# Occurrence of Plasmid encoded ESBLs $bla_{CTX-M}$,$bla_{TEM}$ genes Of E. coli isolated from Clinical cases in Maysan province

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**Abstract:** Escherichiacoli repeatedly causing urinary tract, wound and blood infection resulting in significant morbidity and mortality due to had plasmid encoded ESBLs which in turn lead to treatmentfailure**.** Thepresent study was focused on the estimation of β-lactam antibiotic resistance patterns, the determination of Plasmid ESBLs represented by $bla_{CTX-M}$ and $bla_{TEM}$ gene. So atotal of (291) clinical samples (urine, wound swabs, blood and seminal fluids) were included in thisstudy. All bacterial isolates were subjected to the cultural, microscopical, and biochemical examinations methods, confirmed by API 20E and Vitek2 system. Where the results revealed that 105 of isolates were identified as E. coli. Antibiotic sensitivity was performedby using disk diffusionmethods against β-lactam. Investigation of extended spectrum β-lactamase(ESBL) production for isolates was performed using Initial screening and double discsynergy method (DDST). The results showed that most isolates showed highresistance to ß-lactam antibiotics, while all isolates were sensitive to Imipenem. The results of PCR technique were performed to detect P1asmid encoded ESBLs $bla_{CTX-M}$ and$bla_{TEM}$genes, revealed that (100%) of E. coli isolates carried this genes for both.

**Keyword:** Escherichia coli, β-lactam Resistance, ESBL, $bla_{CTXM}$, $bla_{TEM}$gene.

## 1. Introduction:

Plasmids are main mechanism for the spread of antibiotic resistant genes and confer traits of antibiotic resistance in bacterial populations[1]. The transfer of plasmid-mediated genescan occur either between closely related strains, or between widely related strains from diverse species or generaand can play a significant role in the mobility of resistance genes[2,3].Resistance to β-lactams and other antibiotics in the Enterobacteriaceae is frequently associated with plasmid resistance determinants, β-Lactamase-mediated resistance is increasingly associated with plasmid-encoded extended-spectrum β-lactamases (ESBLs) and carbapenemases, specifically the CTX-M family of ESBLs, these enzymes are now appearing in multiple combinations of ESBLs and carbapenemases, thereby conferring resistance to virtually all β-lactam antibiotics[4,5]**.** Therefore, Plasmids have important role behind the success of the ESBL genes as they both mediate transfer and immobilize maintenance of these genes in new hosts[6]**.**

## 2. MATERIAIS AND METHODS

### 2.1. Bacterial samples collection and identification:

A total of 291 samples, were collected from different clinical cases (urine, wound swabs, blood and seminal fluid)from main Hospitals in Maysan Province during the period from October 2018 till the end of September 2018. The samples were immediately inoculated on blood agar and MacConkey agar, then incubated for overnight at 37°C under aerobic conditions. All E. coli was isolated and identified according to their diagnostic characteristics and then compared with their being reported in MacFaddin(2000).


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The isolates were confirmatively diagnosed by API 20 E system and VITEK2 system by using VITEK®2 GN kit, then stored at maintenance medium until further tests.

### 2.2. Antimicrobial susceptibility testing

Antimicrobial Susceptibility testing for β-lactam group was determined by the agar disk diffusion method[7], and the results were interpreted according to CLSI guidelines[8]. The following antibiotic disks were used: Ampicillin 10 µg,Pipracillin 100 µg, Augmentin20/10µg, Cefoxitin 30 µg, Ceftazidime 30 µg, Cefotaxime30 µg, Ceftriaxone 30 µg, Cefipeme 30 µg,Aztreonam 30 µg, Imipenem 10 µg, (Bioanalyse, Turkey).

### 2.3. Phenotypic detection of ESBLs

All β-lactamase producing bacterial isolates were assayed for ESBL production by initial screening test according toCLSI guidelines[8]. The isolates showing resistance to one or more third generation Cephalosporins (3GCs) were tested for ESBL production by Double Disc Synergy Test (DDST)**[9].**

### 2.4. Extraction of Bacterial DNAPlasmid

Plasmid extraction was done toward all E. coli isolates according to AccuPrep® Plasmid Mini extraction kit protocol (Bioneer, South Korea). The integrity of extracted Plasmid was tested using Agarose Gel Electrophoresis. The Plasmid DNA then subjected to monoplex PCR.

### 2.5. Molecular detection of CTX-MGene Using PCR Technique

The protocol used in accordance with the manufacturer's instructions (Bioneer, South Korea). The sequences of primers and thermal cycler conditionsare shown in the table (1). The amplification achieved productswere separated in 1% agarose gels containing ethidium bromide 3µl(o.5µg), DNA ladder 100bp (Bionear, Korea) wasused for compare with. After electrophoresis, the gel was photograhed under UV light as described by[10].

