Occurrence of CTX-M-I Type β-lactamases Gene in Certain Gram Negative Bacteria

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ABSTRACT:

BACKGROUND:

The CTX-M-type β-lactamases represent a group with a typical extended-spectrum β-lactamase (ESBL)-resistance phenotype. These enzymes, encoded by transferable plasmids. They have a preferential hydrolysis of Cefotaxime over Ceftazidime. The CTX-M-type β-lactamases have been described in species of *Enterobacteriaceae* and *Pseudomonas aeruginosa*.

OBJECTIVE :

This study was designed to investigate of the occurrence of CTX-M-I type in some Gram negative bacteria species isolated from clinical cases of in Iraq.

METHODS:

A group of Gram negative bacteria were isolated from different sources.Plasmid DNA extraction, and electrophoresis were performed. Using specific primers, CTX-M-I enzyme genes were amplified by PCR.

RESULTS:

Plasmid profile of the tested isolates reveals the presence of relatively large plasmids, their Wight was more than 10 kb some isolates posses' 3-4 kb plasmids. The results of PCR amplification showed the presence of CTX-I genes. All isolates of *Salmonella enterica* serovar *Typhimurium* (100%) are negative for CTX-M-I gene as well as most of *P. aeruginosa* isolates (86.7%). In contrast, all of *E. coli* (100%) and most of *Proteus* Spp isolates were positive for CTX-M-I gene.

CONCLUSION:

CTX-M genes are predominant in *E.coli* followed by *Proteus* Spp. while *Salmonella enterica* serovar *Typhimurium* and *P. aeruginosa* isolates showed low incidence of *blaCTX-M* genes occurrence. The alarming situation with dissemination of CTX-M producing isolates highlights the need for their epidemiological monitoring and prudent use of antimicrobial agents.

KEYWORDS: CTX-M-I, Gram negative bacteria, PCR.

INTRODUCTION:

The CTX-M-I type β-lactamases represent a rapidly emerging group with a typical extended-spectrum β-lactamase (ESBL)-resistance phenotype[1]. These enzymes, encoded by transferable plasmids, exhibiting extended-spectrum activities, are capable of hydrolysing some broad-spectrum Cephalosporins and are inhibited by Clavulanic acid and Tazobactam. They have a preferential hydrolysis of cefotaxime over Ceftazidime. This group of CTX-M type β-lactamases (>30 enzymes) have been found predominantly in *Enterobacteriaceae*, most prevalently in *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Salmonella typhimurium*[2]. In some countries, CTX-M-type enzymes are the ESBLs most frequently isolated from *E. coli* strains[3,4]. In clinical isolates, CTX-M encoding genes have been found on a number of plasmids; some of them are part of transposons or constitute cassettes in integrons[2]. This study is design to investigate the prevalence of these genes in some Gram negative bacteria.

MATERIALS AND METHODS:

Twenty seven Gram negative bacteria isolates were isolated from different sources and identified according to Forbes *et al*. (2002) [5]. The isolates were isolated from non hospitalized patients of Al-Kadhymia teaching hospital\Baghdad February to April 2011. Table (1) shows source and number of these isolates.

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Table 1: Source and number of the isolates involved in this study.

Bacteria	Source	Number	Percentage(%)
Salmonella enterica serovar Typhimurium	Diarrhia	2	7.4
E. coli	Otitis media Stool	2 3	7.4 11.1
Proteus Sp.	Wound Urinary tract infections	2 3	7.4 11.1
Pseudomonas aeruginosa	Otitis media	15	55.6
Total		27	100

Plasmids of study isolates were extracted by simple and rapid boiling procedure. Briefly, loopfull bacterial culture were suspended in 200 µl of lysis buffer containing 1% Triton X-100, 0.5% Tween 20, 10 mM Tris-HCl (pH=8.0), and 1 mM EDTA and incubated for 3 minutes in a boiling water bath. After centrifugation for 2 minutes at 10,000 × *g* to sediment the debris, a 10 µl aliquot of the clear supernatant was directly used for agarose gel electrophoresis [6] and 2 µl aliquot transferred to the PCR Master mix of PCR, respectively [7].

