis. Lane 1: control (positive) *bla*GES, Lanes 2 to 5; positive *bla*GES, Lanes 6; control negative: Lane 7; negative *bla*GES, Lane M: 100-bp DNA ladder.

ESBL gene and isolated from 78.6% (n=22)] of the ESBL producing strains, while, *bla*PER (925 bp) was detected in 21.4% (n=6) of the isolates.

According to the statistical analysis, a significant association was observed between the antibiotic resistance to third generation cephalosporin and the presence of *bla*PER-1 and *bla*GES-1 genes ($p < 0.05$), while no association was observed between antibiotic resistance to other classes of antibiotics and presence of any or both *bla*PER-1 and *bla*GES-1 genes ($p > 0.05$).

DISCUSSION

The occurrence of multidrug-resistant *P. aeruginosa* strains is increasing worldwide. Infections caused by *P. aeruginosa* are difficult to treat as *P. aeruginosa* exhibit intrinsic resistance to several antimicrobial agents. *P. aeruginosa* was identified as the fifth most frequently isolated nosocomial pathogen (Lister and Wolter, 2009).

The results showed that ESBL producing *P. aeruginosa* strains had a varied level of resistance to different antibiotic classes such as β-lactams, fluoroquinolones and aminoglycosides. The highest resistance rate reached was 71.4% for ciprofloxacin, while the lowest resistance rate (10.7%) was seen in imipenem followed by colistin (21.6%) (Figure 1). Decreased susceptibility of *P. aeruginosa* to the commonly used antibiotics has also been shown in different studies (Arya et al., 2005; Obritsch et al., 2004). This is due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL enzymes.

As a result of the ability of *P. aeruginosa* to develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection, different studies on ciprofloxacin resistance to *P. aeruginosa* reported ranges between 0 and 89% (Gul et al., 2007), although some researchers reported that more than 90% of isolates were sensitive to ciprofloxacin (Gul et al., 2007). Bashir et al., 2011) reported resistance rate

of 13.4% to imipenem by *P. aeruginosa*. Many researchers reported 100% sensitivity for imipenem and meropenem (Bashir et al., 2011; Shaikh et al., 2015; Aggarwal et al., 2008). Colistin, a polypeptide antibiotic belonging to the polymyxin group, was initially used for the treatment of patients colonized with *P. aeruginosa*; and as a therapy of otitis, conjunctivitis and skin infections (Falagas and Kasiakou, 2005). Despite the risk for nephrotoxicity, colistin has been successfully used to treat ESBL-associated infections, especially when therapeutic choices are seriously limited (Linden et al., 2003). But recent data suggest that resistance to colistin is emerging, and outbreaks of colistin-resistant strains have been reported (Kontopoulou et al., 2010).

In the present study, 67.9% of isolates were ceftazidime-resistant. In similar results, ceftazidime resistance was relatively higher among the isolates from inpatients which are 60.34, 65 and 73.4%, observed in studies done by different researchers (Easwaran et al., 2016; Umadevi et al., 2011; Haider et al., 2014). The resistance to ceftazidime is increasing at an alarming rate, complicating the clinical management of patients infected with *P. aeruginosa* isolates (Easwaran et al., 2016).

The present study showed that 78.6% of ESBLs producer of *P. aeruginosa* strains carried *bla*GES gene (Figure 2), while *bla*PER appeared in 22.4%, (Figure 3). GES is a known class-A ESBL in *P. aeruginosa*. GES-1 was initially described in a *K. pneumoniae* isolate from a neonatal patient just transferred to France from French Guiana (Poirel et al., 2000). GES-1 has hydrolytic activity against penicillins and extended-spectrum cephalosporins, but not against cephamycins or carbapenems, and is inhibited by β-lactamase inhibitors. These enzymatic properties resemble those of other class A ESBLs; thus, GES-1 was recognized as a member of ESBLs. The rate of GES-1 is considered high, as compared to other geographical areas, as in Brazil, Turkey, Saudi Arabia and Egypt (Sidjabat et al., 2009; Castanheira et al., 2004; Er et al., 2015; Tawfik et al., 2012; Azab et al., 2015).

