

Original Research Article

Investigation of the Prevalence of *CTX-M-1* Beta-Lactamase Gene in *Pseudomonas Aeruginosa* Strains Isolated From Urinary Tract Infections in Zanjan Hospitals, Iran

Sasan Dabir, Jamal Mohammadi, Ali Alizadeh, Fatemeh karimi, Khatereh Nori, Sara Mahmoudi, Amir Pournajafi*

Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

***Corresponding Author**

Amir Pournajafi

Article History

Received: 13.04.2020

Accepted: 21.04.2020

Published: 24.04.2020

Abstract: *Background and Aim:* *Pseudomonas aeruginosa* is an important cause of opportunistic infections. Infection caused by *Pseudomonas aeruginosa* is often severe and threatening, and is difficult to treat due to its limited susceptibility to antimicrobial agents and resistance to treatment. Therefore, the aim of this study was to identify the molecular identification of *CTX-M-1* genes in the *P. aeruginosa* strains isolated from urinary tract infections in Zanjan hospitals. *Materials and Methods:* In this descriptive-analytical study of the study of 289 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. The bacterial DNA was then extracted and studied by PCR using specific gene primers. *Results:* The most resistant to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%), respectively. A total of 35 samples were identified as the final ESBL producer. 29 specimens of *Pseudomonas* bacteria had the *CTX-M1* gene. *Conclusion:* The genes studied in this study were all located on the chromosome of *P. aeruginosa* bacteria. Therefore, further investigation of ESBL genes such as *CTX-M1* gene seems necessary to control this bacterium.

Keywords: *CTX-M1* gene, Extended-Spectrum Beta-Lactamases, *Pseudomonas aeruginosa*, Urinary Tract Infection, Antibiotic Resistance.

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, ie 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 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, 6]. 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 [7]. The most important ESBLs

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examined are *TEM* and *CTX*. *CTX* was first identified in Germany in 1989 and is divided into five groups, *CTX M1*, *CTX M2*, *CTXM8*, *CTXM9* and *CTXM15*, based on changes in the amino acid sequence. Generally, family members hydrolyze *CTX-M*, cefotaxime, and ceftriaxone better than ceftazidime. They are more inhibited by tazobactam than clavulanic acid [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]. *Pseudomonas aeruginosa* is an opportunistic bacterium that often leads to dangerous hospital infestations in people with weakened immune systems. Today, an important problem in the treatment of pseudomonas infections is the high resistance of this bacterium to antibiotics that have no structural or functional similarity to each other [14, 15]. Pseudomonas infections have been reported frequently in burns, urinary tract infections, and lung diseases such as cystic fibrosis. This variation in pseudomonas infections is due to the development of various acquired mechanisms, including gene expression regulation. In addition, by forming biofilm, it provides the ability to protect the host immune system and various antimicrobial agents [16]. *P. aeruginosa*, gram-negative bacilli, positive oxidase, no spores and aerobic. These bacteria are inherently resistant to penicillin and most beta-lactam antidotes, but are sensitive to the antibiotics ciprofloxacin, tobramycin, and imipenem [17]. The bacterium is clearly seen in burn patients, respiratory patients, and cancer patients undergoing chemotherapy, people with hereditary fibrocystic disease, bacteremia, septicemia, and many other nosocomial infections [18-20]. The aim of this study was to investigate the *CTX-M-1* gene in the *P. aeruginosa* strains isolated from urinary tract infections in Zanjan.

MATERIALS AND METHODS

In this descriptive study, 289 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. 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).

After determining the phenotypically positive isolates, the DNA of the identified samples was extracted using kits OiaGen, Hilden (Germany). The PCR reaction was performed with a final volume of 25 µl, including 1 µl of each primer, Mr. Mix 12.5 µl, DNA pattern 3.5 µl and 7 µl of distilled water (all consumables were manufactured by Sinagen Iran). Thermal Cycler device program contains 35 cycles with 4 minute temperature conditions and initial return at 94 C, connection at 60 C for 45 seconds, lengthening at 72 C for 1 minute and finally lengthening. The final was done at 72 C for 10 min. The PCR product was then evaluated on 1% agarose gel with electrophoresis and the gel containing PCR products was placed in a tank containing ethidium bromide for 15 to 20 minutes after the end of the electrophoresis period. The *E.coli* ATCC 13911 strain with the *CTX-M-1* gene was used as positive control. Primers used in this study: *CTXM1* F:5' ATGGTTAAAAAATCACTGCGTC 3' and R: 5' TTGGTGACGATTTTAGCCGC 3'. In order to statistically analyze the data, the twentieth version of SPSS software and Chi-square test were used. A significant boundary was set at $p < 0.05$.