The sequence of oligonucleotide primers that were used in PCR to detect the presence of CTX-M-I genes were taken from Mirzaee *et al*., (2009)[8] and synthesized in Alpha DNA Co. (Canada). Table (2) shows primers sequence and product.

Table 2: Primers Used to Access CTX-M PCR.

CTX enzyme group	Primer name	Primer sequence	PCR product (bp)	CTX-M gene
CTX-M-I	CTXM1-Forward CTXM1-Reverse	GACGATGTCACTGGCTGAGC AGCCGCCGACGCTAATACA	499	CTX-M-1,-3,-10-12,-15,-22,-23,-28-30

The gene was amplified using PCR, 2-µl aliquot of the plasmid clear supernatant was directly transferred to the PCR Master mix as a template[7]. The amplification was performed as follows:

In a microcentrifuge reaction tube, 25 µl master mix was prepared for each test. A master mix contained the following components (according to the manufacturer instructions):

Go-Taq green master mix (Promega-USA)	12.5 µl
Each primer (set of 10 picomol\µl each)	1.5 µl
Nuclease free distilled water (Promega-USA)	9 µl
Plasmid DNA template	2µl

The cycling was performed using protocol comprising an initial denaturing step at 94°C for 3 minutes, followed by 25 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extention at 72°C for 1 minute with a final step 72°C for 7 minutes[8]. Sterile distilled water was used instead of DNA template to ensure absence of contaminants in the reaction preparations.

RESULTS:

Plasmid profile of the tested isolates reveals the presence of relatively large plasmids, with molecular weight more than 10kb, as shown in figures 1 and 2 as well as plasmid smaller than that molecular weight.

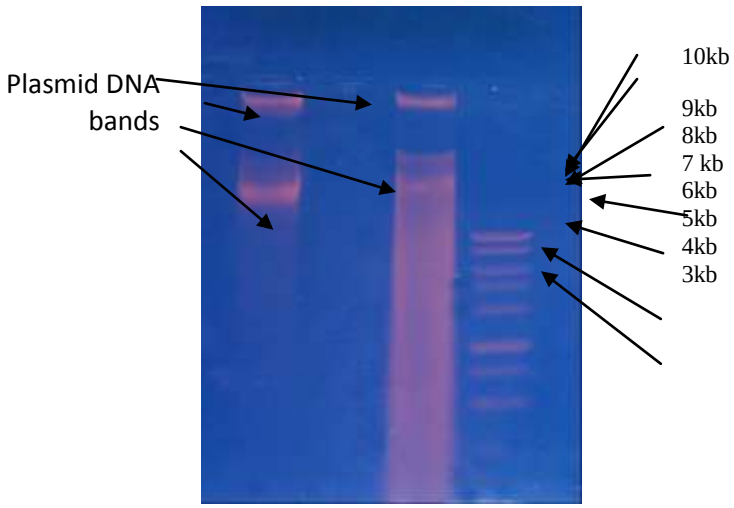

Figure 1: Plasmid profile of *E. coli*

Electrophoresis was carried out in 1.5% agarose gel supplied with Ethidium bromide at (7V/cm) for 90 minutes.

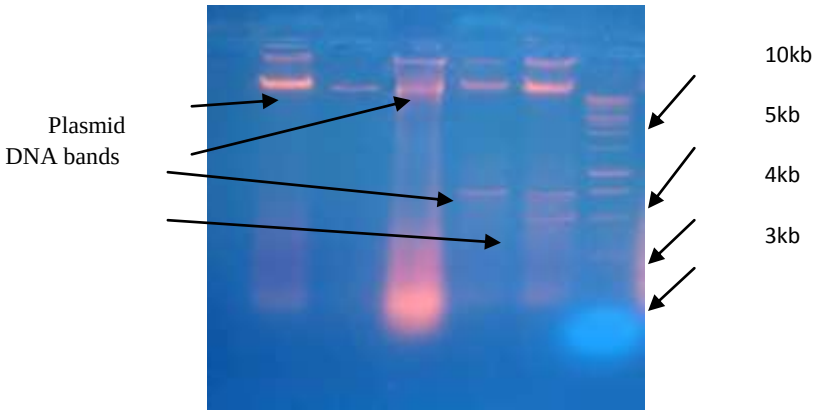

Figure 2: Plasmid profile of *P. aeruginosa*

Electrophoresis was carried out in 1.5% agarose gel supplied with ethidium bromide at (7V/cm) for 90 minutes.

