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

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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 Zanjan hospitals.

**Materials and Methods:** In this descriptive-analytical study of the study of 300 cases of urinary tract infection in Zanjan 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 β-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]. \textit{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 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 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 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 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 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 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 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 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 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 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 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 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 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].

\textit{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, hexachlorophene, soaps and iodinated solutions [20]. The aim of this study was to evaluate clinical isolates of \textit{P. aeruginosa} collected from hospitals in Zanjan in order to present a sensitivity pattern to experimental antibiotics and phenotypic study of ESBL 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 \textit{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 (30\(\mu\)g), cefotaxime (30\(\mu\)g), ceftazidime / clavulanic acid (30\(\mu\)g / 10\(\mu\)g) and Cefotaxime / clavulanic acid (30\(\mu\)g / 10\(\mu\)g). 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 (30\(\mu\)g / 10\(\mu\)g) and cefotaxime / clavulanic acid (30\(\mu\)g / 10\(\mu\)g) discs compared to ceftazidime (30\(\mu\)g) and cefotaxime (30\(\mu\)g) discs) indicates ESBL positive of sample and recorded as positive result. In this experiment \textit{E. coli} ATCC 25922 was used as negative control and \textit{E. coli} ATCC 35218 as positive control. After confirmation of the presence of \textit{P. aeruginosa}, the antibiogram for the samples was recommended by the Clinical and Laboratory Standards Institute. Antibiotic discs used were tetracycline (30 \(\mu\)g), nitrofurantoin (300 \(\mu\)g), ceftazidime (30 \(\mu\)g), ampicillin sulbactam (10 \(\mu\)g), amoxicillin (25 \(\mu\)g), amoxicillin-clavulanic (25 \(\mu\)g), nalidixic acid (30 \(\mu\)g), amikacin (30 \(\mu\)g), tobramycin (10 \(\mu\)g), imipenem (10 \(\mu\)g), ciprofloxacin (5 \(\mu\)g) and gentamicin (10 \(\mu\)g) (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%) \textit{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 Table 1.

| Antibiotics      | Resistance | Intermediate | Sensitive |
|------------------|------------|--------------|-----------|
| Tetracycline     | 48         | 10           | 43        |
| Nitrofurantoin   | 9          | 4            | 87        |
| Ceftazidime      | 26         | 29           | 45        |
Table 1: Frequency of antibiotic resistance pattern of *P. aeruginosa* strains isolated from urinary tract infections.

| Antimicrobial          | Frequency |
|------------------------|-----------|
| Ampicillin Sulbactam   | 75        |
| Amoxicillin            | 45        |
| Amoxicillin-Clavulanic | 47        |
| Nalidixic Acid         | 33        |
| Amikacin               | 10        |
| Tobramycin             | 20        |
| Imipenem               | 22        |
| Ciprofloxacin          | 33        |
| Gentamicin             | 5         |
| Total                  | 10        |

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].

### 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 Khoosravi reported the highest resistance to ceftazidime (71%) [25]. Wesam AH showed the highest resistance to nalidixic acid and tetracycline antibiotics and in another study Taghvae 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.

### References

1. AL-Jasser A (2006) Extended-spectrum beta-lactamases (ESBLs): A global problem, J Kuwwait Medical 38(3): 171-185.
2. Medeiros AA (1997) Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. Clin Infect Dis 24(1): 19-45.
3. Ensor VM, Livermore DM, Hawkey PM (2007) A novel reverse-line hybridization assay for identifying genotypes of CTX-M-type extended-spectrum beta-lactamases. J Antimicro Chemother 59(3): 387-395.
4. Dizaji AS, Fathi R, Sales AJ (2016) Molecular study of extended-spectrum beta-lactamase (TEM-1) gene in...
Escherichia Coli isolates collected from Ostad Alinasaab Hospital in Tabriz Iran. Marmara Medical Journal 29: 35-40.

5. Sales AJ, Shadi-Dizaji A (2018) Molecular analysis of CTX-M genes among ESBL producing in Pseudomonas aeruginosa isolated from clinical samples by Multiplex-PCR. Hozan J Environment Sci 2(5): 17-29.

