



# Frequency of Drug Resistant *Pseudomonas Aeruginosa* Producing Extended Spectrum Beta-Lactamases in Zanzan Hospitals, Iran

Amini M, Rahmati H, Tanhaei S and Pournajafi A\*

Department of Biology, Islamic Azad University, Iran

\*Corresponding author: Amir Pournajafi, Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran; Email: amirpournajafi1990@gmail.com

## Research Article

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## Abstract

**Background and Aim:** *Pseudomonas aeruginosa* is one of the most important pathogenic bacteria causing nosocomial infections that is inherently resistant to many antibiotics. Therefore, the present study was performed to investigate the susceptibility and antibiotic resistance patterns of ESBL-producing *P. aeruginosa* strains isolated from patients referred to Zanzan hospitals.

**Materials and Methods:** In this descriptive-analytical study of the study of 300 cases of urinary tract infection in Zanzan medical centers in 2019, 100 isolates of *P. aeruginosa* were identified by standard bacteriological methods. Antibiotic susceptibility of the isolates was determined by disk diffusion method and ESBL-producing isolates were identified by combined disk method.

**Results:** The most resistant to ampicillin (75%) and tetracycline (48%) were the most sensitive to amikacin (90%) and nitrofurantoin (87%), respectively. A total of 49 samples were identified as the final ESBL producer.

**Conclusion:** Given the high percentage of resistance to third generation cephalosporins, careful antibiograms and avoidance of overuse of antibiotics in infections caused by ESBL-producing organisms is an inevitable necessity.

**Keywords:** Extended-Spectrum Beta-Lactamases; *P. aeruginosa*; Urinary Tract Infection; Antibiotic Resistance

**Abbreviations:** DNA: Deoxyribonucleic Acid; ESBL: Extended spectrum  $\beta$ -lactamases; UTI: Urinary Tract Infection; MDR: Multidrug-Resistant.

## Introduction

Since sulfanamides and penicillins have come into the field, a new opportunity has emerged in the treatment of diseases. In the early days of the use of these drugs, numerous epidemics subsided. However, infections caused by infectious organisms remain a serious problem [1]. There are two important mechanisms through which increased resistance to antibiotics and other drugs. The former is due to spontaneous mutation, in the sense that the mutation occurs at a frequency of about 10 to 5%, altering the susceptibility to the drug, and the drug acts only as a selective agent and promotes the survival of resistant organisms among organisms [2]. The second mechanism of genetic exchange

resistance is the genetic information that controls the drug resistance of the bacterium to both chromosomal DNA and extra-chromosomal DNA, i.e., plasmids, through the transformation, conjugation, and transduction of a (resistant) cell transferred to another (sensitive) cell. Hospitalized patients are exposed to nosocomial infections, especially with multidrug-resistant organisms, and are one of the most important contributors to nosocomial infections and as a result mortality from Gram-negative bacilli infection. Since antibiotics, especially in ICU wards, are usually empirically due to the rush of treatment [3,4].

ESBLs, with the power to hydrolyze the wide range of beta-lactam antibiotics used in clinics, pose a serious problem in medicine. Bacteria producing ESBLs with class C cephalosporinases encoded by the AmpC chromosomal gene have been the most common mechanism of resistance to Gram-negative bacilli against this antibiotic [5-7].

Since the second half of the 1980s, with the reporting of variants of ESBLs and the wide geographical distribution of these enzymes, their release has been discussed as an epidemiological phenomenon [8,9]. Urinary tract infections are one of the most common human-acquired infections. In the United States, urinary tract infections are the second most common cause of upper respiratory tract infections, and many men and women are infected throughout their lives. Different factors such as age, sex and immune system influence the prevalence of UTI [10-13]. *P. aeruginosa* is a pathogenic and opportunistic bacterium that is a major contributor to the mortality of immunocompromised patients.

The intrinsic resistance to antimicrobial agents in this bacterium makes the treatment of infections worse [14]. Lipopolysaccharide, pili and polar flagella in this opportunistic pathogen bind the bacterium to the cell membrane and play an important role in pathogenicity of this bacterium [15]. Beta-lactam is a good drug for the treatment of *Pseudomonas aeruginosa*. However, after some time, some bacteria become resistant to this antibiotic by producing beta-lactamase enzymes [16]. Nosocomial infections are one of the major medical problems in developed and developing countries that cause infectious diseases to spread in society [17]. In recent years, attention has been paid to nosocomial infections, as there were only eighty-eight thousand deaths from nosocomial infections worldwide in 1995 [18].

*P. aeruginosa* is one of the most common causes of nosocomial infection, especially in burn wounds. Infection with this bacterium can lead to septicemia, pneumonia, meningitis and other fatal diseases [19]. *Pseudomonas* has an inherent resistance to a wide range of antimicrobial and antiseptic substances, such as ammonium, hexachlorophen, soaps and iodinated solutions [20]. The aim of this study was to evaluate clinical isolates of *P. aeruginosa* collected from hospitals in Zanjan in order to present a sensitivity pattern to experimental antibiotics and phenotypic study of ESBLs producing isolates.

## Materials and Methods

In this descriptive study, 300 urine samples were collected from outpatients and inpatients of Zanjan hospitals during three months from November to December of 2019 and were cultured on EMB (Merck Company, Germany). Then

routine biochemical tests were performed on the colonies. Also, standard strain of *P. aeruginosa* PTCC 17589 was used as quality control. Combined disk test was used to evaluate ESBL producing strains. This experiment was performed using ceftazidime (30µg), cefotaxime (30µg), ceftazidime / clavulanic acid (30µg / 10µg) and Cefotaxime / clavulanic acid (30µg / 10µg). For this test, the isolates under study were suspended in physiological saline and their turbidity was adjusted to 0.5 McFarland standards. Then, cotton swabs were cultured in Muller Hinton Agar medium in three directions and after 24 h incubation at 37°C, the growth zone diameter was recorded around the discs. Then, cotton swabs were cultured in Muller Hinton Agar medium in three directions and after 24 h incubation at 37°C, the growth zone diameter was recorded around the discs.

