

**DETECTION CTX-M TYPES EXTENDED SPECTRUM  $\beta$  -  
LACTAMASES (ESBLs) IN *ESCHERICHIA COLI* ISOLATION FROM  
PREGNANT URINARY TRACT INFECTIONS (UTIs) IN BASRA,  
PROVINCE, IRAQ**

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**ABSTRACT**

Urinary tract infections (UTIs) who are cause by *E. coli* an important etiologic agent of morbidity in the presently extended spectrum  $\beta$ -lactamases (ESBLs). Aim of present study was detection the types of CTX-M that spreading in the case of urinary tract infection between pregnant women. Current study were included the collection one hundred samples of the urine from pregnant women from, eighty samples given positive cultivation results, Gram-negative distributed after diagnosed using conventional techniques and conformed by using API 20E system to 37(46.25%) *E.coli*, 25(31.25%) *Klebsiella* spp., 12(15%) *Pseudomonas* spp. And 6(7.5%) *Proteus* spp. Results of detected ESBLs production were showed from 37(46.25%) *E.coli*

isolates the 15(40.54%) ESBLs producing isolates, while 22(59.46%) non- ESBLs producing isolates. The second part of this study the amino acid sequencing data results for 15 plasmid DNA that identified (11) plasmid DNA in isolates (No3,7,11,25, No28,34,37,45,49 and No55,66) similarity the plasmid DNA have accession number in genbank (AJW2218.1, AEZ49563.1 and AMM70777.1) respectively as *bla*<sub>CTX-M-1</sub> gene (100%), while (4) plasmid DNA in isolates (No30,72 and No 12,77) similarity the plasmid DNA have accession number in genbank (AGE61862.1 and ACU44517.1) respectively as *bla*<sub>CTX-M-15</sub> gene (100%).

**KEYWORD:** CTX-M, ESBLs, *E.coli*, UTI.

**1. INTRODUCTION**

*Escherichia coli* is one of the common intestinal commensals normal flora , it's a member of Enterobacteriaceae family play important role in diverse hospital infections and community-

acquired infections. This bacteria can be causes infections such as gastroenteritis, urinary tract infection and meningitis.<sup>[1,2]</sup> Frequently use of antibiotics to remedy *E. coli* infectious lead to increased the multidrug resistance (MDR) and hence trouble to treating the infection.<sup>[3,4]</sup>  $\beta$ -lactamase production considered as the more important mechanism between several mechanisms to resistance of antibiotic in *E.coli*.<sup>[2,5]</sup> Urinary tract infections (UTIs) that cause by *E. coli* are an important etiologic agent of morbidity in the presently extended spectrum  $\beta$ -lactamases (ESBLs).<sup>[6,7]</sup> The ability to production ESBLs is contributors in the problem of resistance to antimicrobial.<sup>[6]</sup> Cefotaxim hydrolyzing (CTX-M) ESBL family has been increasing in frequency around the world.<sup>[8,9]</sup> ESBLs originate mainly because of mutations in  $\beta$ -lactamases encoded by the *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes, the variants TEM and SHV are most widespread ESBLs, but strains expressing CTX-M ESBLs have initiate to emerge in many countries.<sup>[12,11]</sup> The non-TEM, non-SHV most frequent ESBLs type,  $\beta$ -lactamases CTX-M type are characterized by selective degradation of cefotaxime.<sup>[11,13]</sup> This study aimed to determine types of *bla*<sub>CTX-M</sub> ESBLs among ESBLs producing *E.coli* isolates from pregnant women which tribulation urinary tract infection (UTI).

## 2. MATERIALS AND METHOD

### 2.1. Bactarial isolation

During September/2015 to November/2015, (100) urine samples were collected from pregnant women which suspected to have urinary tract infection (UTI), reviewers for private laboratories in the Basra province, Iraq. Urine samples transferred to laboratory and culture according to followed the standard methods, after incubation for 24 h at 37°C, the positive culture were identified by conventional techniques and Confirmed by using API 20E system (BioMérieux, Marcy l'Etoile, France).

