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# Distribution of extended−spectrum β−lactamase genes in antibiotic−resistant strains of *Pseudomonas aeruginosa* obtained from burn patients in Ahvaz, Iran

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## ARTICLE INFO

## ABSTRACT

**Objective**: To evaluate the drug susceptibility profiles and the frequency of beta-lactamase encoding genes in *Pseudomonas aeruginosa* (*P. aeruginosa*) obtained from burn patients. **Methods**: Totally 93 non-duplicate clinical isolates of *P. aeruginosa* were recovered from burn patients of Taleghani Burn Hospital of Ahvaz. Antibiotic susceptibility testing was conducted by disk diffusion method according to the CLSI 2017 recommendations. PCR assay was performed by to find beta-lactamase encoding genes. **Results**: In this study, most clinical specimen was obtained *via* wound swabs [65 (69.9%)], followed by blood [14 (15.1%)] and biopsy [7 (7.5%)]. Forty-two (45.16%) patients were male and 51(54.84%) were female. High resistance was observed for most of antibiotics especially for gentamicin and ciprofloxacin (Up to 85%), whereas the highest susceptibility was reported for colistin (100.0%), followed by ceftazidime (66.7%). According to PCR results, 16.1% (15), 9.7% (9) and 14.0% (13) of isolates carried $bla_{DHA}$, $bla_{VEB}$ and $bla_{GES}$ genes, respectively. It also revealed that the $bla_{VEB}$ gene was found to coexist within 2 isolates (2.2%). **Conclusions**: Antibacterial resistance is high among *P. aeruginosa* isolates. Colistin is highly active against multi-drug resistant *P. aeruginosa* isolates. Antimicrobial susceptibility testing can confine indiscriminate uses of antibiotics and resistance increase, and can improve management of treatment.

## 1. Introduction

*Pseudomonas aeruginosa*(*P. aeruginosa*) is one of the most important bacteria in nosocomial infections, particularly in burn units. Infections of this pathogen, especially multidrug-resistant

(MDR) isolates, in burn patients, are difficult to treat and regarded as a common problem[1,2]. The increasing prevalence of MDR strains is associated with prolonged hospitalization and a significant rise in patients' morbidity and mortality[3]. Beta-lactamases can be

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on the chromosome or on plasmids and may be classified as extended-spectrum $\beta$-lactamases (ESBLs), AmpCs, and carbapenemases, among other types. Nowadays, the increasing $\beta$-lactam resistance in high among *P. aeruginosa* isolates illustrates the significance of $\beta$-lactamases-coding genes: ESBLs, AmpC $\beta$-lactamases as well as metalo $\beta$-lactamases, which are commonly related to transmissible genetic elements promoting the resistance development[4].

ESBLs are a rapidly growing group of $\beta$-lactamases that hydrolyze penicillins and expanded-spectrum cephalosporins, and belong to class A of Ambler molecular classification system. Most predominant ESBL genes reported in *P. aeruginosa* include sulfydryl variable (SHV), cefotaximase (CTX-M) and temoneira (TEM) types. Other less common ESBLs, such as Guiana extended spectrum (GES), Vietnamese extended-spectrum beta-lactamase (VEB) and *Pseudomonas* extended-spectrum beta-lactamase (PER) types, have now been isolated on several continents[5,6].

*P. aeruginosa* possesses an inducible AmpC $\beta$-lactamase that hydrolyzes almost all beta-lactam antibiotics including penicillins, cephalosporins, and monobactams and may be chromosomally- or plasmid-encoded. Although plasmid-mediated AmpC $\beta$-lactamases are less prevalent than ESBLs, they've been found in several areas of the globe. There are different types of plasmid-mediated AmpC $\beta$-lactamases: Ambler class C (ACC), AmpC type (ACT), MIR (Miriam hospital in Providence), cephamycins (CMY), moxalactam (MOX), cefoxitin (FOX) and DHA (Dhahran hospital in Saudi Arabia (DHA)[7,8]. For the first time, the DHA enzyme was identified in 1992 in the city of Dhahran, Saudi Arabia, in Salmonella enterica isolate from a stool sample of a patient with lung cancer. Consequently, $bla_{DHA}$-1 producers pathogens have been found worldwide[9,10].

