

Antibiotic resistance patterns in *Pseudomonas aeruginosa* isolates producing ESBL in Zahedan 2014

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Abstract

Introduction:

Pseudomonas aeruginosa is one of the most important causes of opportunistic infections. As *Pseudomonas aeruginosa* is prevalent, its resistance to antibiotics is especially significant. This study aimed to determine the prevalence of antibiotic resistance in extended spectrum of beta lactamase (ESBL) strains.

Material and Method:

From a total of 499 samples of urine cultures, blood cultures, wounds, pulmonary secretions and other samples, 88 isolates of *P. aeruginosa* were collected in hospitals of Zahedan in 2014. Initially, ESBL-producing strains were identified and their antibiotic resistance was determined using disk diffusion method according to CLSI standards.

Results:

Of the 88 isolates of *Pseudomonas aeruginosa*, 51 isolates (96.62%) were beta-lactamase producing, with the highest resistance to Cefoxitin (30µg) and the least resistance to Aztreoname (30µg). Most ESBL strains were separated from women, that is, a significant relationship was observed between the presence of resistant strains and gender of patients admitted to hospital. Furthermore, a significant relationship was observed between resistance to some antibiotics and strains of *Pseudomonas aeruginosa*. However, no relationship was found between isolates taken from patients in various wards, types of sample and the isolated bacterium ($P \leq 0.05$).

Conclusion:

Because the prevalence of resistant strains of *Pseudomonas aeruginosa* is high, it is necessary to prescribe medication for and treat infections with greater care.

Keywords: *Pseudomonas Aeruginosa*, Beta Lactamase, ESBL, Antibiotic Resistance

Introduction

Some hospital bacteria always pose a risk to inpatients and hinder recovery by creating secondary infections (1). Antibiotic resistance among hospital bacteria and its spread has incurred many difficulties for the treatment process (2). *Pseudomonas aeruginosa* is one of the non-fermenting

gram-negative bacilli with a high prevalence in health-care settings (3). The presence of this bacterium is increasing in inpatients, especially those who stay longer periods (4, 5). However, most of these bacteria have become resistant to various antibiotics making them Multiple Drug

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Resistant (MDR) (6, 7). Antibiotics used for treating *Pseudomonas* infections include broad-spectrum penicillin (carbenicillin, ticarcillin, and piperacillin), broad-spectrum cephalosporin (ceftazidime and cefepime), carbapenems, aminoglycosides, fluoroquinolones, and aztreonam. The *Pseudomonas aeruginosa* isolates that are resistant to these factors are increasing (5 and 6). Among different types of drug resistance, resistance to beta-lactam antibiotics has posed a serious concern in the treatment of bacterial infections. Various genes contribute to the resistance in *Pseudomonas aeruginosa* that are carried both on chromosomes and plasmids (8). These genes cause a variety of resistance in the gram-negative bacilli by affecting different parts. One of them is the expression of beta-lactamases that directly affect beta-lactam antibiotics (9). Plasmid-based genes are usually located on plasmids and can easily be transmitted among species, strains, and even different genera, which shows the importance of examining the dependence of the genes (10). Beta-lactamases are bacterial enzymes that can hydrolyze beta-lactam antibiotics and make these compounds inactive (11, 12). Over the past 20 years, a large number of beta-lactam antibiotics were produced, but the bacteria have also produced new beta-lactamases, which have led to the ineffectiveness of these antibiotics. It is believed that the overuse of these antibiotics in the treatment of patients leads to the production of various types of broad-spectrum beta-lactamase by the bacteria (13, 14). There are no precise and comprehensive definitions for these enzymes, but in summary, broad-spectrum beta-lactamases are a group of enzymes that protect beta-lactamase-producing bacteria against penicillin, cephalosporin (first,