The PER-1 ESBL confers resistance to most β-lactams, and may be carried on a plasmid that has been transferred *in vitro* from PER-1-positive *P. aeruginosa* to PER-1-negative strains of the same species (Luzzaro et al., 2001). Shacheraghi et al. (2010) reported that *bla*PER-1 and *bla*GES-1 genes were detected in the 68.3 and 24.4% of the ESBL producing isolates respectively. Similarly, Picao et al. (2009), reported GES-1 gene of 16.3% from *P. aeruginosa*.

The rate of PER-1 is considered higher than that reported in Iran, Turkey and Bolivia (Bavasheh and Karmostaji, 2017; Vahaboglu et al., 1997; Celenza et al., 2006). The antibiotic resistance in *P. aeruginosa* is due to a combination of factors either through the acquisition of resistance genes on mobile genetic elements (plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms

in addition of the low permeability of its cell wall. The two most common strategies considered to address this need are through optimizing therapy of basic antibacterial pharmacodynamic principles and treating *P. aeruginosa* with a combination of antibacterial drugs.

One of the limitations of this study is unreliability of the current ESBL detection method for *P. aeruginosa*, because according the CLSI, the screening tests for ESBLs (including disk diffusion) can be used for *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli* and *Proteus mirabilis*. The method used in the study is not yet standardized for *P. aeruginosa*.

It could be concluded that the ESBL producing *P. aeruginosa* is a major challenge to hospitals in Makkah city due to the emergence and spread of isolates with decreased susceptibilities to several antibiotics. The imipenem and colistin are of highest antimicrobial activity and *bla* GES gene was the most common ESBL genes. The results of the present study can help to prevent the mortality and morbidity associated with *Pseudomonas* infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

REFERENCES

Aggarwal R, Chaudhary U, Bala K (2008). Detection of extended-spectrum β-lactamase in *Pseudomonas aeruginosa*. Indian J. Pathol. Microbiol. 51(2):222-224.

Ahmed OB, Asghar AH, Elhassan MM (2014) Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. Afr. J. Microbiol. Res 8:598-602.

Arya M, Arya P, Biswas D, Prasad R (2005). The antimicrobial susceptibility pattern of the bacterial isolates from post-operative wound infections. Indian J. Pathol. Microbiol. 48(2):266-269.

Azab M, Shehata A, Mohamed M (2015). OXA- 10 and GES-1 Extended-spectrum β-lactamases Play A Major Role in Causing Antibiotic Resistance of Pseudomonas aeruginosa Isolated from Nosocomial Infections in Ismailia, Egypt. Egypt. J. Med. Microbiol. 24(4):81-88.

Bashir D, Thokar MA, Fomda BA, Bash G, Zahoor D, Ahmad SA, Toboli AS (2011). Detection of metallobeta- lactamases (MBL) producing *Pseudomonas aeruginosa* at a tertiary care hospital in Kashmir. Afr. J. Microbiol. Res. 5:164-172.

Bavasheh N, Karmostaji A (2017). Antibiotic Resistance Pattern and Evaluation of blaOXA-10, blaPER-1, blaVEB, blaSHV Genes in Clinical Isolates of Pseudomonas aeruginosa Isolated from Hospital in South of Iran in 2014-2015. Infect. Epidemiol. Med. 3(1):1-5.

Bodey GP, Bolivar R, Fainstein V, Jadeja L (1983). Infections caused by Pseudomonas aeruginosa. Rev. Infect. Dis. 5:279-313.

Castanheira M, Mendes RE, Walsh TR (2004). Emergence of the

extended-spectrum beta-lactamase GES-1 in a *Pseudomonas aeruginosa* strain from Brazil: Report from the SENTRY Antimicrobial Surveillance Program. Antimicrob. Agents Chemother. 48:2344-2345.

Celenza G, Pellegrini C, Caccamo M, Segatore B, Amicosante G, Perilli M (2006). Spread of bla(CTX-M-type) and bla(PER-2) beta-lactamase genes in clinical isolates from Bolivian hospitals. J. Antimicrob. Chemother. 57(5):975-978.

CLSI-Clinical and Laboratory Standards Institute (2012). NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; 15th information supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.

Easwaran S, Yerat RC , Ramaswamy R (2016). A study on detection of extended-spectrum beta-lactamases (ESBLs) and comparison of various phenotypic methods of AmpC detection in Pseudomonas aeruginosa from various clinical isolates in a tertiary care teaching hospital. Muller J. Med. Sci. Res. 7(1):35-39.