RESULTS

In this study, 289 urine samples were collected from 100 (34.60%) *P. aeruginosa*. 60 specimens were isolated from the inpatients ward and 40 samples from the outpatients ward. Based on the results of the combined disk test, 40 samples were identified as final ESBL producers. Of the 35 strains of ESBL producing *P. aeruginosa*, 29 samples had *CTX-M-1* genes. The results of the sensitivity test against the 12 selected antibiotics are shown in Table 1.

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

Antibiotics	Resistance	Intermediate	sensitive
Tetracycline	49	10	41
Nitrofurantoin	7	4	89
Ceftazidime	29	29	42
Sulbactam Ampicillin	73	10	17
Amoxicillin	43	16	41
Amoxicillin-Clavulanic	45	0	55
Acid Nalidixic	30	18	52
Amikacin	8	0	92
Tobramycin	18	2	80
Imipenem	21	4	75
Ciprofloxacin	31	3	66
Gentamicin	10	5	85

DISCUSSION

Broad-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]. In the present study, from 100 *P. aeruginosa* isolates, 60 samples from the inpatient ward and 40 samples from the outpatients ward were isolated. Based on the results of the combined disk test, 35 samples were identified as final ESBL producers. 29 specimens of *Pseudomonas* bacteria had the *CTX-M1* gene. The highest resistance to ampicillin (73%) and tetracycline (49%) were the most sensitive to amikacin (90%) and nitrofurantoin (89%), respectively. Wesam *et al.* have examined the resistance pattern of *Pseudomonas* to the antibiotics and the results showed that this bacterium has the highest resistance to the antibiotics of nalidixic acid and tetracycline [22]. In another study in Arak in 2013, the resistance of this bacterium to the following antibiotics has been reported: ceftazidime (33.3%), imipenem (22.2%), amikacin (20.3%), ciprofloxacin (15.7%) and gentamicin (19.4%) [23]. In the study by Ahadi *et al.*, the resistance of it to imipenem (55%) and ceftazidime (57%) has been reported [24]. Rajat Rakesh *et al.* have reported the resistance of it to the antibiotics of ciprofloxacin (49%), gentamicin (63%) and imipenem (14%) in their study. Chander Anli has reported the resistance of it to the antibiotics of amikacin (25%), ciprofloxacin (75%) [25]. Kianpour *et al.* have reported the resistance of it to the antibiotics of amikacin (58.14%), ciprofloxacin (42.58%) and imipenem (14.8%) [26]. The production of ESBL in the isolates of *P. aeruginosa* is on the rise in the last few years. The rate of its increase was 20.6% in Thailand in 2003 [27], 25.4% in 2005 in Korea [28], 23.4% in 2006 in Bolivia and 45.3% in China in 2006 [29]. In the study by Mirsalehian *et al.*, the production of ESBL in the clinical strains isolated in Tehran, has been reported 40% which is consistent with the results of present study [30]. Shakibaie *et al.* [31] have been reported that 41 (34%) of 120 isolates of *P. aeruginosa* were ESBL-producing strains. Shahcheraghi *et al.* have been reported that 234 (39%) of 600 isolates of *P. aeruginosa* were Extended-Spectrum beta-lactamases producing strains [32].

In the study by Shahcheraghi *et al.* [2010], the presence of the genes of VEB, OXA-10, CTX-M, PER-1, GES-1, OXA-1, OXA-4 in the *P. aeruginosa* strains isolated from the hospitals in Iran has been confirmed (33). Performed in Shiraz, the frequencies of CTX-M1, CTX-M2 and CTX-M3 have been reported 49.9%, 135%, 23.1%, respectively [34]. This difference may be due to the types of samples. In other countries, different results have been reported for the prevalence of ESBL. For example, based on the studies performed in Brazil 2010, the highest prevalence of the enzymes of ESBL was related to CTX-M2 (19.6%) [35]. In the studies performed in France and Japan, the frequency of the enzyme of CTX-M in the *P. aeruginosa* strains has been reported 0.0% that is probably due to the appropriate use of beta-lactam antibiotics, especially cephalosporins in these countries [36,37].

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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