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آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Antimicrobial Resistance Patterns and Prevalence of blaPER-1 and blaVEB-1 Genes Among ESBL-producing Pseudomonas aeruginosa Isolates in West of Iran

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Background: Pseudomonas aeruginosa is a leading cause of nosocomial infections worldwide. Resistance of P. aeruginosa strains to the broad-spectrum cephalosporins may be caused by extended-spectrum β-lactamases (ESBLs).

Objectives: The aim of this study was to determine the antimicrobial resistance patterns and prevalence of PER-1 and VEB-1 type genes among ESBL producing strains of P. aeruginosa.

Material and Methods: A total of 106 P. aeruginosa isolates were collected from two university hospitals in Hamadan, Iran, during 7-month study (2009). The antimicrobial susceptibility of isolates was determined by disc diffusion method and interpreted according to the clinical and laboratory standards institute (CLSI) recommendations. Production of ESBL was determined by combined disk test and presence of PER-1 and VEB-1 type ESBL genes was identified by PCR.

Results: The resistance against broad-spectrum cephalosporins and monobactams were: cefepime (97%), cefotaxime (92.5%) ceftazidime (92.5%) and aztreonam (27%). Ciprofloxacin (91.5%), imipenem (84.9%) and meropenem (82.1%) were the most effective anti-pseudomonas agents in this study. The results revealed that 88.7% of the isolates were multiresistant, 58.25% of those were ESBL positive. Sixteen (26.6%), 9 (15%) and 3 (5%) strains among ESBL-producing strains contained blaPER-1, blaVEB and blaPER-1+blaVEB, respectively.

Conclusions: This study highlighted the need to establish antimicrobial resistance surveillance networks for P. aeruginosa to determine the appropriate empirical treatment regimen. The high prevalence of multidrug resistance and production of ESBLs in P. aeruginosa isolates confirms the necessity of protocols considering these issues in the hospitals.

Keywords: Pseudomonas aeruginosa; Antimicrobial Drug Resistance; Beta-lactamase

1. Background

Pseudomonas aeruginosa is an important cause of nosocomial infections, including pneumonia, burn infection, urinary tract infections, meningitis and bacteremia. The infections can be particularly severe in patients with immune deficiency (1). Antibiotics have been used successfully for several decades, but resistance genes have emerged and disseminated particularly in the last few years (2).

Extended-spectrum β-lactamases (ESBLs) mediate resistance to various broad-spectrum cephalosporins, including cefotaxime, ceftiraxone, ceftriaxide, and aztreonam (3). These enzymes originally collected from Klebsiella pneumoniae and Escherichia coli and recently from P. aeruginosa (4-7). Most of the methods for detection of ESBLs are used in bacterial species such as Klebsiella and E. coli lacking chromosomal β-lactamase activity (8, 9). However, the detection of ESBL production in P. aeruginosa has some difficulties, because this bacterium not only has an inducible AmpC enzyme but also has an efflux-mediated resistance and a higher degree of impermeability than Enterobacteriaceae (10, 11). The PER-1 and VEB-1 type ESBLs belong to class A of β-lactamases and is associated with high level of resistance to cephems, monobactams and cefazidime (12, 13).

2. Objectives

The aim of this study was to determine the antibacterial resistance patterns and prevalence of PER-1 and VEB type ESBLs among P. aeruginosa isolated from patients in west of Iran.

3. Materials and Methods

3.1. Bacterial Isolates

A total of 106 isolates of P. aeruginosa recovered from...
various clinical specimens (Table 1) in Beasat Teaching Hospital at the Hamadan University of Medical Sciences during a 7-month study in 2009. The identification were carried out by colonial morphology, positive oxidase test, pigment formation; growth test at 42°C on nutrient agar, Gram staining and motility test.

Table 1. Distribution of P. seudomonas aeruginosa by Site of Isolation

| Source       | Isolates, No. (%) |
|--------------|-------------------|
| Burn wounds  | 55 (51.9)         |
| Trashes      | 20 (18.9)         |
| Urine        | 12 (11.1)         |
| Blood        | 7 (6.6)           |
| Feces        | 6 (5.7)           |
| Sputum       | 5 (4.7)           |
| CSF<sup>a</sup> | 1 (0.9)         |
| **Total**    | **106 (100)**     |

<sup>a</sup> Abbreviation: CSF, Cerebral spinal fluid

3.2. *Antibiotic Susceptibility Test*

Antibiotic susceptibility of the isolates was determined by standard disk diffusion method (14). The following antibiotics were used: gentamicin (30 µg), aztreonam (30 µg), meropenem (10 µg), imipenem (10 µg), amikacin (30 µg), tobramycin (30 µg), piperacillin (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), cefepime (30 µg) and cefotaxime (30 µg) (Himedia, India). P. aeruginosa ATCC 27853 was used as control. The results were interpreted according to the clinical and laboratory standards institute (CLSI) (15).

