Gene Reports 13 (2018) 19–23

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

# The occurrence of *bla*TEM, *bla*SHV and *bla*OXA genotypes in Extended-Spectrum β-Lactamase (ESBL)-producing *Pseudomonas aeruginosa* strains in Southwest of Iran

Ali Nazari Alam[a,b], Jamal Sarvari[a], Mohammad Motamedifar[a], Hossein Khoshkharam[a], Masoud Yousefi[c], Rezvan Moniri[b], Abdollah Bazargani[a,*]

[a] Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[b] Department of Microbiology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
[c] Department of Microbiology, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran

ARTICLE INFO

*Keywords:*
*Pseudomonas aeruginosa*
Antibiotic resistance
Nosocomial infection
ESBL

ABSTRACT

*Pseudomonas aeruginosa* is considered as a potential nosocomial pathogen, which shows a great tendency for infecting immune-compromised individuals, particularly burn patients. Producing extended spectrum beta-lactamases (ESBL) enzymes by these bacteria resulting inactivation of penicillins and cephalosporins which renders drug resistance. The aims of this study were to assess the antimicrobial susceptibility pattern and the prevalence of resistance determinants in ESBL producing *P. aeruginosa* in hospitalized patients. A total of 165 isolates of *P. aeruginosa* which included in this study isolated from teaching Shiraz hospitals, southwest of Iran. Antimicrobial susceptibility of isolates to antibiotics was determined using disk agar diffusion test. For detecting ESBL producing bacteria, all *P. aeruginosa* isolates that were resistant to Cefotaxime and/or Ceftazidime were examined for resistant to combined disks of Ceftazidime + Clavulanic acid and/or Cefotaxim + Clavulanic acid. The presence of antibiotic resistance determinants were investigated by PCR method. The results revealed that 99 (60%) of the isolates were multiple-drug resistant (MDR) and 21 (12.7%) of the isolates were ESBL positive. Among 21 ESBL-producing strains, 19 (90.5%), 11 (52.4%) and 7 (33.3%) strains were carrying *bla*OXA-10, *bla*SHV and *bla*TEM gene, respectively. The association between beta-lactamase genes with origin of isolates and drug resistance in this study indicates that further work with larger sample size is necessary to find out the actual involvement of *P. aeruginosa* strains carried these genes in the course of infections in burn patients.

## 1. Introduction

*Pseudomonas aeruginosa* is a non-fermentative Gram-negative aerobic rod, ubiquitous in the environment. *P. aeruginosa* is considered as a potential nosocomial pathogen, which shows a great tendency for infecting immune-compromised individuals, particularly burn patients (Lari et al., 2015; Ebrahimpour et al., 2018). *P. aeruginosa* is the most common causes of ventilator-associated pneumonia and takes the one the main causes of mortality among hospital-acquired infections (Lari et al., 2015; Ebrahimpour et al., 2018). There is an increasing reports of *Gram-negative* bacteria with resistance to several antibiotics, especially in *P. aeruginosa* (*Sarhangi* et al., 2013; *Asgharzadeh Kangachar and Mojtahedi*, 2017; *Malekzadegan* et al., 2017; *Hosseinzadeh* et al., 2018; *Soltani* et al., 2018). A diversity of mechanisms of antibiotic resistance in *P. aeruginosa* has been explained, including AmpC beta-lactamase (Livermore, 1992), extended-spectrum beta-lactamase, multi-drug efflux pump (Sobel et al., 2003) and biofilm formation (Emami et al., 2015).

The first beta-lactamase enzyme was produced by the isolation of *Escherichia* in 1940, which caused the penicillin hydrolysis. So far, > 890 beta-lactamase enzymes have been identified. Beta-lactamase are categorized into four groups of A, B, C, D based on Ambler classification (Tavakoly et al., 2018). Classes A, C and D are groups which act by serine mechanisms and Group B needs zinc for activity (Abdollahzadeh et al., 2012; Shanthi et al., 2013). Class A chromosome consists of penicillinase in Gram-negative bacteria which include extended-spectrum β-lactamase (ESBL). It consists of beta-lactamase SHV, TEM, CTX-based substrate, which are classified into a lot of subtypes. Oxacillinase of