### 2.2 Detection extended spectrum $\beta$ -lactamase (ESBL).

Double disk synergy test (DDST) method was using to detection of ESBLs production. The identified *E.coli* were tested by spreading on Mueller -Hinton agar plates, then (30 $\mu$ g) disk of cefotaxime (and /or ceftazidime and /or ceftriaxone and /or aztreonam) and a disk of amoxicillin -clavulanate (20  $\mu$ g amoxicillin / 10  $\mu$ g clavulonic acid) positioned at a distance of (30mm) (centre to centre). Positive resulting in a characteristic shape-zone referred to as 'cham-pagne-cork' or 'keyhole'.<sup>[14,15]</sup>

### 2.3 Identification of *bla*<sub>CTX-M</sub> Gene

The extraction of plasmid DNA was conducted by using a (Pure Yield™ Plasmid Miniprep System, Promega, USA). The plasmid DNA was extracted from (15) isolates of *E.coli* bacteria. The *bla*<sub>CTX-M</sub> gene were detected through polymerase chain reaction techniques (PCR), the gene were amplified with primers *bla*<sub>CTX-M</sub> F (5'-GCGTGATACCACTTCACCTC-3') and *bla*<sub>CTX-M</sub> R (5'-TGAAGTAAGTGACCAGAATC-3').<sup>[16]</sup> Reactions of PCR were carried out in (25 µl) volumes, containing (1 µl) of each primer and extracted plasmid DNA, (12.5 µl) of GoTaq Thermo master mix (Thermo, England), completed volume to (25 µl) with (9.5 µl) Nuclease-free water. PCR Program was preformed with following: (1) cycles of (5 min.) denaturation at (95°C), followed by (35) cycles of (45 sec.) denaturation at (95°C), (45 sec.) annealing at (55°C), (90 sec.) extension at (72°C) and a final extension step of (15 min.) at (72°C).

### 2.4 Purification and Sequencing *bla*<sub>CTX-M</sub> Gene

PCR Products of the correct size were purified with a QIAquick PCR purification Kit (Qiagen, USA). The sequences of amino acids were obtained then aligned with known *bla*<sub>CTX-M</sub> sequences of amino acids in Genbank using the basic local alignment search tool BLAST [www.blast.ncbi.nlm.nih.gov/Blast.cgi](http://www.blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences  $\geq 99\%$  similarity were depended for the diagnosis<sup>[17]</sup> the nucleotide sequence was converted to amino acid sequence by depending on the expasy translate program [www.expasy.org/translate/](http://www.expasy.org/translate/). Phylogenetic tree were constructed by using Multiple alignment (MAFFT) program version 7 <http://mafft.cbrc.jp/alignment/server/>.<sup>[18]</sup>

## 3. RESULTS AND DISCUSSIONS

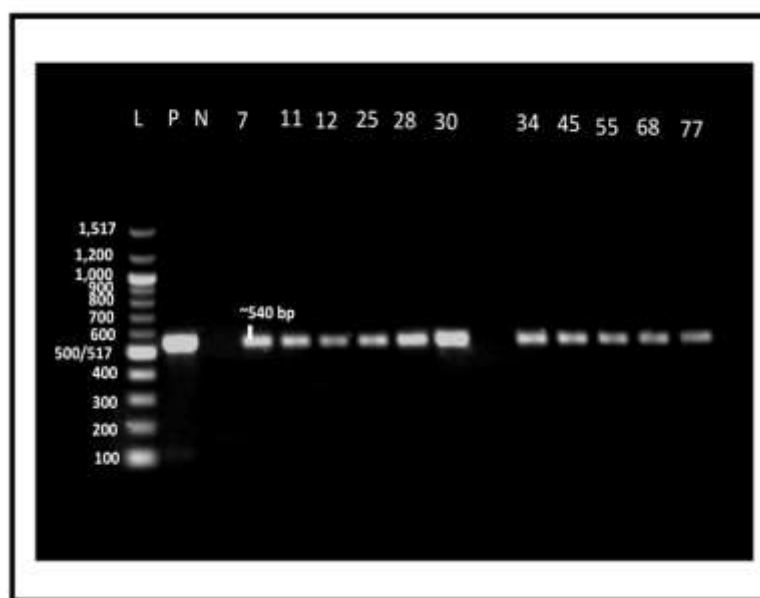
From one hundred samples of the urine that has been collected eighty samples given positive cultivation results, Gram-negative distributed after diagnosed using conventional techniques and conformed by using API 20E system to 37(46.25%) *E.coli*, 25(31.25%) *Klebsiella* spp., 12(15%) *Pseudomonas* spp. And 6(7.5%) *Proteus* spp. A high frequency of UTI its commonly accepted during pregnancy.<sup>[19]</sup> Among pregnant women the UTI bacterial infection is the predominant type, the physiological and hormonal changes in urinary tract include changes in ureteral dilatation, tone and bladder volume that may promote the infection in pregnant women.<sup>[20]</sup>

### - Detection extended spectrum $\beta$ -lactamases (ESBLs)

The results of detected ESBLs production were showed from 37(46.25%) *E.coli* isolates the 15(40.54%) ESBLs producing isolates, while 22(59.46%) non- ESBLs producing isolates. The multidrug resistant isolates of *E. coli* have been increasing around the world and is associated with the acquisition of mobile element-encoded  $\beta$ -lactamases. The CTX-M  $\beta$ -lactamases production is very important mechanism to resistant for many antibiotic groups in *E.coli* isolates. This family of plasmid-mediated ESBLs of Ambler class A that has been detected mainly in Asian countries, Europe and America.<sup>[21,22,23]</sup>

### - Identification of *bla*<sub>CTX-M</sub> Gene

The second part in this study showed that individual band of *bla*<sub>CTX-M</sub> gene were characterized in (540bp) by comparison with the standard molecular DNA ladder (1517bp) figures (1).