6. Sales AJ, Fathi R, Mobaiyen H, Bonarf FR, Kondlaji KB (2017) Molecular Study of the Prevalence of CTX-M1, CTX-M2, CTXM3 in Pseudomonas aeruginosa Isolated from Clinical Samples in Tabriz Town, Iran. Electronic J Bio 13(3): 253-259.

7. Sales AJ, Hosein-Nezhad P, Shahniani A (2020) Antibiotic susceptibility assessment of Escherichia coli isolated from traditional cheeses in Marand, Iran. International J Advanced Biological and Biomedical Research 8(3): 236-241.

8. Sales AJ, Mobaiyen H, Zoghi JFN, Shadad BN, Kaleybar PV (2014) Antimicrobial Resistance Pattern of Extended-Spectrum β-Lactamases (ESBLs) producing Escherichia coli isolated from Clinical Samples in Tabriz city, Iran. Adv Environ Biol 8(16): 179-182.

9. Sales AJ, Bagherizadeh Y, Khalifehpour M, Abdoli-Senejan M, Helali-Pargali R, et al. (2019) Antibiotic resistance pattern and bla-TEM gene expression in Acinetobacter baumannii isolated from clinical specimens of Tabriz hospitals. Zanko Journal of Medical Sci 20(65): 20-29.

10. Sales AJ, Bagherizadeh Y, Arzani-Birgani P, Shirali M (2018) Study of Antibiotic Resistance and Prevalence of bla-TEM gene in Klebsiella pneumoniae Strains isolated from Children with UTI in Tabriz Hospitals. Focus On Medical Sciences Journal 4(1).

11. Tarbiat-Nazloo D, Sales AJ, Bagherizadeh Y (2019) Identification of phylogenetic groups of Escherichia coli isolated from colibacillosis in poultry by multiplex-PCR. New Findings in Veterinary Microbiology 1(2): 89-94.

12. Sales AJ, Mobaiyen H (2017) Frequency and resistance patterns in clinical isolates of Escherichia coli Extended Spectrum Beta Lactamase producing treatment Centers in Marand city, Iran. New Cellular and Molecular Biotechnology Journal 7(26): 19-26.

13. Jafari-Sales A, Rasi-Bonab F (2017) Detection of the antibiotic resistance pattern in Escherichia coli isolated from urinary tract infections in Tabriz City. J Mol Microbiol 1(1): 1-3.

14. Doosti M, Haj Ojagh Faghihi M, Ramazani A, Saini MR (2011) Comparison of culture and PCR for the diagnosis of Pseudomonas aeruginosa and prevalence antibiotic resistance in clinical samples. J Isfahan Med Sch 30(192): 780-786.

15. Fazli H, Fatahi Bafghi M, Faghi M, Akbari R (2012) Molecular Study of PER and VEB Genes is Multidrug Resistant Pseudomonas aeruginosa Isolated From Clinical Specimens in Isfahan/Iran and their Antibiotic Resistance Patterns. J Kerman Univ Med Sci 19(4): 345-353.

16. Mirsalehi A, Nakhjavani F, Bahador A, Ameli FJ, Bigverdi R, et al. (2011) Prevalence of MBL-producing Pseudomonas aeruginosa isolated from burn patients. Tehran Univ Med J 68(10): 563-569.

17. Ahadi A, SharifZadeh A, Golshani Z (2012) Identification of antibiotic resistance patterns of Pseudomonas aeruginosa isolated from patients admitted with multiple resistance. J Veterinary Lab Res 4(1): 119-122.

18. Lister PD, Wolter DJ, Hanson ND (2009) Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev 22(4): 582-610.

19. Tavajjohi Z, Moniri R, Khoeshidi A (2011) Frequency of extended-spectrum beta-lactamase (ESBL) multidrug-resistance produced by Pseudomonas aeruginosa isolated from clinical and environmental specimens in Kashan Shahid Beheshti hospital during 2010-11. FEYZ 15(2): 139-145.