3.3. Phenotypic Detection of Beta-Lactamase

The isolates were tested for the ESBLs production by using combine disk test (CDT) as CLSI recommendations. CDT were performed on ceftazidime, cefotaxime, cefepime and aztreonam resistant strains by placing disks of ceftazidime, and cefotaxime (30 µg each) at a 20 mm distance from a disk containing ceftazidime-clavulanic acid (30/10 µg), cefotaxime-clavulanic acid (30/10µg) and cefepime-clavulanic acid (30/10 µg) (16). ESBL production was inferred when the cephalosporin inhibitory zones were expanded by the clavulanate.

3.4. *PCR Amplification*

PCR amplifications were done using specific primers for the β-lactamases PER-1, and VEB-1 genes, as described previously (17). PCR was performed for all ESBL-producers which their resistance to cephalosporins and phenotypic was identified by confirmatory tests. DNA was extracted by the boiling method as previously described (17). The DNA amplification program consisted of an initial denaturation (94 °C, 5 minutes) followed by 35 cycles of denaturation (94 °C, 60 seconds), annealing (50 °C for PER-1 and 55 °C for VEB-1, 60 seconds), extension (72 °C, 45 seconds) and a single final extension for 5 minutes at 72 °C. Reaction mixtures for PCR contained 1.5mM MgCl₂, 0.5 mM of each primer, 0.2 mM of dNTPs, 1 U of Taq polymerase, 1X PCR buffer and 2 µl of DNA. Primers PER-F (5'-AATTTGGGCTTAGGGCAGAA-3') and PER-R (5'-ATGAATAGTCATTATAAAAC-3') were used for blaPER-1; and VEB-F (5'-CGACTTCCATTTCCCGATGC-3') and VEB-R (5'-GGACTCTGCAACAATACGC-3') were used for blaVEB-1 amplification.

4. Results

4.1. *Antimicrobial Susceptibility Test*

Table 2 shows the antimicrobial susceptibility pattern of *P. aeruginosa* strains. Ciprofloxacin (91.5%), imipenem...
(84.9%) and meropenem (82.1%) were the most active antimicrobial agents followed by ofloxacin (67.9% susceptibility). Aztreonam, a monobactam, and amikacin showed antibiotic activity against 66% of the strains. Susceptibility to the cephalosporins was reduced to 37.7% for cefazidine, followed by cefotaxime (9.4%) and Cefepime (0.9%).

ESBL Production: Among 106 isolates, 94 (88.7%) were multidrug resistant and 60 (58.3%) were putative ESBL producers using phenotypic confirmatory tests (Table 3). Sixteen (26.6%), 9 (15%) and 3 (5%) strains among 60 ESBL-producing strains had blaPER-1 (Figure 1), blaVEB-1 (Figure 2), and blaPER-1-blaVEB related genes, respectively (Table 4).

### Table 3. Susceptibility Pattern to Antimicrobial Agents in ESBLs and non ESBLs-producing P. aeruginosa Strains

| Antimicrobials | ESBL Positive (n=60) | ESBL Negative (n=46) |
|---------------|----------------------|----------------------|
|               | Resistant, No. (%)    | Susceptible, No. (%)  | Intermediate, No. (%) |
| Amikacin      | 29 (48.3)            | 30 (50)              | 1 (1.7)               |
| Ceftazidime   | 40 (66.7)            | 18 (30)              | 2 (3.3)               |
| Aztreonam     | 25 (41.7)            | 32 (53.3)            | 3 (5)                 |
| Cefepime      | 58 (96.7)            | 0                    | 2 (3.3)               |
| Cefotaxime    | 41 (68.3)            | 6 (10)               | 13 (21.7)             |
| Ciprofloxacin | 5 (8.3)              | 50 (83.3)            | 5 (8.3)               |
| Gentamicin    | 32 (53.3)            | 28 (46.7)            | 0                    |
| Imipenem      | 7 (11.7)             | 45 (75)              | 8 (13.3)              |
| Meropenem     | 13 (21.7)            | 44 (73.3)            | 3 (5)                 |
| Ofloxacin     | 10 (50)              | 29 (48.3)            | 1 (1.7)               |
| Piperacillin  | 54 (90)              | 6 (10)               | 0                    |
| Tobramycin    | 31 (51.6)            | 28 (46.7)            | 1 (1.7)               |