second, and third generations) and aztreonam, but are not able to hydrolyze cephamycins and carbapenems. In fact, these enzymes are mutations resulting from the replacement of one or more amino acids in the amino acid sequence of primary beta-lactamases (SHV-1, TEM-1, TEM-2). Although the amino acid replacement rate is less than 2%, the very small change is enough to transform the active site of the enzyme (15). The first broad-spectrum beta-lactamase gene known as SHV-2 was obtained from an isolate of *lebsiella ozaenae* (Germany, 1983), which was in fact derived from SHV-1 (16). The widespread use of beta-lactam antibiotics and the prevalence of vast resistance to these antibiotics in Iran has become one of the most important health concerns in recent years. Various studies carried out in different years have shown that beta-lactam resistance has expanded. Fooladi et al. reported that the prevalence of beta-lactam resistance in different strains of *Pseudomonas aeruginosa* and ESBL enzyme producer in 2010 was below 40% in Tehran, Iran (17). That is while Taghvayi et al. reported a 50% increase in this amount in a study in 2013 in Arak, Iran (18). This amount reached 70% in a study by Torabi et al. in 2016 in Isfahan, Iran (19). The high prevalence of *Pseudomonas aeruginosa* producing ESBL enzymes is always one of the dangers that puts hospitalized patients at risk of contamination. Therefore, it is important to review and provide an overview of the prevalence of broad-spectrum beta-lactam resistance in clinical isolates of *Pseudomonas aeruginosa*. The aim of this study was to investigate the detection of *Pseudomonas aeruginosa* producing broad-spectrum beta-lactamases and also to determine the antibiotic resistance pattern in these isolates.

Materials and Methods

Sample collection

In this descriptive cross-sectional study, 499 samples including urine, blood, wound discharge, mucus, tracheal tube, chest tube, and catheter end were collected by convenience sampling from patients admitted in different wards and outpatients of educational hospitals in Zahedan, Iran, during a 9-month period from December 2014 to September 2015. The inclusion and exclusion criteria in this study were patients who were suspected of having different bacterial infections. In all stages of the study, the identity of patients was recorded confidentially, and none were entered in the main reports. Then, the collected samples were inoculated with 10% glycerol inside the plastic microtubes containing BHI transport medium (Merck Germany) and transferred to the Microbiology Laboratory of the Faculty of Medicine of Zahedan University of Medical Sciences for precision diagnostic tests. Samples were stored at -20 °C until the tests were carried out.

Isolation, identification, and culture of the bacteria

All samples obtained to determine the definite identity were cultured on the Cetremide (Merck Germany) agar medium and incubated at 42 °C. Then the obtained isolates were investigated in terms of oxidase production, OF test and growth in TSI and Simon Citrate Agar culture media (Merck, Germany). In addition, isolates underwent catalase, SH₂ and gas production, pigment production, indole and methyl red, and movement in SIM media tests. After the final identification of the bacterial genus and species by reserved culture in Trypticase Soy Broth (Merck Germany) culture media, they were stored

with 15% glycerol in the freezer at -20 °C for the next steps (20).

Determination of strains producing broad-spectrum beta-lactamases

The disk-diffusion method with clavulanic acid according to the CLSI guidelines was used to determine the strains of *Pseudomonas aeruginosa* producing broad-spectrum beta-lactamase enzymes. First, the identity of the isolates was determined, a dilution of 0.5 McFarland was prepared, and they were lawn cultured on the surface of the plate containing the Mueller-Hinton (Merck Germany) agar medium with a thickness of 4 mm. Then the ceftazidime 30µg disks were placed within 30 mm of ceftazidime/clavulanic acid - 30/10 µg disc, and cefpodoxime disc 30 µg disk within 30 mm of cefpodoxime/clavulanic acid 10 µg disk on the medium (all disks from MAST England). The diameter of the holes was checked in accordance with the CLSI standard. A diameter of a no-growth halo around each of the clavulanic acid disks greater than or equal to 5 mm against the clavulanic acid disks indicates the presence of ESBL in the obtained isolates. The *Klebsiella pneumoniae* ATCC700603 was used a positive control sample and the *Pseudomonas aeruginosa* ATCC27853 as a negative control sample.

Antibiotic resistance pattern of ESBL strains of *Pseudomonas aeruginosa*

In order to determine the antibiotic sensitivity pattern of the isolates, the primary suspension of imipenem (12 µg), meropenem (12 µg), aztreonam (32 µg), ceftriaxone (32 µg), cefepime (32 µg), ceftazidime (32 µg), cefotaxime (32 µg), cefpodoxime (12 µg), gentamicin (32 µg), amikacin (12 µg), ciprofloxacin (5 µg), norfloxacin (12 µg), and levofloxacin (5

µg) antibiotic disks (all from MAST, England) were prepared and placed on the intended medium for antibiogram. The results were investigated according to CLSI guidelines after 18 to 24 hours of incubation at 37°C.

Statistical analysis

Data was analyzed using SPSS V.16 statistical software in this study. The relationship between isolates producing broad-spectrum beta-lactamases was evaluated based on the sample type and gender of patients using the Chi square test. The resulted data was analyzed using descriptive statistics (absolute frequency, relative frequency and mean). The statistical significance level was considered as $P < 0.05$.