*Abbreviations:* ESBL, Expanded Spectrum Beta-Lactamases; MDR, Multidrug resistant; TSB, Trypticase soy broth; CLSI, Clinical and Laboratory Standards Institute

* Corresponding author at: Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Zand St, Shiraz, Iran.
*E-mail address:* bazargania@sums.ac.ir (A. Bazargani).

https://doi.org/10.1016/j.genrep.2018.08.002
Received 5 June 2018; Received in revised form 26 July 2018; Accepted 6 August 2018
Available online 09 August 2018

class D (OXA) are of the plasmid-born origin (Poirel et al., 2001; Branger et al., 2005; Rezaei et al., 2018).

TEM, SHV and OXA types are the most common ESBLs found in *P. aeruginosa* isolates (Shacheraghi et al., 2010; Lin et al., 2012; Tawfik et al., 2012). For the establishment of the appropriate antimicrobial therapy and for assessment and control of the spread of drug resistant *P. aeruginosa*, the molecular detection and surveillance of resistance genes are becoming increasingly important (Bedenic et al., 2010). The aim of this study was to determination of *bla*TEM, *bla*SHV and *bla*OXA genotypes in ESBLs producing *P. aeruginosa* isolates among Iranian hospitalized patients.

## 2. Material and methods

### 2.1. Study design and bacterial isolates

In this cross-sectional study, a total of 165 clinical isolates of *P. aeruginosa* were collected consecutively from hospitalized patients from four teaching hospitals in Shiraz including Nemazee, Faghihi, Ghotbedin, and Aliasghar hospitals during a 9-month period from January to October 2012. The isolates were taken from patients' wounds ($n = 80$), sputum ($n = 20$), blood ($n = 6$), urine ($n = 35$) and etc. ($n = 27$). Isolates were identified according to Gram staining, colony morphologies, McConkeys agar, TSI test, oxidase reaction, growth at 42 °C and pigment production. The isolates preserved in trypticase soy broth (TSB) with glycerol (30%) and stored at −70 °C until used (Imani Foolad et al., 2010; Tavajjohi and Moniri, 2011).

### 2.2. Detection of antibiotic resistance

Antibiotic susceptibility pattern of the isolates was determined using the disk diffusion assay on Mueller-Hinton agar (Merck, Germany) in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2016). *P. aeruginosa* strains were classified as susceptible, intermediate or resistant to antibiotics based on CLSI breakpoints. The used antibiotics disks including Ceftazidime (30 μg), Imipenem (10 μg), Gentamicin (10 μg), Piperacillin (100 μg), Aztreonam (30 μg), and Tobramycin (10 μg) were purchased from Rosco-Denmark. *P. aeruginosa* ATCC 27853 was used as quality controls in each susceptibility test (Wayne, 2016). Multiple-drug resistant (MDR) isolates were estimated according to previously described definitions. The isolates non-susceptible to ≥1 agent in ≥3 different antimicrobial categories were considered as MDR (Magiorakos et al., 2012).

### 2.3. Phenotypic detection of ESBL

In this method Combination of Ceftazidime and Cefotaxime disks alone and Ceftazidime (30 μg) and Cefotaxime (30 μg) + Clavulanic acid (10 μg) were put at a distance of 2 cm on the Muller Hinton agar. After incubation for 18 h at 35C° ESBL production was determined by increasing the size of the diameter around the disk of Ceftazidime/Clavulanic acid and or Cefotaxime/Clavulanic acid by 5 mm or more (Ghaffarian et al., 2018).

