



**MOLECULAR DETECTION OF CTX-M- $\beta$ -LACTAMASES IN *PSEUDOMONAS AEROGENOSA* STRAINS ISOLATED FROM CORNEAL INFECTIONS**

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Article Received on 27/01/2017

Article Revised on 16/02/2017

Article Accepted on 08/03/2017

**ABSTRACT**

Bacteria producing CTX-M- $\beta$ -lactamases are emerging around the world as a source of resistance to oxyiminocephalosporins such as cefotaxime (CTX). This study was aimed to detect the presence of *bla*CTX-M gene among *Pseudomonas aerogenosa* (*P. aerogenosa*) strains isolated from corneal infections in Ibn- Al haitham teaching hospital for eyes in Baghdad. Eleven *P. aerogenosa* strains were isolated from specimens of corneal infections of patients at Ibn- Al haitham teaching hospital during four months (April- July, 2016).. The results of antibiotic susceptibility of *P. aerogenosa* strains showed most strains were resist to most antibiotics under test and the highest resistant antibiotic was Cefazolin which gave 11(100%) resistance rate followed by Ampicillin, Aztreonam, Ampicillin-Sulbactam and Amoxicillin-clavulanic acid which gave 9(81.81%) resistance rate. On the other hand the most effective antibiotic under test was Amikacin which it gave the lowest resistance rate 2(18.18%) followed by Chloramphenicol which it gave 4(36.36%) resistance rate. Phenotypic detection of ESBL producing strains was done by screening test and the results showed that out of 11 *P. aerogenosa*, 7(63.64%) were positive ESBL producing strains while 4(36.36%) *P. aerogenosa* strains were negative ESBL producing strains. Molecular detection of *bla*CTX-M showed that out of 11 *P. aeruginosa* strains, 3(27.27%) were harbored the *bla*CTX gene (544bp) and those 3 strains were also positive ESBL producing strains by phenotypic detection, while 8(72.72%) strains not harbored *bla*CTX-M gene.

**KEYWORDS:** *Pseudomonas aerogenosa*, Extended-Spectrum  $\beta$ -Lactamases (ESBL), CTX-M gene and corneal infections.

**INTRODUCTION**

Eye diseases are common worldwide, sometimes to an epidemic degree Ocular infections can cause damage to structures of the eye, which can lead to reduced vision and even blindness if left untreated.<sup>[1]</sup> Pathogenic microorganisms cause ocular disease and the most frequently affected parts of the eye are the conjunctiva, lid and cornea which are external part of the eye.<sup>[2]</sup> Bacteria are major causative agents that frequently cause infections in eye and possible loss of vision.<sup>[3]</sup>

*Pseudomonas aeruginosa* is an opportunistic bacterial pathogen capable of causing sight-threatening corneal ulcers. It can also cause infections in immunocompromised people or in those suffering from burns or cystic fibrosis.<sup>[4]</sup> The management of bacterial eye infections may involve treatment with broad spectrum antibiotics. The indiscriminate use of antibiotics led to the development of resistance to many

commonly used antimicrobial medications. The emergence of bacterial resistance towards topical antimicrobial agents may increases the risk of treatment failure with potentially serious consequences.<sup>[5]</sup>

Antibiotics can be purchased without prescription in less development countries, which leads to misuse of antibiotics. This may led to the emergence and spread of antimicrobial resistance.<sup>[1]</sup> Resistance to the expanded-spectrum cephalosporins can occur in *Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa* via the production of extended-spectrum  $\beta$ -lactamases (ESBLs).<sup>[6]</sup> CTX-M-type enzymes are a lineage of molecular class A extended-spectrum  $\beta$ -lactamases (ESBLs) that are capable of hydrolyzing the oxyiminocephalosporins and monobactams, with an overall preference for cefotaxime and ceftriaxone.<sup>[7]</sup> This resistance mechanism is widespread throughout the world, with reports of clinical isolates producing these  $\beta$ -

lactamases from Europe, Africa, Asia, South America, and most recently North America.<sup>[6]</sup> The aim of this study was to detect the ESBL producing strains by phenotypic method and also molecular detection of CTX-M gene in *Pseudomonas aerogenosa* strains isolated from corneal infections in Ibn- Al haitham teaching hospital for eyes in Baghdad.