Results

In this study, 499 clinical samples were collected from Ali ibn Abi Talib Hospital over a period of 9 months. After initial experiments and biochemical tests, 88 isolates were identified as *Pseudomonas aeruginosa*. Of the 88 clinical samples of *Pseudomonas aeruginosa* isolated from the educational hospitals of Zahedan, Iran, 61 samples were taken from Ali ibn Abi Talib

hospital, 23 from Khatam-ol-Anbia hospital and 4 from Bu-Ali hospital. A total of 61 isolates (69.31%) were obtained from women and 27 isolates (30.68%) from men (Table 1, Figure 1). The highest frequency of the isolates from different hospital wards was that of the pediatric and intensive care units, with 27 (30.68%) and 25 isolates (28.4%), respectively. The neonatal ward was ranked third with 14 samples (15.9%) (Figure 1).

Most samples isolated for *Pseudomonas aeruginosa* bacterial contamination were from urine and blood culture (Figure 2). In terms of antibiotic resistance patterns, more than 90% of isolates were resistant to cefoxitin and cefpodoxime. The isolates had the least resistance to aztreonam and ciprofloxacin antibiotics (Table 2). Of the 88 studied isolates, 51 (57%) had the ability to produce beta-lactamase enzymes (Table 2). According to the Chi-square test, there was a significant relationship between the gender of the patients and the distribution of broad-spectrum beta-lactamase enzymes ($P < 0.05$). All of the antibiotics examined in this study were somehow related to the presence of broad spectrum beta-lactamase enzymes (Table 2).

Table 1: Frequency of clinical isolates of *Pseudomonas aeruginosa* based on patient's gender

Gender	Pseudomonas aeruginosa (n=88)		P-value
	ESBL isolates Number (percent)	Non-ESBL isolates Number (percent)	
Male	14 (51.85)	13 (48.15)	0.019
Female	37 (60.65)	24 (39.35)	

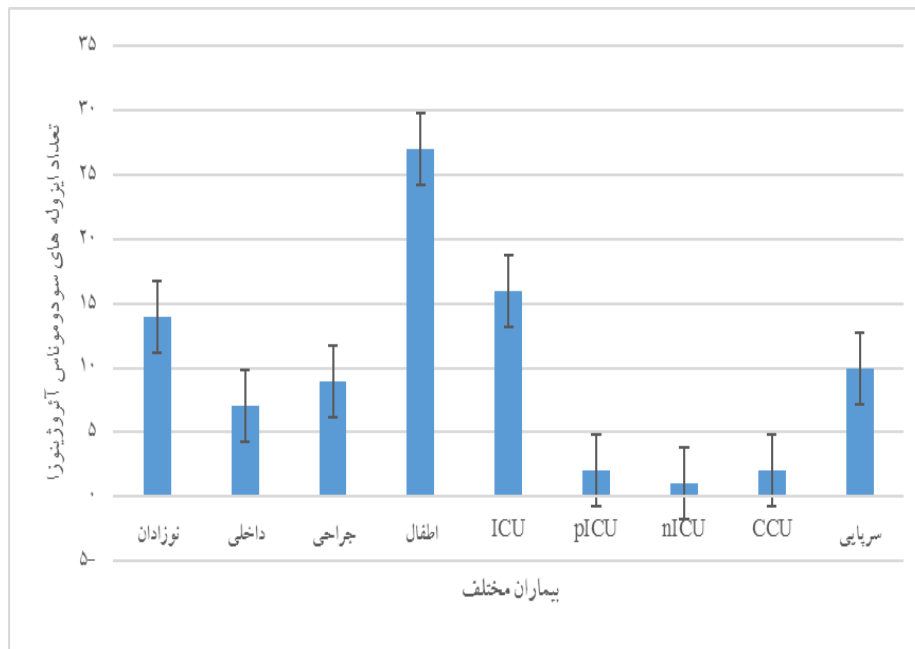


Figure 1: Frequency of clinical isolates of *Pseudomonas aeruginosa* based on patients in different hospital wards