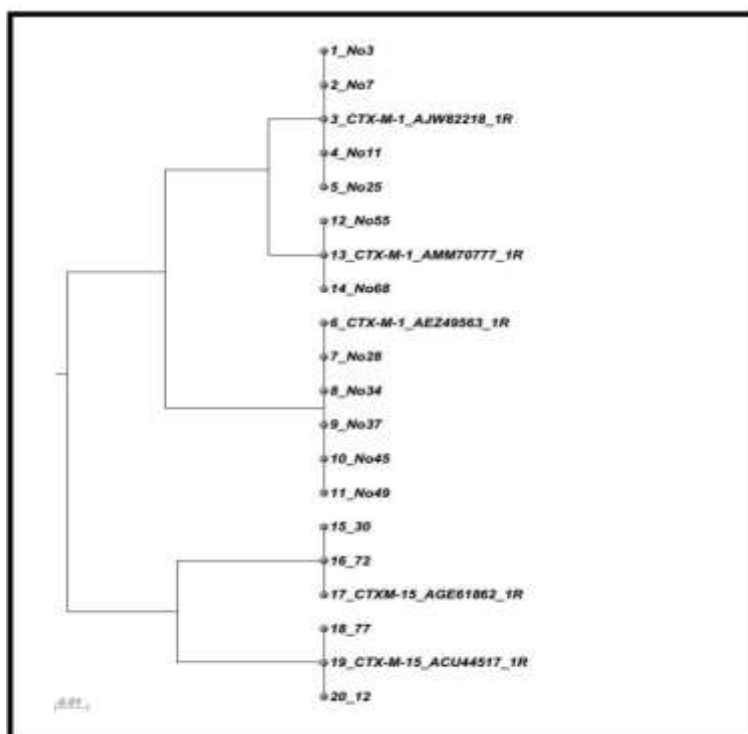


**Figure(1) Agarose electrophoresis patterns showed PCR amplified products of *bla*<sub>CTX-M</sub> gene Lane (no.1):(1517bp DNA ladder), lane (no.2), Positive control to *bla*<sub>CTX-M</sub>. lane (no.3) Negative control and Lane: (no. 7-77) plasmid DNA band of bacterial isolates.**

The *bla*<sub>CTX-M</sub> gene protein sequencing data results for fifteen alignments of plasmid DNA isolates were identified in this study were compared with (5) reference plasmid proteins from genbank, that identified (11) plasmid DNA in isolates (No3,7,11,25, No28,34,37,45,49 and No55,66) similarity the plasmid DNA have accession number in genbank (AJW2218.1, AEZ49563.1 and AMM70777.1) respectively as *bla*<sub>CTX-M-1</sub> gene (100%), while (4) plasmid

DNA in isolates (No30,72 and No12,77) similarity the plasmid DNA that have accession number in genbank (AGE61862.1 and ACU44517.1) respectively as *bla*<sub>CTX-M-15</sub> gene (100%). The *bla*<sub>CTX-M-1</sub> was conceder as common type of *bla*<sub>CTX-M</sub> that detected from Asia, Europe, North and South America among multidrug-resistant in *E.coli*.<sup>[24,25]</sup> The *bla*<sub>CTX-M-15</sub> was predominant ESBL In Arab countries, the first description of CTX-M was in Egypt and then in the United Arab Emirates and Kuwait.<sup>[26-27]</sup>

The result of protein sequence data for the (15) isolates in this study were concatenated producing a amino acid sequence length (395) amino acid depend on the shorter one among the sequences. The root phylogenetic tree was constructed and displayed in figure (2). These tree show the distribution of phylogenetic relationships among the studied DNA plasmid and their identical reference DNA plasmid.



**Figure (2).** Neighbour Joining phylogenetic tree the amino acid sequences derived from an alignment of CTX-M gene by using the MAFFT alignment and visualized using forester version1038. This N-J tree showing the distribution and phylogenetic relationships between 15 CTX-M protein were detected on *E.coli* by using 5 reference protein (R). All vertical branch lengths were drawn to scale. Bootstrap values after 1000 repetitions are indicated.

## 5. CONCLUSION

This study detected two types *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-15</sub> spreaded between the *E.coli* isolates that causes urinary tract infection in pregnant women.

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