

## Short Communication

## Molecular investigation of CTX-M gene in Extended Spectrum $\beta$ Lactamases (ESBLs) producing *Pseudomonas aeruginosa* isolated from Iranian patients with burn wound infection

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### Abstract

**Background:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is one of the most important causes of infection in burns and intensive care units. Extended-spectrum  $\beta$ -lactamases (ESBLs) production in *P. aeruginosa* is a major factor in the antibiotic resistance and is thought of as a serious threat to the currently available antibiotic armory. The purpose of this study was to determine the prevalence of CTX-M gene in ESBL-producing *P. aeruginosa* isolates in burn wound samples. **Materials and Methods:** In this cross-sectional survey, a total of 60 clinical isolates of *P. aeruginosa* were obtained from patients suffering from burn wound infection referred to major hospitals of Tehran, Iran. After verification by biochemical tests and antimicrobial susceptibility testing, CTX-M gene was identified using PCR method. **Results:** The results of the molecular analysis of CTX-M gene showed that the prevalence of isolates of *P. aeruginosa* harboring CTX-M gene was 20% (12/60). **Conclusion:** The results from this study showed high levels of antibiotic resistance and CTX-M gene among *P. aeruginosa* isolated samples of burn-wound infections which condition may result in the increased the emergence of multidrug-resistant strains and the failure of therapy This study suggests that detailed data on the CTX-M gene frequency can be useful to achieve the best therapy for infections caused by ESBLs producing *P. aeruginosa*.

**Keywords:** *Pseudomonas aeruginosa*, CTX-M, polymerase chain reaction, antibiotic resistance, ESBL.

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### Introduction

*Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic bacterial pathogen, considered as one of the main causes of death and morbidity in burn wards (1-3). Treatment of burn wound infection caused by *P. aeruginosa* is becoming difficult, due to its notable intrinsic mechanisms of resistance (4). One of the most common drug resistance mechanisms is the production of Extended-Spectrum Beta-

Lactamase enzymes (ESBLs) in the bacteria. The ESBLs consist of a heterogeneous molecular cluster of plasmid-mediated bacterial enzymes that indicate substantial resistance to oxyimino-cephalosporin and monobactam antimicrobials, but they cannot hydrolyze carbapenemases or cephamycins (5, 6).

Pathogenic strains of *P. aeruginosa* produce three main classes of ESBLs: TEM, SHV, and CTX-M (6-8). In the 1990s, ESBL-producing bacteria were defined essentially as closely related components of

the TEM and SHV- $\beta$ -lactamase families, in *Klebsiella pneumoniae* and *Escherichia coli* causing outbreaks of nosocomial infection (6, 9). One of the most important enzymes is the CTX-M, which stands for 'active on cefotaxime, first isolated in Munich' and reflects the potent hydrolytic activity of the enzyme against cefotaxime (10). CTX-M gene has disseminated among a wide range of clinical bacteria within and across the species in the world since 1995 (11).

ESBLs producing *P. aeruginosa* has become a major global challenge in burn research and management and there is ample evidence regarding the prevalence of ESBLs in the range of 6 – 88% in health care centers around the world (6). Furthermore, systemic infections induced by ESBL producing *P. aeruginosa* are linked to higher healthcare costs, severe adverse clinical consequences, and mortality (7, 12). Given these, the rapid increase of ESBL-producing *P. aeruginosa* in hospital-acquired infections is now a serious concern for the therapeutic development for infection by resistant bacterial pathogens (13).

Knowledge on antibiotic resistance patterns and the frequency of CTX-M gene between *P. aeruginosa* isolated samples in different regions could help in the selection of antimicrobial therapy and improve the outcome of infections. With this background, the aim of this study was to determine the prevalence of the ESBL phenotype and CTX-M gene in *P. aeruginosa* isolated from patients suffering from burn wound infection in Tehran, Iran.

## Methods

**Isolation of bacteria.** A total of 60 consecutive multidrug-resistant *P. aeruginosa* isolates were obtained from different burn wards in affiliated hospitals in Tehran city, Iran, in the years 2016 and 2017. The isolates were detected as *P. aeruginosa* by the standard bacteriological methods and biochemical testing including oxidase tests, H<sub>2</sub>S production, movement, indole production, urease, culturing in agar starter and Tsi and carbohydrate fermentation. The isolates were stored at -20 °C in the media containing Tryptic Soy Broth (TSB) medium with 10% glycerol.

### *Extraction of plasmid DNA of ESBL*

**producing *Pseudomonas aeruginosa*.** Pure colonies of freshly bacteria were inoculated into 2 ml of Luria Bertani medium containing 100  $\mu$ g ampicillin in sterile conditions. The tubes were inseminated after incubation at 37 °C at 170 rpm for 15 hours. Plasmid DNA was isolated from bacterial cells using SinaClon Preps kit (Cat. No: PR881612) according to the company's instructions. The purified plasmid was stored at -20°C.