## MATERIALS AND METHODS

### Collection of specimens

The specimens were collected from patients with corneal infections at Ibn- Al haitham teaching hospital for eyes in Baghdad / Iraq. The period of collection was extended from April, 2016 till July, 2016.

### Identification of bacterial strains

All specimens from corneal infections were cultured and the bacteria were isolated according to standard

microbiology methods<sup>[8]</sup>, then the *Pseudomonas aerogenosa* strains were identified to species level by using API 20 NE (Analytic Profile Index ) system (Bio-Merieux, France), which is a standardized system for the identification of bacteria belong to non Enterobacteriaceae family.

### Antimicrobial Susceptibility Test

Susceptibility of *Pseudomonas aerogenosa* strains to different types of antibiotics was performed according to Kirby-Bauer (disk diffusion) technique<sup>[9]</sup>, by using Muller-Hinton agar and different types of antimicrobial discs which supplied commercially (Table-1). Inhibition zones developed around the discs were measured by millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute.<sup>[10]</sup> Results were read according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS).<sup>[11]</sup>

**Table 1: Antibiotic discs used in the present work.**

Antibiotic discs	Code	Disc potency (µg/disc)	Manufacturing Company/ Origin
Ampicillin	AMP	10	Bioanalyse/ Turkey
Amikacin	AK	30	
Ampicillin-Sulbactam	SAM	10/10	
Amoxicillin-Clavulanic acid	AMC	20/10	
Aztreonam	ATM	30	
Cefazolin	CZ	30	
Cefotaxime	CTX	30	
Ceftazidime	CAZ	30	
Ceftriaxone	CRO	30	
Chloramphenicol	C	30	
Ciprofloxacin	CIP	5	
Gentamicin	CN	10	

### Phenotypic Detection of ESBL producing strains

Phenotypic detection of ESBL producing *Pseudomonas aerogenosa* strains was done by screening test.<sup>[11&12]</sup> The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs ceftazidime (30 µg) (Bioanalyse/ Turkey) and cefotaxime (30 µg) (Bioanalyse/ Turkey) were placed on the surface of the Mueller-Hinton agar and then incubated at 37°C for 24 h. *Escherichia coli* ATCC 25922 was obtained from Teaching Laboratories at Medical city in Baghdad and used as negative control. If the inhibition zone diameter of  $\leq 22$  mm for ceftazidime and  $\leq 27$  mm for cefotaxime was recorded, the strain was considered as 'suspicious' for ESBL, producer.

### Genotypic detection of blaCTX-M gene by polymerase chain reaction (PCR)

PCR assay was performed in order to amplify the fragments of bla gene in a monoplex pattern for all *Pseudomonas aerogenosa* strains under study.

### DNA extraction

DNA was extracted by suspending 2-3 colonies of each *Pseudomonas aerogenosa* strain grown on MacConkey agar plates in 500 µl of nuclease-free water (Promega,

USA) and heating for 10 min at 90°C by using a water bath. Samples were spun for 10 min at 10000 rpm. These samples were then used as the bacterial DNA template for PCR assay.<sup>[13]</sup>

### Primers and amplification reaction used in this study

The sequence of blaCTX-M Forward primer was (5-TTTGCGATGTGCAGCACCA GTAA-3) and the sequence of blaCTX-M Reverse primer was (5-CGATATCGTTGGTG GTGCCATA-3) (Alpha DNA , Canada).<sup>[14]</sup>

Amplification reactions (25µl for each sample) were performed using the Master mix 2X (Kapa , India) (12.5µl), forward primer (1.5µl), reverse primer (1.5µl), nuclease-free water (4.5µl) and DNA sample (5µl). Temperature cycling conditions included an initial denaturation at 95°C for 5 min followed by 30 cycles of 94°C for 30 sec., annealing at 55°C for 40 sec. and extension at 72°C for 50 sec. Cycling was followed by a final extension at 72°C for 10 min.