Empel J, Filczak K, Mro´wka A, Hryniewicz W, Livermore DM, Gniadkowski M (2007). Outbreak of Pseudomonas aeruginosa Infections with PER-1 Extended-Spectrum _-Lactamase in Warsaw, Poland: Further Evidence for an International Clonal Complex. J. Clin. Microbiol. 45(9):2829-2834.

Er H, Altindis M, Asik G, Demir C (2015). Molecular epidemiology of beta-lactamases in ceftazidime resistant Pseudomonas aeruginosa isolates. Mikrobiyol. Bul. 49(2):156-165.

Falagas ME, Kasiakou SK (2005). Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin. Infect. Dis. 40:1333-1341.

Frase H, Smith CA, Toth M, Champion MM, Mobashery S, Vakulenko SB (2011). Identification of products of inhibition of GES-2 beta-lactamase by tazobactam by x-ray crystallography and spectrometry. J. Biol. Chem. 286(16):14396-14409.

Garza-Ramos U, Barrios H, Reyna-Flores F, Tamayo-Legorreta E, Catalan-Najera JC, MorfinOtero R, Rodríguez-Noriega E, Volkow P, Cornejo-Juarez P, González A, Gaytan-Martinez J, Del Rocío Gónzalez-Martínez M, Vazquez-Farias M, Silva-Sanchez J (2015). Widespread of ESBL- and carbapenemase GES-type genes on carbapenemresistant Pseudomonas aeruginosa clinical isolates: a multicenter study in Mexican hospitals. Diagn. Microbiol. Infect. Dis. 81(2):135-137.

Gul AA, Ali L, Rahim E, Ahmed S (2007). Chronic suppurative otitis media; frequency of Pseudomonas aeruginosa in patients and its sensitivity to various antibiotics. Prof. Med. J. 14:411-415.

Haider M, Rizvi M, Fatima N, Shukla I, Malik A (2014). Necessity of detection of extended spectrum beta-lactamase, AmpC and metallo-beta-lactamases in gram-negative bacteria isolated from clinical specimens. Muller J. Med. Sci. Res. 5:23-28.

Kontopoulou K, Protonotariou E, Vasilakos K, Kriti M, Koteli A, Antoniadou E, Sofianou D (2010) Hospital outbreak caused by *Klebsiella pneumoniae* producing KPC-2 β-lactamase resistant to colistin. J. Hosp. Infect. 76:70-73.

Lee S, Park YJ, Kim M, Lee HK, Han K, Kang CS, Kang MW (2005). Prevalence of Ambler class A and D beta-lactamases among clinical isolates of Pseudomonas aeruginosa in Korea. J. Antimicrob. Chemother. 56(1):122-127.

Libisch B, Poirel L, Lepsanovic Z, Mirovic V, Balogh B, Paszti J, Hunyadi Z, Ak AD, Ak MF, Nordmann P (2008). Identi¢cation of PER-1extended-spectrumb-lactamase producing Pseudomonas aeruginosa clinical isolates oftheinternational clonal complex CC11from Hungary and Serbia. FEMS Immunol. Med. Microbiol. 54:330-338.

Linden PK, Kusne S, Coley K, Fontes P, Kramer DJ, Paterson D (2003). Use of parenteral colistin for the treatment of serious infection due to antimicrobial-resistant *Pseudomonas aeruginosa*. Clin. Infect. Dis. 37:e154-e160.

Lister PD, Wolter DJ, Hanson ND (2009). Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin. Microbiol. Rev. 22(4):582-610.

Livermore DM (1995). Beta-lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. 8:557-584.

Livermore DM (2002). Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin. Infect. Dis.

34:634e40.

Luzzaro F, Mantengoli E, Perilli M, Lombardi G, Orlandi V, Orsatti A, Amicosante G, Rossolini GM, Toniolo A (2001). Dynamics of a nosocomial outbreak of multidrug-resistant Pseudomonas aeruginosa producing the PER-1 extended-spectrum beta-lactamase. J Clin Microbiol. 39(5):1865-1870.