The current study was performed to identify the occurrence of selective genotypes of $\beta$-lactamase (VEB, GES, and DHA) genes, as well as antibiotic resistance profiles in *P. aeruginosa* isolates obtained from hospitalized burn patients in this geographic region of Iran.

## 2. Materials and methods

### 2.1. Ethics

This research was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Ethic of Number: IR.AJUMS.REC.1396.1011).

### 2.2. Bacterial isolates

In this cross-sectional study, between March and August 2017, 93 non-duplicate clinical isolates of *P. aeruginosa* were collected from patients attending Taleghani Burn Hospital in Ahvaz. The specimens yielded these isolates included wound, blood, biopsy, urine, ear, stool and, catheter. These isolates related to different wards of hospitals, including ICU, Gynaecology, Andrology, Pediatrics, and Plastic surgery departemt. Bacterial identification was performed by using standard culture and biochemical methods as described previously[11].

### 2.2. Antimicrobial susceptibility testing

Antibiotic susceptibility was carried out using the Kirby-Bauer disk diffusion test on Mueller-Hinton agar (Merck, Germany) plates according to the Clinical and Laboratory Standards Institute recommendations[12]. The antimicrobial agents were ciprofloxacin (5 μg), pipracillin-tazobactam (100/10 μg), meropenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), imipenem (10 μg), and colistin-sulfate (10 μg)(Mast Co., UK). MDR *P. aeruginosa* was defined as isolate resistance to three or more classes of antimicrobial agents. *P. aeruginosa* ATCC 27 853 was used as a control strain.

### 2.3. DNA extraction and detection of $bla_{VEB}$, $bla_{GES}$ and $bla_{DHA}$ genes by PCR

DNA extraction was performed by boiling method as described previously[13]. The DNA quantity and quality were assessed using Nano Drop Spectrophotometer PROMO (Thermo Scientific, USA) and electrophoresis on 1.5% gel agarose, respectively. PCR amplification was conducted for the detection of $bla_{VEB}$, $bla_{GES}$ and $bla_{DHA}$ genes using the set of primers described previously (Table 1) [14]. PCR mix was prepared in a final volume of 50 μL, containing 10 × buffer 5 μL, dNTPs mixture (2.5 mmol/L) 4 μL, primer (25 μmol/L) 1 μL, template 1 μL, Taq enzyme 0.5 μL, ddH₂O 37.5 μL. Amplification involved an initial denaturation at 93 ℃ for 2 min, followed by 35 cycles of denaturation at 94 ℃ for 1 min, annealing at 55 ℃ for 1 min and extension at 72 ℃ for 1 min, with a final extension step at 72 ℃ for 5 min. PCR products (5 μL) were separated by electrophoresis (80 V, 40 min) using a 1% agarose gel (Sinaclon, Iran) in TBE buffer 1 × and then visualized using an ultraviolet light after staining with DNA safe stain (CinnaGen Co., Tehran, Iran). *Klebsiella pneumoniae* ORI-1 containing $bla_{GES}$ and *P. aeruginosa* containing $bla_{VEB}$ were used as controls.

**Table 1**

Primers used in this study.

| Primer | Oligonucleotide sequence (5' to 3') | Product size |
|--------|-------------------------------------|--------------|
| *VEB*-F | GCGGTAA TT TAACCAGA | 961 |
| *VEB*-R | GCC TATGAGCCAGTG TT | |
| *DHA*-F | AAC T TTC AC AGGTG TGCTGGGT | 405 |
| *DHA*-R | CCG TACGCAT AC TGGC T TTGC | |
| *GES*-F | ATGCGCT TCAT TCACGCAC | 846 |
| *GES*-R | C TA TT TGTCCG TGC TCAGG | |

## 3. Results

Out of 93 samples, the most common clinical specimen received in the microbiology laboratory was wound swabs [65 (69.9%)] followed by blood [14 (15.1%)] and biopsy [7 (7.5%)]. Forty-two (45.16%) patients were male and 51 (54.84%) were female. The hospital wards involved in the *P. aeruginosa* infection was ICU [48 (51.6%)], Gynaecology Department [22 (23.7%)], Plastic Surgery Department [12 (12.9%)], Andrology Department 8 (8.6%) and Pediatrics Department 3 (3.2%).