### Agarose gel electrophoresis

Gel electrophoresis was used for the detection of PCR products which visualized with the aid of ethidium

bromide and UV transilluminator documentation system.<sup>[15]</sup>

## RESULTS

### Isolation and Identification of *Burkholderia cepacia* strains

Eleven *Pseudomonas aerogenosa* strains were isolated from specimens of corneal infections of patients at Ibn-Al haitham teaching hospital for eyes in Baghdad during four months (April- July, 2016). All strains of *Pseudomonas aerogenosa* were identified by API 20 NE (Analytic Profile Index ) system (Bio-Merieux, France).

### Antimicrobial Susceptibility Test

The antibiotic susceptibility test of eleven *Pseudomonas aerogenosa* strains under study toward 12 different

antibiotics was showed in figure-1. From this figure, showed that *P. aerogenosa* strains were resist to most antibiotics under test and the highest resistant antibiotic was Cefazolin which gave 11(100%) resistance rate followed by Ampicillin, Aztreonam, Ampicillin-Sulbactam and Amoxicillin-clavulanic acid which gave 9(81.81%) resistance rate. On the other hand the most effective antibiotic toward *P. aerogenosa* strains under test was Amikacin which it gave the lowest resistance rate 2(18.18%) followed by Chloramphenicol which it gave 4(36.36%) resistance rate. Also from figure-1, the resistance rate of Cefotaxime, Ceftazidime and Ceftriaxone was 8(72.72%) and the resistance rate of Gentamicin and Ciprofloxacin was 7(63.63%).

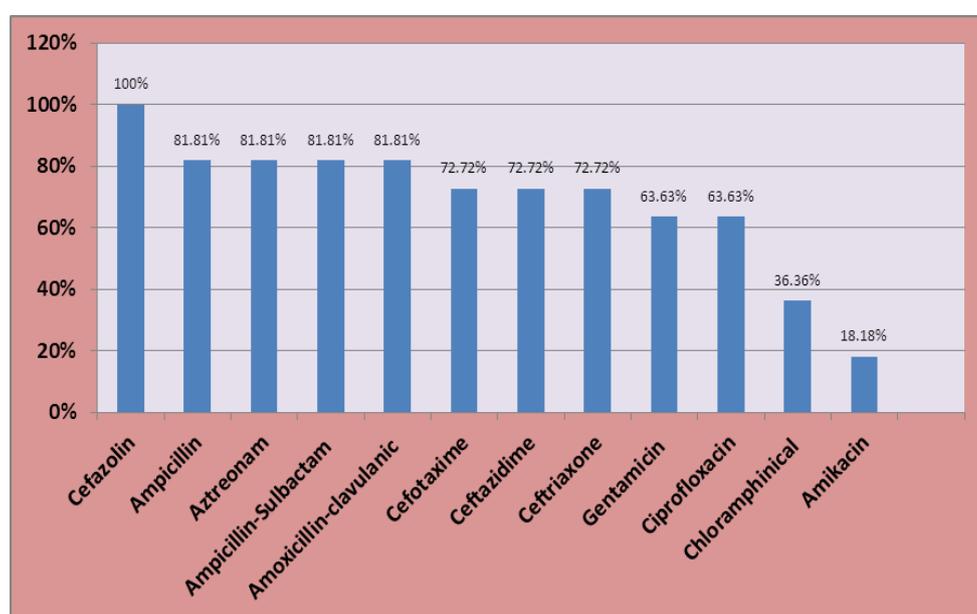


Figure-1: The resistance rate of 11 *P. aerogenosa* strains isolated from corneal infections toward 12 types of antibiotics.