**CTX-M gene detection using Polymerase Chain Reaction (PCR).** Polymerase chain reaction (PCR) was performed to amplify type-specific CTX-M gene. 10  $\mu$ l of DNA template used in the PCR reaction containing a 1X buffer, MgCl<sub>2</sub>, dNTP, Taq polymerase, and primers for detection of the CTX-M gene (593 bp). The primer sequences were as follows: CTX-M-F 5'-ATGTGCAGYACCAGTAARGTKATGGC-3' and CTX-M-R 5'-TGGGTRAARTARGTSACCAGAAAYCAGCGG-3'. The PCR conditions for amplification of the CTX-M gene were done as follows: Initial denaturation at 95 °C for 3 minutes, denaturation at 95 °C for 30 seconds, 30 seconds for annealing at 55 °C, 1 minute at 72 °C for elongation, and the final extension was conducted at 72 °C for 10 minutes. A negative control was used in every PCR assay. The amplified products were analyzed after electrophoresis in 1.5% agarose gel at 120 V, for 35 minutes. The gel was visualized by staining with ethidium bromide and a 100 – 5000 bp ladder molecular weight marker (Fermentas, Vilnius, Lithuania) was used. The images of stained DNA bands were documented using the UV gel documentation system.

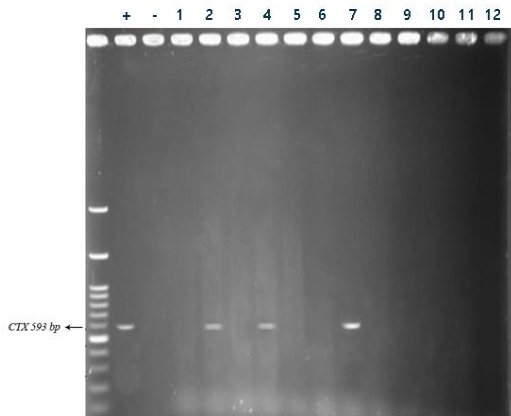
**Statistical analysis.** Statistical analysis was performed by the Chi-Square test ( $\chi^2$ ) using SPSS version 18 (SPSS Inc., Chicago, IL, USA). All values, in which the probability of the null hypothesis was  $P \leq 0.05$ , were regarded as statistically significant.

**Ethical Considerations.** The present study was accepted by the ethics committees of the Islamic Azad University, Central Tehran Branch, Iran. Written informed consents were obtained from all the study patients or their parents.

## Results

During the study period, 60 clinical isolates

were isolated consecutively from wounds at burn wards. The ESBL-producing *P. aeruginosa* were subjected to PCR using CTX-M specific primers. Of the ESBL-producing *P. aeruginosa* isolates 12 (20%) were positive for ESBL genes and 48 isolates (80%) were negative. The results of PCR-products electrophoresis indicated genomic patterns related to CTX-M (593 bp) (Figure 1).



**Figure 1.** Electrophoresis of PCR products on a 1% agarose gel. Lane 1. Ladder 100 bp. Lane 2. Positive control. Lane 3. Negative control. Lanes 2, 4, 7. Positive samples that indicated a 593 bp PCR product.

## Discussion

Burn-wound infectious complications are the important causes of death and morbidity in serious thermal injuries. The most common agents responsible for burn-wound infections are bacteria, with *P. aeruginosa* being the most important species (14-16). Increasingly, resistance to antimicrobials among the bacteria causing the infections, practically *P. aeruginosa*, has limited treatment choices. The prevalence of ESBL among clinical isolates of *P. aeruginosa* impressively varies between geographical areas and is changing rapidly with time. Therefore, identification of beta-lactamase is critical for a reliable molecular epidemiological analysis of antimicrobial resistance (17-19). On the other hand, insights into local antimicrobial resistance trends among isolated samples are important for evidence-based recommendations in the empirical antibiotic therapy of the infections (6).

Due to the important role of *P. aeruginosa* in burn wound infections and the necessity of identifying antibiotic resistance patterns the CTX-M

gene was identified by PCR. High prevalence of ESBL-producing *P. aeruginosa* within our population might be caused by the extensive use of third generation cephalosporins in hospitals. As mentioned, the distribution and occurrence of ESBL vary greatly worldwide among different species and countries (20). Our findings indicate that CTX-M type  $\beta$ -lactamases are prevalent in the studied population (20%). We figured out the higher prevalence of CTX-M type  $\beta$ -lactamase than that described in countries such as China (2). Similar results were reported in Brazil (21) (19.6%), Iran (in a different area) (22) (17.3%) and Saudi Arabia (23) (22.4%) of *P. aeruginosa*.

High levels of antibiotic resistance in ESBL-producing *P. aeruginosa* in clinical isolates of burn wounds is alarming (24). ESBLs enzymes, especially CTX-M, have increased sharply in recent years as showed in the review of Paterson and Bonomo (7). Consistent with findings of recent studies by other researchers, it can be concluded that the excessive use of antibiotics causes the higher percentage of drug-resistance in ESBL-producing *P. aeruginosa* and leads to the transmission of the resistance genes through the pathogenic bacteria more quickly. In the current study, the frequency of the  $\beta$ -lactamase enzyme, CTX-M, was 20%, which could be a rational indicator for high-level resistance of isolates to third and fourth generation cephalosporins.

## Conclusion

In summary, our findings indicate a high prevalence of CTX-M gene among the ESBL-producing *P. aeruginosa* isolates obtained from burn wards. The evidence from this study enhanced our knowledge of the prevalence of ESBL enzymes and suggested the need for improved surveillance of additional ESBL, CTX-M, in ESBL-producing *P. aeruginosa*. In the case of suspected ESBLs producer clinical isolates of *P. aeruginosa*, PCR based molecular methods could help to detect the gene and the approach may help in preventing the development of drug resistance.

## Conflicts of Interest

The authors have no conflict of interest to declare.

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