Patterns of antibiogram testing of *P. aeruginosa* isolates have been showed in detail in Table 2 and they indicated that the highest antibiotic resistance rates were recorded for gentamicin 94.6%, ciprofloxacin 93.5% and meropenem 90.3%, while all isolates (100.0%) were sensitive to colistin.

**Table 2**

Antibiotic susceptibility test results of *P. aeruginosa* isolated from different clinical specimens [$n$(%)].

| Antibiotics | Resistant | Intermediate | Susceptible |
|---|---|---|---|
| Gentamicin | 88(94.6) | 0 | 5(5.4) |
| Ciprofloxacin | 87(93.5) | 0 | 6(6.5) |
| Meropenem | 84(90.3) | 1(1.1) | 8(8.6) |
| Ceftriaxone | 82(88.2) | 3(3.12) | 8(8.6) |
| Amikacin | 82(88.2) | 1(1.1) | 10(10.8) |
| Pipracillin/tazobactam | 81(87.1) | 3(3.2) | 9(9.7) |
| Imipenem | 78(83.9) | 5(5.4) | 10 (10.8) |
| Ceftazidime | 28(30.1) | 3(3.2) | 62(66.7) |
| Colistin | 0 | 0 | 93(100) |

Eighty-three (89.24%) of the *P. aeruginosa* isolates were resistant to at least three classes of antibiotics ( β -lactams, fluoroquinolones, aminoglycosides) and classified as MDR. Moreover, there was a severe antibiotic resistance among the 48 isolates of *P. aeruginosa* collected from burn ICU ward in comparison with other wards isolates. According to PCR results, 16.1%(15), 9.7%(9) and 14.0%(13) of isolates carried $bla_{DHA}$, $bla_{VEB}$ and $bla_{GES}$ genes, respectively. It also revealed that the $bla_{VEB}$ gene was found to coexist with in 2 isolates (2.15%).

## 4. Discussion

A main colonizer in burn patients is *P. aeruginosa*, which may increase the risk of infections[15]. Higher resistance to antibacterial agents and ESBL production were observed in the MDR *P. aeruginosa* isolates from burn wards, where the patients were immunocompromised, and had a longer hospital stay, with a longer chemotherapy course and invasive therapeutic procedure[15,16].

The fewer incidence rates have been reported from Asian, European, and Latin America countries (16.4%-45.9%)[17-21]. In Iran, limited information is available on concerning the prevalence of MDR *P. aeruginosa*[22]. A recent meta-analysis and systematic review in Iran conducted by Vaez *et al.*[22] revealed that the prevalence of MDR *P. aeruginosa* was 58%. The highest and lowest prevalence of MDR *P. aeruginosa* reported in Tehran (100%), and Zahedan (16%), respectively. Also, the highest resistance rate was against ceftazidime (50%) and amikacin (50%).

Our findings revealed that the prevalence of MDR *P. aeruginosa* was greatly reduces treatment options. Farshadzadeh *et al.*[23] and Khosravi *et al.*[24] in Ahvaz reported that the MDR of *P. aeruginosa* isolates rapidly increased from 95.1% in 2010–2011 to 100% in 2016. In Asia, various studies have reported a significant increase in MDR *P. aeruginosa* rates in Pakistan[25], India[26], and Thailand[27], with resistant rates of 30%, 50%, and ≥20%, respectively, which is lower than our findings. In 2015, annual report of the European antimicrobial resistance surveillance network in thirty participated countries reported the rates of MDR *P. aeruginosa* isolates was < 50%[28].