***Table (1)*** *Primers sequences and thermal cycler conditions*

| Name | Primers sequences ( (5'-3') | | Size bp | PCR Condition Temp/time Cycles No. | Reference |
|---|---|---|---|---|---|
| $bla_{TEM}$ | F<br>R | -ACTGCGGCCAACTTACTTCTG<br>-CGGGAGGGCTTACCATCTG | 374 | 95C/5min 1X<br>94C/30s<br>62C/30s 30X<br>72C/30s<br>72C/10min 1X | [11] |
| $bla_{CTX-M}$ | F<br>R | -CGCTTTGCGATGTGCAG<br>-ACCGCGATATCGTTGGT | 550 | 94C/5min1X<br>94C/1min<br>55C/1min 34X<br>72C/1min<br>72C/5min 1X | [12,13] |

## 3. RESULTS AND DISCUSSION

### 3.1. Isolation and identification of Bacterial Isolates

The results of this study showed that among 291 clinical samples 235 gave positive growth and 105 (44.7%) were identified as E. coli. As compared with other studies, our findings in line with studies reported by[14,15],where identified E.coli was (44%) for both,while the result of present study was contrary with study conducted by[16] where the result was (22.27%), and higher than other studies reported in India and Ethiopia implemented by[17,18],where the results were (21.96%) and (25.4%) respectively.

### 3.2. The antibiotic susceptibility pattern

The resistance patterns of E. coli towards various antibiotics were determined using disc diffusion method. Data in (Table 2) exhibited that isolates of E. coli have the highest level of resistance to Ampicillin where up to (98.1%) were resist to this antibiotic, followed by (96.2%) for Pipracillin and (93.3%) for Ceftazidime, Cefpodoxime, and Cefotaxime was (94.3%), whereas in (92.4%) was the resistance for Cefepime, while to Amoxicillin/Clavulanic acid the percent of resistance was(90.5%). The resistance to the Ceftriaxone was (87.6%), (86.7%) for Cefoxitin and Aztreonam (81.9%).On the otherhandthis study recorded that all isolates (100%) were sensitive to Imipenem antibiotic. Over all these results were directly line with local studies conducted by[19],in Iraq, also in compatible with[20],where they found high resistant to β-lactam antibiotic and they considered the antibiotic Imipenem and Meropenem should be preferred drugs for E. coli infection isolated from clinical samples. The observed high resistance rates in most antibiotic may be due to uncontrolled consumption, consequence of easy access to inefficient and cheap antibiotics moreover could be justified by insufficient adherence to guidelines for infection control as well as inappropriate use of antibiotics.

***Table(2):*** *Results of Antibiotics susceptibility test for Escherichia. coli isolated from different clinical cases(n=105).*

| No | Antibiotic | Sensitive n (100%) | Intermediated n (100%) | Resistance n (100%) |
|---|---|---|---|---|
| **1** | Ampicillin | 2 (1.9) | - | 103 (98.1) |
| **2** | Pipracillin | 4 (3.8) | - | 101 (96.2) |
| **3** | Amoxicillin/ Clavulanic acid | 8 (7.6) | 2 (1.9) | 95 (90.5) |
| **4** | Cefoxitin | 8 (7.6) | 6 (5.7) | 91 (86.7) |
| **5** | Ceftazidime | 7 (6.7) | - | 98 (93.3) |
| **6** | Ceftraxone | 11 (10.5) | 2 (1.9) | 92 (87.6) |
| **7** | Cefotaxime | 6 (5.7) | - | 99 (94.3) |
| **8** | Cefepime | 8 (7.6) | - | 97 (92.4) |
| **9** | Aztronam | 11 (10.5) | 8 (7.6) | 86 (81.9) |
| **10** | Imipenem | 105 (100) | - | - |

### 3.3. Molecular detection of Plasmid encoded ESBL genes

[21]reported that plasmid carried ESBL-associated genes were prevalent in E. coli. Also study conducted by[22]found high prevalence of plasmid-mediated ESBLs was detected among E. coli in Diabetic foot infections(DFIs) in Egypt. In addition to[23]who mentioned in their studies that CTX-M β -lactamases form a new and rapidly rising family of plasmid-mediated ESBLs that currently replace the transformed TEM or SHV ESBL families with high greater penetration into E. coli. In current study the result revealed that **(100%)**of E. colicarried $bla_{CTX-M}$ gene on plasmid as shown in figure (1), these findings were greatly similar to study for[22,24]where the results were **(100%)** for both**,** and in

concordance with study of[25]who found that**(86.7%0)**of E. coli carried **bla$_{CTX-M}$** geneon plasmid. The elevated rate of CTX-M β-lactamases in E. coli isolates suggest that the horizontal transfer of **bla$_{CTX-M}$**genes mediated by plasmid and/ or mobile genetic element, contributes to ease with which these enzymes are spreading in E. coli isolates and the dissemination of **CTX-M**enzymes.