Amplification of common sequence of CTX-I genes produces amplicon of 499bp, figure 3 and 4 show this amplicon.

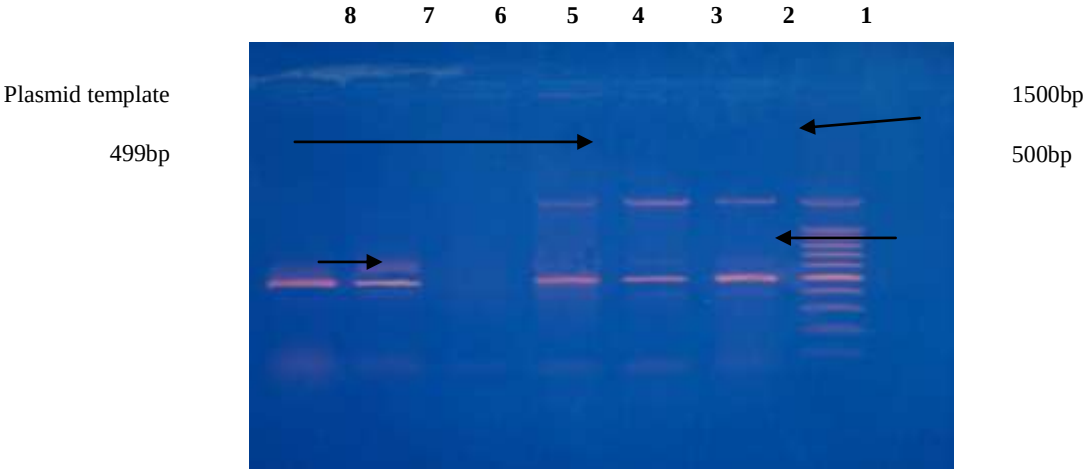

Figure 3: Agaroe gel electrophoreticogram of CTX-I PCR product.

Lane 1: no template negative control, lane 2: 100bp ladder, lanes 3,7and 8: CTX-I PCR products of plasmids of *E.coli* from otitis media and from two stool samples, respectively. Lanes 4,5 and 6: CTX-I PCR products of plasmids of *Proteus* Spp from urinary tract infections. Electrophoresis was carried out in 1.5% agarose gel supplied with ethidium bromide at (7V/cm) for 90 minutes.

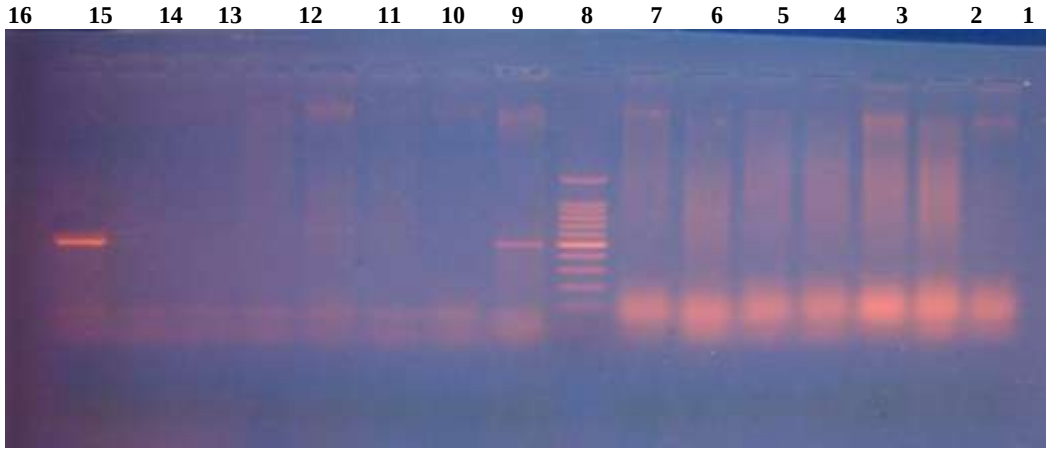

Figure 4: Agaroe gel electrophoreticogram of *P. aeruginosa* CTX-I PCR product. Lane 8: 100bp ladder, lanes 1-7 and 9-16 are CTX-I PCR products only two isolates (lanes 9 and 16) were positive. Electrophoresis was carried out in 1.5% agarose gel supplied with ethidium bromide at (7V/cm) for 90 minutes.