20. Okesola AO, Oni AA (2012) Occurrence of Extended-Spectrum Beta-Lactamase-Producing Pseudomonas aeruginosa Strains in South-West Nigeria. Res J Med Sci 6(3): 93-96.

21. Falagas ME, Karageorgopoulos DE (2009) Extended-spectrum β-lactamase-producing organisms. Journal of Hospital infection 73(4): 345-354.

22. Wirth FW, Picoli SU, Cantarelli VV, Gonçalves ALS, Brust FR, et al. (2009) Metallo-β-Lactamase-producing Pseudomonas aeruginosa in two hospitals from Southern Brazil. Braz J Infect Dis 13(3): 170-172.

23. das Neves MT, de Lorenzo MEP, Almeida RAMB, Fotezales CMCB (2010) Antimicrobial use and incidence of multidrug-resistant Pseudomonas aeruginosa in a teaching hospital: An ecological approach. Rev Soc Bras Med Trop 43(6): 629-632.

24. Salehi M, Hekmatdoost M, Hosseini F (2014) Quinolone
resistance associated with efflux pumps mexAB-oprM in clinical isolates of *Pseudomonas aeruginosa*. Journal of Microbial World 6(4): 290-298.

25. Mihani F, Khosravi A (2007) MBL-producing *Pseudomonas aeruginosa* strains isolated from patients with burn wound infections and PCR methods to identify blaVIM, blaIMP genes. Iran J Microbiol 1(1): 23-31.

26. Wesam AH (2009) Molecular Identification of Antibiotics Resistant *Pseudomonas aeruginosa* Wt. Aust J Basic Appl Sci 3(3): 2144-2153.

27. Taghvae R, Shojapour M, Sadeghi A, Pourbabaie AA (2013) The study of antibiotic resistance pattern and the frequency of Extended spectrum beta-lactamases (ESBL) in *Pseudomonas aeruginosa* strains isolated from medical centers in Arak city, Iran. Qom Univ Med Sci J 7(4): 36-41.

28. Rakesh MR, Govind LN, Kalpesh M, Rosy P, Kanu P, et al. (2012) Antibiotic Resistance Pattern in *Pseudomonas aeruginosa* Species Isolated at a Tertiary Care Hospital, Ahmadabad. National J Medic Resea 2(2): 156-159.

29. Kianpour F, Havaei SA, Hosseini MM (2010) Evaluation of *Pseudomonas aeruginosa* isolated from cutaneous infections and determination of drug resistance pattern in patients of Alzahra hospital in Isfahan. J Isfahan Med Sch 28(110): 503-509.

30. Pitout JDD, Gregson DB, Poirel L, McClure JA, Le P, et al. (2005) Detection of *Pseudomonas aeruginosa* producing metallo beta lactamases in a large centralized laboratory. J Clin Microbiol 43(7): 3129-3135.

31. Lee S, Park YJ, Kim M, Lee HK, Han K, et al. (2005) Prevalence of Ambler class A and D beta-lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. J Antimicrob Chemother 56(1): 122-127.

32. Celenza G, Pellegrini C, Caccamo M, Segatore B (2006) Spread of bla(CTX-M-type) and bla(PER-2) beta-lactamase genes in clinical isolates from Bolivian hospitals. J Antimicrob Chemother 57(5): 975-978.

33. Strateva T, Ouzounova-Raykova V, Markova B, Todorova A, Marteva-Proevska Y, et al. (2007) Problematic clinical isolates of *Pseudomonas aeruginosa* from the university hospitals in Sofia, Bulgaria: current status of antimicrobial resistance and prevailing resistance mechanisms. J Med Microbiol 56(7): 956-963.

34. Mirsalehian A (2008) Broad-spectrum Of beta-lactamase in *Pseudomonas aeruginosa* isolates in burn patient. J Tehran University of Medical Sciences 66(5): 333-337.

35. Shirehjini FF, Amini K, Fatahi H (2017) Identification of blaCTX-M, blaSHV, and blaTEM Genes in *Pseudomonas aeruginosa* Strains Isolated from Human and Animal Samples Using Multiplex-PCR Method. Qom Univ Med Sci J 10(11): 51-60.