### 2.4. Polymerase chain reaction

Using specific primers (Bioneer, Korea) which their sequences are shown in Table 1, the polymerase chain reaction (PCR) was performed to detect *bla*TEM, *bla*SHV, *bla*OXA-10 genes. Two or three grown bacterial colonies on Muller-Hinton agar plates were suspended in 180 μL PBS. Then total DNA from *P. aeruginosa* isolates was extracted by phenol-chloroform method (Tavajjohi and Moniri, 2011). PCR tests were carried out as follows: 10 min initial denaturation at 94 °C, 35 cycles of denaturation at 94 °C for 30 s, annealing (at 52 °C for 1 min, 60 °C for 1 min and 55 °C for 30 s for TEM, SHV and OXA genes respectively), extension at 72 °C for 50 s and final extension at 72 °C for 5 min. Amplification reactions were performed in a final volume of 25 μL containing 1× PCR buffer (CinnaGen, Iran), 200 μM concentrations of dNTPs (CinnaGen, Iran), 10 μmol/L of each primer (Bioneer, Korea), 1.5 mM MgCl2 (CinnaGen, Iran), 1 U Taq polymerase (CinnaGen, Iran) and 3 μL DNA templates. Amplified samples were done electrophoresis on 2% agarose gel in TAE buffer. The gel was stained with ethidium bromide 0.5 mg/mL. The amplified bands were visualized under ultraviolet light and photographed. A 100-bp ladder was used to distinguish between different sized amplicons. Positive controls were obtained from Pasteur Institute, Tehran, Iran (Karakoc and Gerceker, 2001; Tawfik et al., 2012).

**Table 1**
The sequences of primers and PCR product size of *bla* TEM, *bla* SHV and, *bla* OXA-10 genes.

| Primer | Primer sequence | Amplicons size (bp) |
|---|---|---|
| TEM-A | 5′-GAGTATTCAACATTTCCGTGTC-3′ | 861 |
| TEM-B | 5′-TAATCAGTGAGGCACCTATCTC-3′ | |
| SHV-A | 5′-AAGATCCACTATCGCCAGCAG-3′ | 231 |
| SHV-B | 5′-ATTCAGTTCCGTTTCCCAGCGG-3′ | |
| OXA-10-A | 5′-TCAACAAATCGCCAGAGAAG-3′ | 276 |
| OXA-10-B | 5′-TCCCACACCAGAAAAACCAG-3′ | |

### 2.5. Statistical analysis

The statistical analysis of data was conducted using Version 19.0 (SPSS Inc., Chicago, IL, USA). The chi-square test was used to compare antibiotic resistance rates. $P < 0.05$ was considered as statistically significant.

## 3. Results

Overall, 165 isolates of *P. aeruginosa* specimens were collected from wound 80 (45.8%), respiratory samples (sputum, throat, trachea), 35 (21.2%), urine 35 (21.2%), blood 6 (3.6%), and others 9 (5.5%).

The highest rate of resistance (resistant and intermediate-resistant isolates) was seen against aztreonam (69.7%), while the lowest rate was seen toward tobramycin (53.3%). The full results of antibiotic susceptibility profile of *P. aeruginosa* isolates were summarized in Table 2. Also, of 165 tested isolates 99 (60%) were found as MDR.

Twenty one isolates (12.7%) were identified as ESBL-producing isolates (Table 3). The > 40% of ESBL-producing isolates were collected from wound samples. TEM, SHV, OXA-10 genes were detected of 19 ESBL-producing isolates (90.4%), 11 isolates (52.4%) and 7 isolates (33.3%), respectively (Fig. 1). Moreover, the overall prevalence of TEM, SHV, and OXA-10 genes were 13.9% ($N = 23$), 15.2% ($N = 25$), and 33.9% ($N = 56$), respectively. Totally, 40 out of 90 imipenem-resistant isolates (44.4%) carried OXA-10 gene.

**Table 2**
The antibiotic susceptibility testing results of 165 clinical isolates of *P. aeruginosa*.

| Class | Antibiotic | Susceptible no. (%) | Intermediate-resistant no. (%) | Resistant no. (%) |
|---|---|---|---|---|
| Carbapenems | Imipenem | 71 (43) | 4 (2.4) | 90 (54.5) |
| Penicillins | Piperacillin | 66 (40) | 7 (4.2) | 92 (55.8) |
| Cephalosporins | Ceftazidime | 71 (43) | 2 (1.2) | 92 (55.8) |
| Aminoglycosides | Gentamicin | 66 (40) | 3 (1.8) | 96 (58.2) |
| | Tobramycin | 77 (46.7) | 24 (14.5) | 88 (55.3) |
| Monobactams | Aztreonam | 50 (30.3) | 4 (4.3) | 91 (55.2) |