### Table 4. Association of Antimicrobial Resistance Pattern and ESBL Genotypes

| Antimicrobials | PER-1:16 (26.6%) | VEB-9 (15%) |
|---------------|------------------|-------------|
|               | Resistant, No. (%) | Susceptible, No. (%) | Intermediate, No. (%) | Resistant, No. (%) | Susceptible, No. (%) | Intermediate, No. (%) |
| Amikacin      | 10 (62.5)         | 5 (31.25)    | 1 (6.25)              | 5 (55.5)          | 2 (22.2)          | 2 (22.2)              |
| Ceftazidime   | 12 (75)           | 4 (25)       | 0                     | 9 (100)           | 0                | 0                    |
| Aztreonam     | 10 (62.5)         | 4 (25)       | 2 (12.5)              | 9 (100)           | 0                | 0                    |
| Cefepime      | 16 (100)          | 0            | 0                     | 9 (100)           | 0                | 0                    |
| Cefotaxime    | 87.5 (14)         | 0            | 2 (12.5)              | 9 (100)           | 0                | 0                    |
| Ciprofloxacin | 2 (12.5)          | 13 (81.25)   | 1 (6.25)              | 2 (22.2)          | 6 (66.6)         | 1 (11.1)              |
| Gentamicin    | 10 (62.5)         | 6 (37.5)     | 0                     | 6 (66.6)          | 3 (33.3)         | 0                    |
| Imipenem      | 5 (31.25)         | 5 (31.25)    | 6 (37.5)              | 1 (11.1)          | 6 (66.6)         | 2 (22.2)              |
| Meropenem     | 7 (43.75)         | 8 (50)       | 1 (6.25)              | 6 (66.6)          | 2 (22.2)         | 1 (11.1)              |
| Ofloxacin     | 11 (68.8)         | 5 (31.25)    | 0                     | 8 (88.8)          | 1 (11.1)         | 0                    |
| Piperacillin  | 16 (100)          | 0            | 0                     | 9 (100)           | 0                | 0                    |
| Tobramycin    | 10 (62.5)         | 6 (37.5)     | 0                     | 6 (66.6)          | 3 (33.3)         | 0                    |
5. Discussion

*P. aeruginosa* has a high resistance to antibiotics and is a common cause of morbidity and mortality in hospitalized and immunocompromised patients (18). Treatment of *P. aeruginosa* infections is complicated by the inherited and acquired resistance to the most of commonly used antimicrobial agents (19). The results from this study showed the high resistance of *P. aeruginosa* to most of the used antimicrobial agents. It was also demonstrated that the prevalence of antibiotic resistance of the isolates was very high in comparison to other studies and most of *P. aeruginosa* isolates (88.7%) were multi-drug resistant (resistant to ≥ 3 different antibiotic classes) (20-25).

The prevalence of ESBL-producing *P. aeruginosa* isolates in this study was also higher than other investigations (5, 20, 26, 27). Among 60 ESBL-positive strains, 16 (26.6%), 9 (15%) and 3 (5%) contained *PER-I*, *VEB-I* and *PER-I-VEB-I* genes, respectively. These results indicated that the prevalence of *VEB-I* gene in our area, is higher than Turkey and Korea, but the prevalence of *VEB-I* and *PER-I* genes, is lower than in Thailand (94.4% *blaVEB-I*) and Italy (34.61% *blaPER-I*) (5, 13, 26). Data on the prevalence of ESBL-producing *P. aeruginosa* strains in our area is limited. In the study performed by Shahcheraghi and colleges on *P. aeruginosa* isolates in Tehran, the rate of *blaVEB* and *blaPER* ESBLs were reported 24% and 17%, respectively, that was similar to our results (28). The high prevalence of *PER-I* and *VEB-I* indicated the high resistance to penicillins, ceftazidime and cefotaxime, as reported by other studies (26, 29).

This is the first report about the presence of these enzymes in *P. aeruginosa* isolates from west of Iran. It has shown that ESBL production in strains of *P. aeruginosa* can greatly complicate the clinical management of infection if advanced care is not taken. However, further studies are needed to determine other ESBL-types in *P. aeruginosa* strains in this area and their role in resistance to other antibiotic classes. The results of this study emphasizes on the need for a surveillance network to monitor the trends and emerge of new resistance mechanism in *P. aeruginosa* from different geographic regions. Therefore, the improvement in antibiotic prescription policies and infection control programs are of high necessity to prevent the spread of such resistant infectious agents.

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Authors’ Contribution

All authors contributed in the research equally.

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