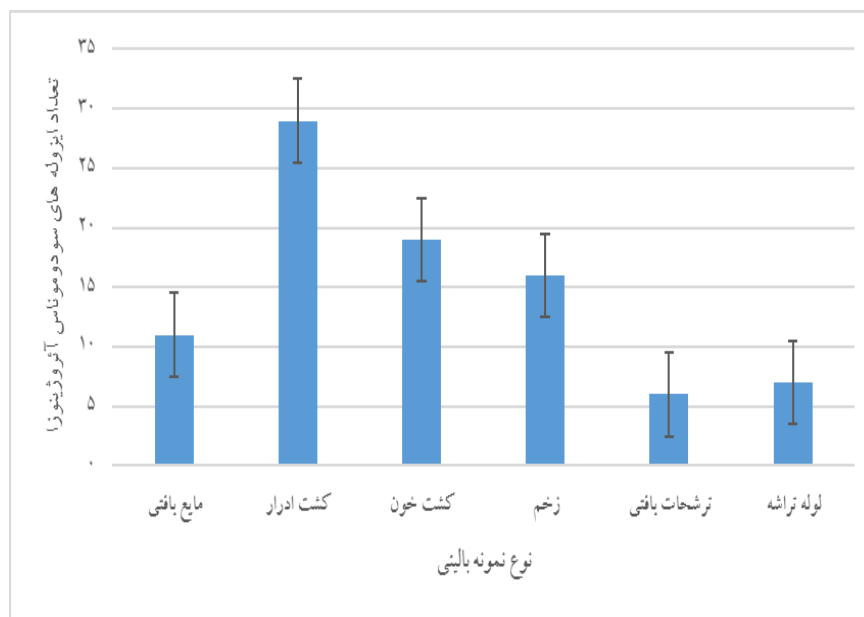
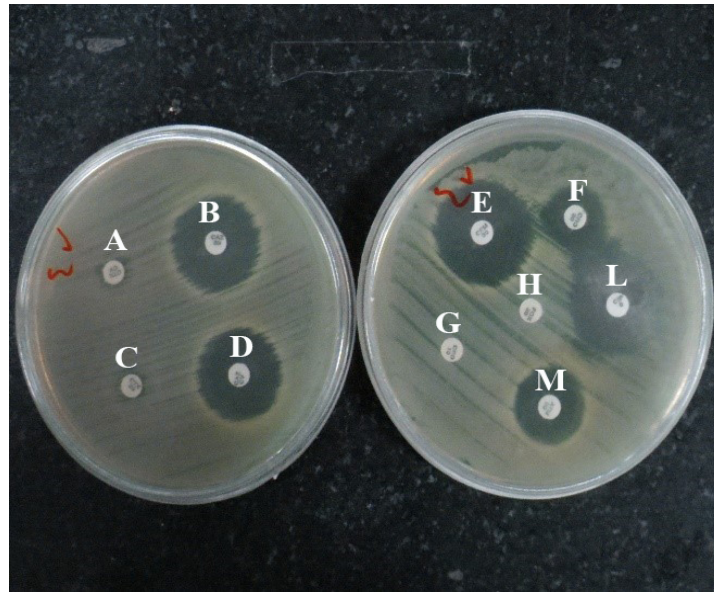


Figure 2: Frequency of clinical isolates of *Pseudomonas aeruginosa* based on the type of the clinical sample



Picture 1: Disk diffusion test to isolate ESBL-producing strains among *Pseudomonas aeruginosa* isolates. *Klebsiella pneumoniae* ATCC700603 as Positive control and *Pseudomonas aeruginosa* ATCC27853 as Negative control. A: Ceftazidime; B: Ceftazidime-Clavulanic acid; C: Cefpodoxime; D: Cephupodoxime-clavulanic acid; E: Cefotetan; F: Aztreonam; G: Cefotaxime; H: Cefoxitin; L: Ceftriaxone; M: Ciprofloxacin.

Table 2: Antibiotic resistance spectrum of ESBL-producing and non-ESBL strains in clinical samples of *Pseudomonas aeruginosa*

Antibiotics	Pseudomonas aeruginosa (n=88)						P.Value
	ESBL (n=51)			Non-ESBL (n=27)			
	Resistant Number (percent)	Semi- sensitive Number (percent)	Sensitive Number (percent)	Resistant Number (percent)	Semi- sensitive Number (percent)	Sensitive Number (percent)	
Cefoxitin	49 (96)	2 (4%)	0 (0.0)	0 (0.0)	3 (14)	24 (88)	0.032
Ceftazidime	8 (15)	0 (0.0)	43 (85)	0 (0.0)	2 (8)	25 (92)	0.041
Ceftriaxone	27 (52)	16 (31)	9 (17)	1 (4)	1 (4)	25 (92)	0.012
Cefotaxime	49 (96)	2 (4)	0 (0.0)	0 (0.0)	2 (8)	25 (92)	0.019
Cefotetan	15 (30)	2 (3)	34 (67)	0 (0.0)	6 (23)	21 (77)	0.039
Cefpodoxime	50 (99)	1 (1)	0 (0.0)	0 (0.0)	2 (8%)	25 (92)	0.05
Aztreonam	4 (8)	5 (9)	42 (83)	0 (0.0)	0 (0.0)	27 (100)	0.029
Ciprofloxacin	4 (8)	3 (7)	43 (84)	0 (0.0)	4 (15)	23 (85)	0.038