Mirsalehian A, Feizabadi M, Nakhjavani FA, Jabalameli F, Goli H, Kalantari N (2010). Detection of VEB-1, OXA-10 and PER-1 genotypes in extended-spectrum β-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients. Burns 36(1):70-74,

Obritsch MD, Fish DN, Maclaren R, Jung R (2004). The national surveillance of antimicrobial resistance in the Pseudomonas aeruginosa isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob. Agents Chemother. 48:4606-4610.

Olowe OA, Adefioye S (2014). Understanding extended spectrum betalactamases in clinical settings. Review update. Int. J. Med. Appl. Sci. 3(3):51-61.

Picao RC, Poirel L, Gales AC, Nordmann P (2009). Diversity of beta-lactamases produced by ceftazidime-resistant *pseudomonas aeruginosa* isolates causing bloodstream infections in Brazil. Antimicrob. Agents Chemother. 53:3908-3913.

Poirel L, Thomas IL, Naas T, Karim A, Nordmann P (2000). Biochemical sequence analyses of GES-1, a novel class A extended-spectrum betalactamase, and the class 1 integron In52 from Klebsiella pneumoniae. Antimicrob. Agents Chemother. 44:622-632.

Poirel L, Weldhagen GF, Naas T, Champs C, Dove MG, Nordmann P (2001). GES-2, a class A β- lactamase from Pseudomonas aeruginosa with increased hydrolysis of imipenem. Antimicrob. Agents Chemother. 45(1):2598-2603.

Shacheraghi F, Shakibaie MR, Noveiri H (2010). Molecular Identification of ESBL Genes *bla*GES-1, *bla*VEB-1, *bla*CTX-M *bla*OXA-1, *bla*OXA-4, *bla*OXA-10 and *bla*PER-1 in *Pseudomonas aeruginosa* Strains Isolated from Burn Patients by PCR, RFLP and Sequencing Techniques. Int. Sch. Sci. Res. Innov. 4(1):1009-1013.

Shaikh S, Fatima J, Shakil S, Mohd S. Rizvi D, Kamal M A (2015). Prevalence of multidrug resistant and extended spectrum beta-lactamase producing Pseudomonas aeruginosa in a tertiary care hospital. Saudi J. Biol. Sci. 22:62-64.

Sidjabat HE, Paterson D L. , Adams-Haduch J M, Ewan L, Pasculle AW, Muto CA, Tian G-B, Doi Y (2009). Molecular Epidemiology of CTX-M-Producing Escherichia coli Isolates at a Tertiary Medical Center in Western Pennsylvania. Antimicrob. Agents Chemother. 53(11):4733-4739.

Tavajjohi Z, Moniri R, Zarrabi M (2013). Detection of GES-2, a Class A β-Lactamase Produced by Pseudomonas aeruginosa in a Teaching Hospital in Iran. Jundishapur J. Microbiol. 6(10):e8166.

Tawfik AF, Shibl AM, Aljohi MA, Altammami MA, Al-Agamy MH (2012). Distribution of Ambler class A, B and D beta-lactamases among Pseudomonas aeruginosa isolates. Burns 38(6):855-860.

Umadevi S, Joseph NM, Kumari K, Easow JM, Kumar S, Stephen S, Srirangaraj S, Raj S (2011). Detection of extended spectrum beta lactamases, ampc beta lactamases and Metallobetalactamases in clinical isolates of ceftazidime resistant *Pseudomonas Aeruginosa*. Braz. J. Microbiol. 42:1284-1288.

Vahaboglu H, Oztürk R, Aygün G, Coşkunkan F, Yaman A, Kaygusuz A, Leblebicioglu H, Balik I, Aydin K, Otkun M (1997). Widespread detection of PER-1type extended-spectrum β-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. Antimicrob. Agents Chemother. 41:2265-2269.

Weldhagen GF, Poirel L, Nordmann P (2003). Ambler class A extendedspectrum beta-lactamases in Pseudomonas aeruginosa: novel developments and clinical impact. Antimicrob. Agents Chemother. 47(8):2385-2392.

Yetkin G, Otlu B, Cicek A, Kuzucu C, Durmaz R (2006). Clinical, microbiologic, and epidemiologic characteristics of Pseudomonas aeruginosa infections in a university hospital, Malatya, Turkey. Am. J. Infect. Control 34:188-192.