Table (3) illustrates the results of PCR amplification of CTX-I genes. The two isolates of *Salmonella enterica* serovar *Typhimurium* are failed to produce a product as well as most of *P. aeruginosa* isolates, only two out of 15 were positive. In contrast, all of *E. coli* shows positive, 3 out of 5 of *Proteus* Spp isolates were positive.

Table 3: Number and percentage of positive and negative CTX-M-I genes of some Gram negative isolates.

Bacteria	Source	Number Of tested isolates	CTXM-I positive isolates	Percentage (%)	CTXM-I negative solates	Percentage (%)
Salmonella enterica serovar Typhimurium	Diarrhia	2	0	0	2	100
E. coli	Otitis media Stool	2 3	2 3	100 100	0 0	0 0
Proteus Spp	Wound Urinary tract infections	2 3	1 2	50 66.6	1 1	50 33.4
Pseudomonas aeruginosa	Otitis media	15	2	13.3	13	86.7

DISCUSSION:

Extended-spectrum-β-lactamases (ESBLs) are enzymes produced by bacteria which impart resistance against advanced-generation-cephalosporins. CTX-M enzymes have become the most prevalent ESBLs[9] . A new family of ESBL that preferentially hydrolyzes cefotaxime has arisen. It has been found in isolates of *E.coli* mainly and some other species of *Enterobacteriaceae*. Amplification of common sequence of CTX-M-I genes in this study was higher than that of other study. Punpanich *et al.* (2008)[10] reported that *bla*CTX-M-I genes were detected in 78% of the ESBLs producing *E.coli*, while Rajesh *et al.* (2010)[11] reported that CTX-M-I was detected in 28.57% of the *E.coli* and this percentage is lower than the result of this study. Mirzaee *et al.* (2009)[8] finding illustrated that 89% of the *bla* CTX-M belong to CTX-M-I and they reported that this group is disseminated in the ESBLs producing *E.coli* isolates. Sasaki *et al* (2010)[12] showed that, a markedly high number (58.2%) of the healthy stool specimens showed the presence of CTX-M beta-lactamase-producing *Enterobacteriaceae.* The majority of the CTX-M beta-lactamase-producing bacteria were *E. coli* (85.1%). This study revealed a wide dissemination of CTX-M beta-lactamase-producing *Enterobacteriaceae* in the healthy population especially *E. coli*. This result is agreed with our study hence, all of stool *E.coli* isolates were positive. In Baghdad, Al-Kaabi (2011)[13] found the following results: The CTX-M-I type enzyme was detected in 78.94% of *E.coli* isolates, 100% in *P.mirabilis* and 83.33% in *P. aeruginosa*. The differences between the results that obtained in this study and these studies may be attributed to the low number of tested isolates in this study, as well as the source of isolates may create such differences.

In a global survey of CTX-M-I, many of *E. coli* strains were strongly associated with urinary tract infections. Interestingly, the CTX-M-I genes were always found on one of three plasmids. The *bla*CTX-M-15 gene, one of the CTX-M-I genes, was located on a 63-kb transferable plasmid [14]. Insertion sequences, especially ISEcp1, have repeatedly been found adjacent to genes encoding some of these enzymes[15,16,17]. Eckert *et al.* (2006)[18] confirmed the predominant role of ISEcp1 in the mobilization of *blaCTX-M* genes of the CTX-M-1 cluster.

CONCLUSION:

blaCTX-M genes are predominant in *E.coli* followed by *Proteus* Spp. while *Salmonella enterica* serovar *Typhimurium* and *P. aeruginosa* isolates showed low incidence of *blaCTX-M* genes occurrence. The alarming situation with dissemination of CTX-M producing isolates highlights the need for their epidemiological monitoring and prudent use of antimicrobial agents.

Plasmid DNA bands

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