### Phenotypic Detection of ESBL producing strains

Phenotypic detection of ESBL producing *Pseudomonas aerogenosa* strains was done by screening test and the result showed that out of 11 *P. aerogenosa* strains, 7(63.64%) were positive ESBL producing strains as shown in figure-2 and table-2, while 4(36.36%) *P. aerogenosa* strains were negative ESBL producing strains.



Figure 2: Screening test for ESBL producing *Pseudomonas aerogenosa* strains.

### Genotypic detection of *bla*CTX-M gene among *Pseudomonas aeruginosa* strains by PCR

All eleven *P. aeruginosa* strains were subjected to PCR assay to detect the presence of *bla*CTX-M gene. The results in figure-3 and table-2, showed the presence of *bla*CTX gene (544bp) in 3(27.27%) strains (strains number 4, 5 and 11), out of 11 *P. aeruginosa* strains, while 8(%) strains not carried *bla*CTX-M gene. The

three *P. aeruginosa* strains that harbored *bla*CTX-M gene were also positive ESBL producing strains by phenotypic detection method. On other hand strains that were positive ESBL producing strains by phenotypic detection method and not carried *bla*CTX-M gene may be resist to cephalosporin antibiotics by another mechanism rather than CTX-M enzyme.



**Figure-3:** Gel electrophoresis of PCR product of *bla*CTX-M (544 bp) in *Pseudomonas aeruginosa* strains isolated from corneal infections. Lane M: 100 pb DNA ladder (Kapa , India); lanes 1-11: *Pseudomonas aeruginosa* strains; lane C: Negative control (had all PCR mixture including water instead of DNA template). Detection was done on agarose gel (1%) at 5 V/cm for one hour, stained with ethidium bromide and visualized on a UV transilluminator documentation system.

**Table 2:** Results of phenotypic detection of ESBL and genotypic detection of *bla*CTX-M gene among *Pseudomonas aeruginosa* strains isolated from ocular infections.

Strain NO.	Phenotypic Detection of ESBL producing strains	Genotypic detection of <i>bla</i> CTX-M gene
1	Negative	Negative
2	Positive	Negative
3	Negative	Negative
4	Positive	Positive
5	Positive	Positive
6	Positive	Negative
7	Negative	Negative
8	Positive	Negative
9	Positive	Negative
10	Negative	Negative
11	Positive	Positive

### DISCUSSION

The organisms that cause ocular infection are generally exogenous. However, in certain circumstances they gain access to enter the eye and cause infection. *P. aeruginosa* is the predominant Gram-negative bacterium that infects the cornea.<sup>[16]</sup> ESBL -producing bacteria are

major causes of mortality and morbidity. Therefore, detection of ESBL -producing *P. aeruginosa* would be critical to control the spreading infectious diseases with antibiotic resistance bacteria.<sup>[17]</sup>

Rapid use of antibiotics for severe ocular infections is routine in ophthalmic practice resulting in increased drug resistance (1). In this study, all 11(100%) strains were resist to Cefazolin and 9(81.81%) of all *P. aeruginosa* strains were resist to Ampicillin, Aztreonam, Ampicillin-Sulbactam and Amoxicillin-clavulanic acid, also 8(72.72%) of all *P. aeruginosa* strains were resist to Cefotaxime, Ceftazidime and Ceftriaxone and 7(63.63%) of all *P. aeruginosa* strains were resist to Gentamicin and Ciprofloxacin. The reason for increased resistance for some antibiotics may be earlier exposure of the isolates to these drugs. Moreover, these drugs are very common due to low cost and often purchased without prescription in different areas(1). Also our study showed the most effective antibiotic under test was Amikacin followed by Chloramphenicol and this is in agreement with the study conducted by Shahaby *et al.* (1).

Our study showed a high prevalence of ESBL-producing *P. aeruginosa* strains among ocular clinical strains in

which out of 11 *P. aeruginosa* strains, 7(63.64%) were positive ESBL producing strains by phenotypic detection also the result of molecular detection showed the presence of *bla*CTX gene in 3(27.27%) strains, out of 11 *P. aeruginosa*.

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