Increase in diameter of more than 5 mm in diameter growth zone around ceftazidime / clavulanic acid (30µg / 10µg) and cefotaxime / clavulanic acid (30µg / 10µg) discs compared to ceftazidime (30µg) and cefotaxime (30µg) discs) Indicates ESBL positive of sample and recorded as positive result. In this experiment *E. coli* ATCC 25922 was used as negative control and *E. coli* ATCC 35218 as positive control. After confirmation of the presence of *P. aeruginosa*, the antibiogram for the samples was recommended by the Clinical and Laboratory Standards Institute. Antibiotic discs used were tetracycline (30 µg), nitrofurantoin (300 µg), ceftazidime (30 µg), ampicillin sulbactam (10 µg), amoxicillin (25 µg), amoxicillin-clavulanic (25 µg), nalidixic acid (30 µg), amikacin (30 µg), tobramycin (10 µg), imipenem (10 µg), ciprofloxacin (5 µg) and gentamicin (10 µg), (Media Companies). After 24-hour incubation at 37°C using a ruler, the growth zone around the discs was measured and compared to the CLSI standards. According to the manufacturer's instructions, the results were based on sensitivity (S) and resistance (R) was reported and semi-susceptible halos were recorded as (I).

## Results

In this study, 300 urine samples were collected from 100 (33.33%) *P. aeruginosa*. 65 specimens were isolated from the inpatients ward and 35 samples from the outpatients ward. Based on the results of the combined disk test, 49 samples were identified as final ESBL producers. The results of the sensitivity test against the 12 selected antibiotics are shown in Table1.

Antibiotics	Resistance	Intermediate	Sensitive
Tetracycline	48	10	43
Nitrofurantoin	9	4	87
Ceftazidime	26	29	45

Ampicillin Sulbactam	75	10	15
Amoxicillin	45	16	39
Amoxicillin-Clavulanic	47	0	53
Nalidixic Acid	33	18	49
Amikacin	10	0	90
Tobramycin	20	0	80
Imipenem	22	4	74
Ciprofloxacin	33	3	64
Gentamicin	5	10	85

**Table 1:** Frequency of antibiotic resistance pattern of *P. aeruginosa* strains isolated from urinary tract infections.

## Discussion

Extended -spectrum beta-lactamases are a group of beta-lactamase enzymes that are of particular importance in antimicrobial therapy. The rate of ESBL production among Enterobacteriaceae varies worldwide [21]. Resistant *P. aeruginosa* strains are a serious public health threat that has raised a great deal of concern in the medical community, particularly in the treatment of multidrug-resistant infections (MDR), in immunocompromised individuals [22]. In the present study, from 100 *P. aeruginosa* isolates, 65 samples from the inpatient ward and 35 samples from the outpatients ward were isolated. Based on the results of the combined disk test, 49 samples were identified as final ESBL producers. The highest resistance to ampicillin (75%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (87%), respectively. The most resistant to ampicillin (75%) and tetracycline (48%) were the most sensitive to amikacin (90%) and nitrofurantoin (87%), respectively.

The results showed that there was a significant relationship between the use of anti-pseudomonas drugs (amikacin, ciprofloxacin, ceftazidime and imipenem, etc.) and the spread of resistant strains of *P. aeruginosa* [23]. Salehi M, et al. (2014) reported 86.54% and 79.81% resistance of *P. aeruginosa* to Nalidixic acid and ceftazidime, respectively [24]. Mihani and Khosravi reported the highest resistance to ceftazidime (71%) [25]. Wesam AH showed the highest resistance to nalidixic acid and tetracycline antibiotics and in another study Taghvaei R, et al. Ceftazidime 33.3, imipenem 22.2, amikacin 3.20, ciprofloxacin 15.7. And gentamicin reported 19.4% [26-27]. Rakesh MR, et al. reported 49% ciprofloxacin resistance, 63% gentamicin and 14% imipenem, and Kianpour F, et al. reported 58.14% amikacin, 42.85% ciprofloxacin and 14.8% imipenem [28-29]. In a similar study by Ahadi A, et al. imipenem and ceftazidime resistance rates were 55 and 57%, respectively [17].

The discrepancies of the results with the present findings

can be explained by the sample size, sampling method and seasons. *Pseudomonas aeruginosa*, due to its genetic nature, accepts a variety of genes through plasmids and transposons, perhaps because this bacterium can rapidly become resistant to a variety of antibiotics [30]. ESBL production in *P. aeruginosa* isolates has been increasing in recent years. In 2003 in Thailand 20.6% [31], in 2005 in Korea 25.4% [32], in 2006 in Bolivia 23.4% [33] and in 2006 in China 45.3% [34] was. In 2017, Shirehjini FF, et al. and Mirsalehian A reported ESBL production in clinical isolates of 60.8 and 40%, respectively [35].

## Conclusion

Due to the increased antibiotic resistance among the strains, it is recommended that antibiogram testing be performed before treatment. Also, preventing bacterial strains and therapeutic failures that lead to complication of the infection can be prevented by proper use of existing medicines, completing the course of treatment and avoiding as many antibiotics as possible. Further research in this field will increase our knowledge and more effective exposure to the antibiotic resistance of emerging microorganisms.

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