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

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*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 antipseudomonal 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 lactamas (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...
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 cephemycins (cefotaxin and cefototan) and the carbapenemides (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, 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, blaGES-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 (Tavajohi 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 blaGES and blaPER 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, spumut 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 blaGES and blaPER 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), Imipenem (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 1. Primers used in the study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
<th>Size</th>
<th>References</th>
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<tbody>
<tr>
<td>GES-1(F)</td>
<td>ATGCCCTTTATCCAGCAGC</td>
<td>860</td>
<td>(Poirel et al., 2001)</td>
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<tr>
<td>GES-1(R)</td>
<td>CTATTTGTCGTGTCAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PER-1(F)</td>
<td>AATTGGGCGATTAGGAAGA</td>
<td>925</td>
<td>(Lee et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>PER-1(R)</td>
<td>ATGAATGTATTATAAGG</td>
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<td></td>
<td></td>
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</tbody>
</table>

### References


**Table 2.** Samples distribution.

<table>
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<th>Specimen source</th>
<th>No.</th>
<th>%</th>
</tr>
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</tr>
<tr>
<td>KAH</td>
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</tr>
<tr>
<td>Total</td>
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<td>100</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Males</td>
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<td>64.3</td>
</tr>
<tr>
<td>Females</td>
<td>10</td>
<td>35.7</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less 20</td>
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<td>10.7</td>
</tr>
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<tr>
<td>41-60</td>
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<td>28.6</td>
</tr>
<tr>
<td>Above 60</td>
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<td>35.7</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>Ward</td>
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</tr>
<tr>
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<td>14</td>
<td>50</td>
</tr>
<tr>
<td>FW</td>
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<td>14.3</td>
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<tr>
<td>SW</td>
<td>3</td>
<td>10.7</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>100</td>
</tr>
</tbody>
</table>

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


**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 *blaPER* and *blaGES* genes are shown in Figures 2 and 3, respectively. The *blaGES* (860 bp) was the most frequent
ESBL gene and isolated from 78.6% (n=22) of the ESBL producing strains, while, blaPER (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 blaPER-1 and blaGES-1 genes ($p < 0.05$), while no association was observed between antibiotic resistance to other classes of antibiotics and presence of any or both blaPER-1 and blaGES-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 \textit{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 \textit{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 patients 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 \textit{P. aeruginosa} isolates (Easwaran et al., 2016).

The present study showed that 78.6% of ESBLs producer of \textit{P. aeruginosa} strains carried \textit{blaGES} gene (Figure 2), while \textit{blaPER} appeared in 22.4%, (Figure 3). GES is a known class-A ESBL in \textit{P. aeruginosa}. GES-1 was initially described in a \textit{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 \textit{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., 2006; Castanheira et al., 2004; Er et al., 2015; Tawfik et al., 2012; Azab et al., 2015).

The PER-1 ESBL confers resistance to most \textit{β}-lactams, and may be carried on a plasmid that has been transferred \textit{in vitro} from PER-1-positive \textit{P. aeruginosa} to PER-1-negative strains of the same species (Luzzaro et al., 2001). Shacharabgh et al. (2010) reported that \textit{blaPER}-1 and \textit{blaGES}-1 genes were detected in the 68.3 and 24.4% of the ESBL producing isolates respectively. Similarly, Picar et al. (2009), reported GES-1 gene of 16.3% from \textit{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 \textit{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 \textit{P. aeruginosa} with a combination of antibacterial drugs.

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

It could be concluded that the ESBL producing \textit{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 \textit{ 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 \textit{Pseudomonas} infections.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

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