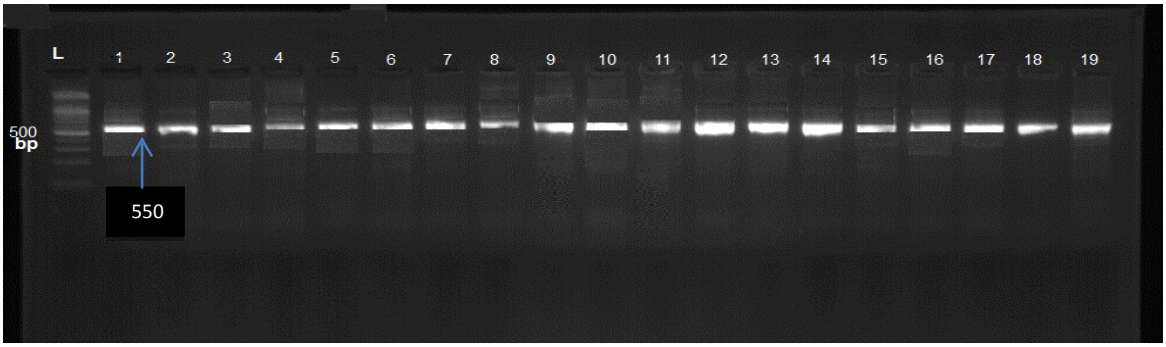


***Figure (1):*** *Agarose gel electrophoresis of PCR plasmid encoded bla$_{CTX-M}$ gene, amplicon (550bp), where L: ladder (100bp) , Lane(1-19) positive results ,the gel stained by ethidium bromide (0.5 µg/ml) and ran at (65) volts for one hour.*

Current study exhibited that**(100%)** of E. coli isolates had bla$_{TEM}$ gene on plasmid, this rate went beyond the study conducted by[26]where the findings was **(75.6%)** and other study in a Swedish achieved by[27]where the frequency of **bla$_{CTX-M}$**genewas **(63%)**among E. coli isolates, while these results contrary to the findings of[22,28,25,24]where the ratio reached to **(50%), (56.8%) (32.5%) and (34.6%)** respectively.[29]explained that TEMtype ESBLs are the first plasmid-mediated β-lactamasethat is often found in general of Enterobacteriaceae. Additionally,[30]who mentioned that TEM is a broad spectrum β-lactamase that is always combined with CTX-M in the same plasmid and the combinations of these genes are frequently seen in the ESBL producing strains**,** this conclusion may be one cause of prevalence **bla$_{TEM}$** gene in our survey.

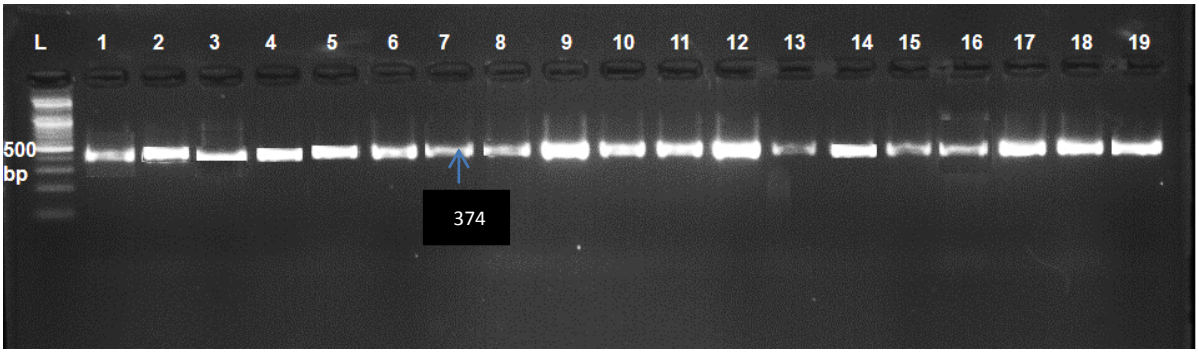


***Figure (2):*** *Agarose gel electrophoresis of PCR plasmid encoded **bla$_{TEM}$** amplicon (374bp), where L: ladder (100bp) , Lane(1-19) positive results ,the gel stained by ethidium bromide (0.5 µg/ml) and ran at (65) volts for one hour.*

## 4. Conclusion

In this study isolated E. coli showed high levels of resistance to most antibiotics of β-lactam group and considered as multidrug resistant bacteria. Furthermore, E. coli produced **bla$_{TEM}$** and **bla$_{CTX-M}$** genes carried on Plasmid in high rate of occurrence reached to (100%) for both. High prevalence of plasmid-mediated ESBLs was detected among clinical cases in Maysan province. Therefore, new guidelines should be undertaken in this area to restrict or prohibit the misuse and abuse of antimicrobial agents. The presence of plasmid-mediated antibiotic resistance encoded ESBLs genes shows that these genes can be disseminated to bacteria of the same or different species, and can play a significant role in the mobility ofresistance genes.

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