In this study, highly resistance of *P. aeruginosa* to ciprofloxacin compared with 26.8% in Latin America[29] and 10%-32% in Europe[30,31]. de Almeida *et al.*[15] in a hospital-based survey in Brazil, observed the highest resistance rates of *P. aeruginosa* isolates against ciprofloxacin (94.3%), and gentamicin (88.6%), which is consistent with our findings. Fazeli *et al.*[32] showed that 21.56% and 39.21% of the *P. aeruginosa* strains were resistant to ciprofloxacin and gentamicin, respectively, which was lower to our results. Fluoroquinolones and aminoglycosides represent the highest resistance rate, and the increasing resistance against of *P. aeruginosa* isolates, respectively[15].

Various studies have been reported *P. aeruginosa* carrying $bla_{GES}$ gene[15,23,33]. One of the most important drug of choice for treatment of MDR *P. aeruginosa* infections is carbapenem[34]. Our results showed a highly carbapenem-resistance in *P. aeruginosa* (≥87.1%), and is significantly higher than European countries[28].

In current study, resistance to ceftazidime is higher than the percentage reported from European countries (0%-6.8%)[28]. However, ceftazidime-resistance (50.4%) of *P. aeruginosa* in different provinces of Iran is higher than the percentage reported from our study[22]. Additionally, in comparison with most European countries[28], resistance to other antibiotics is high. In current study, the prevalence of ESBL-producing *P. aeruginosa* strains was about 13.26%.

In several previous studies performed by Tavajjohi *et al.*[35] and Bokaeian *et al.*[36] in Kashan and Zahedan, it was 9.2% and 6.8%. Woodford *et al.*[37] in UK and Lim *et al.*[38] in Malaysia reported it as 3.7% and 4.2%, respectively, which was lower to our results. ESBL-positive *P. aeruginosa* strains that produce ESBLs are frequently isolated[39]. In the study conducted by Gad *et al.*[40] reported that 97% of *P. aeruginosa* isolates were beta-lactamase producers. In

the human isolates, the frequency of $bla_{DHA}$ in *P. aeruginosa* is 21.56%[32].

However, in the present study, we found that $bla_{DHA}$ and $bla_{GES}$ were most prevalent ESBLs in *P. aeruginosa*. The production of $bla_{GES}$ has been related to expanded spectrum cephalosporin resistance[41]. An analysis performed in a hospital in Riyadh, Saudi Arabia[42] between January to April 2010 indicated that 25 (16%) were ESBL producers *P. aeruginosa* isolates, with 5 (20%) carrying $bla_{GES}$ genes.

Another study from Riyadh conducted by Al-Agamy *et al.*[43] also reported $bla_{GES}$ in 5 (22%) of ESBL-positive *P. aeruginosa* isolates. The first report of $bla_{VEB}$ in Iran declared by Shahcheraghi *et al.*[44]. They displayed the frequency of $bla_{VEB}$, and $bla_{GES}$ among the ESBL isolates (MIC $\geqslant$16 mg/L) were 24%, and 0%, respectively. Our findings showed 9/93 (9.7%) of isolates carried $bla_{VEB}$ gene.

Additionally, in a hospitals-based survey[36] conducted for investigation the prevalence of ESBLs –producing *P. aeruginosa* isolated from various clinical samples (wound, tracheal tube, urine, blood, ear discharge) of patients hospitalized in Zahedan. Their results revealed that frequency of MDR and ESBL-positive of the isolates were 19/116 (16.37%), and 8/116 (6.89%), respectively, and 4 isolates (3.4%), amplified $bla_{VEB}$-1. Finally, our data indicated colistin presented an excellent activity against MDR *P. aeruginosa* isolates. The results of our study showed that most antibiotics used are unsuitable for the treatment of *P. aeruginosa* infections. Also, the frequency of ESBL producing *P. aeruginosa* isn't significant in our study. Performance of antimicrobial susceptibility testing confines indiscriminately uses of antibiotics and resistance increase and improve treatment programs.

## Conflict of interest statement

The authors report no conflict of interest.

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