Discussion

Beta-lactamases are an important defense system for bacteria against beta-lactam antibiotics. This resistance system is also abundant in both gram-negative and gram-positive bacteria. Resistance to beta-lactam

antibiotics emerged since the use of these antibiotics in the treatment of bacteria-infected infections became widespread, and after a short time, extensive resistance to this group of antibiotics was observed in

gram-negative and gram-positive bacteria. The active presence of *Pseudomonas aeruginosa* in a wide range of patients admitted to hospitals can be attributed to the unique characteristics of this bacterium. In the present study, the presence of this bacterium was also found in patients admitted in different hospital wards, such that they were observed in blood and urine culture samples. This indicates the role of *Pseudomonas aeruginosa* in urinary tract diseases. The results regarding broad-spectrum of these bacteria were consistent with the studies of Tavajjohi et al. in 2010 in Kashan and Hashemi et al. in 2015. In both studies, *Pseudomonas aeruginosa* had the highest prevalence in urine and blood samples' culture (21, 22). The prevalence of *Pseudomonas aeruginosa* and also strains that produce broad-spectrum beta-lactamase enzymes in women indicates the active role of this bacterium in urinary tract infections in women. Cefoxitin resistance is one of the main criteria for determining the strains of *Pseudomonas aeruginosa* in the ESBL group. In this study, the prevalence of antibiotic resistance was investigated in 88 isolates of *Pseudomonas aeruginosa*. The prevalence of resistance to ceftazidime, ciprofloxacin, cefotetan, cefoxitin, cefpodoxime, ceftriaxone, and aztreonam antibiotics was 15%, 8%, 30%, 96%, 99%, 52%, and 8%, respectively. High levels of resistance in cefoxitin and cefpodoxime antibiotics in this study due to the detection of ESBL strains is important. The rate of resistance to ceftazidime and ciprofloxacin antibiotics in this study was 15% and 9%, respectively; while it was 20.6% and 59.8% in the study of Ebino et al. on *Pseudomonas aeruginosa* strains, indicating less antibiotic resistance in the present study (23). Studies conducted in different countries show increased resistance to ceftazidime from

9% to 15% and 32% over different periods, which is consistent with the present study (27-24). Resistance to ciprofloxacin in different countries and time periods has been 9%, 23% and 97%, some of which were close to the present study (28, 29). However, resistance to ciprofloxacin in some studies was higher than the present study. For example, in a study by Behera et al., resistance to this antibiotic was over 75% (30).

Resistance to beta-lactam has been reported in many studies with a high prevalence in *Pseudomonas aeruginosa* strains. This is while the presence of ESBL strains also shows an increasing incidence. In this study, 96% and 99% resistance of cefuroxime and cefpodoxime antibiotic were consistent with reports by Upadhyay et al. (2009), which revealed a high prevalence of ESBL strains of *Pseudomonas aeruginosa*. In this study, resistance to cefoxitin and frequency of ESBL strains was 100% (31). In terms of resistance to ceftriaxone, the results of the present study were almost identical with those of Fazeli et al. (32); while Rubin et al. reported 39% resistance, which is lower than the amount obtained in this study (33). In the present study, cefotaxime resistance was 96%, compared to 26% in other reports (34). Also, in a study by Rabani et al. on antibiotic resistance of *Pseudomonas aeruginosa*, resistance to cefotaxime was about 10%, which is much less than the result of this study (34). The 30% and 8% resistance to cefotetan and aztreonam antibiotics in this study were much less than that of Lee et al. in Korea which was more than 60% (35).

Conclusions

Regarding the relative prevalence of broad-spectrum beta-lactamase enzymes in

clinical strains of *Pseudomonas aeruginosa* and a high prevalence of some types of antibiotic resistance, a more focused and targeted monitoring of antibiotic use in hospitals is needed. On the other hand, the transfer of beta-lactam resistance among strains and even other species can lead to problems in the treatment of simple and controlled infections. In addition, the prevalence of several antibiotic-resistant strains and the role of these strains in the emergence of some important infections should be considered. Due to the cross-sectional nature of this study and some limitations, including the number and type of samples, as well as the sensitivity of the

subject, larger studies with longer time periods are needed.

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Conflict of interest

The authors have no conflict of interest with regard to the compilation or publication of this study.

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