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# Molecular Detection of Cephalosporin Resistance Genes in *Escherichia coli* Isolated from Urinary Tract Infections in Baghdad Hospitals

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**Abstract:** *Escherichia coli* is a normal flora in the human and animals gut, but also it is pathogenic in the patient with immune system disorder ,*is* the leading cause of enteritis, urinary tract infection, septicemia and other Medical infections One hundred and eighty (180) midstream urine samples (MSU) were collected from patients with urinary tract infection (UTI) of all age and both sexes. Samples were collected from Al-Karma Teaching Hospital and Al-Yarmouk Teaching Hospital from the beginning of November 2021 until the end of February 2022. All isolates were diagnosed based on microscopic examinations and morphological characteristics using Eosin methylene blue (EMB), Blood agar and MacConkey agar. The diagnosis was confirmed through Vitek system and API E kit. Where the diagnostic results showed that *E.coli* had the highest percentage, representing 44.7%, followed by *Klebsiella pneumonia* with 16% and *Pseudomonas aeruginosa*. 8% and *Proteus mirabilis* 2.7%, *Staphylococcus aureus* 14.3%, *Enterococcus spp*, 6.3% and Fungi 8%. As for age, the age groups of (11-20) years had the highest rate of infection with 29.5%, followed by (21-30) years with a rate of 16.1, while the age groups over 60 and (1-10) were at 12.5% and ( 51-60) and (31-40) were 10.7%, and the lowest percentage of infection was (31-40), representing 8.0% .while was the injuries, 58% were in women, 42% in men Testing antibiotic sensitivity against 14 different antibiotics showed that *E. coli* was highest resistance Ceftriaxone (82%), Cefotaxime and Tetracyclic (78%), Cefixime (76%), Ceftazidime and Ampicillin (74%). While there is less resistance at Cefepime (68%) On the other hand, moderate resistance Cephalothin (50%), Cephalexin and Azetrenam (40%) The current study demonstrated that *E.coli* possessed a low-level resistance against Amikacin (34%), Ciprofloxacin (28%), Imipenem (16%), and Gentamycin (52%). In addition, PCR was used to identify cephalosporin resistance genes. It was concluded that all isolates were resistant to cephalosporin through the presence of the *CTX-M*-1 gene by (100%), while the results of the presence of the *OXA-48* genes were (23.3%). Three resistant and three intermediate resistant isolates were selected for gene expression study.

**Keywords**: *OXA-48, CTX-M-1,* UTI, PCR, *E. coli*

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## Introduction

Global population growth is accompanied by a wide spread of diseases caused by multidrug-resistant bacteria. Urinary tract infection (UTI) is the most common community-acquired and hospital-acquired bacterial infection typically characterized by a high rate of treatment failure and recurrence of infection (1). Bacterial infection typically characterized by a high rate of treatment failure and recurrence of infection (2). They cause urinary tract infections, including *Escherichia coli*, *Staphylococcus saprophyticus*, *Klebsiella spp*, Proteus *spp*, and *Enterococcus* spp. (3).

The main pathogen responsible for infectious diseases in UTIs worldwide is the pathogenic *Escherichia coli* (UPEC). Urinary tract is successfully colonized by UPEC. UPEC isolates show different virulence factors that increase pathogenicity (4).

*Escherichia coli* is a Gram-negative bacteria, motile or non- motile a member of the Enterobacteriaceae, rod shaped, facultatively anaerobic, lactose fermentative. It is motile as it has peritrichous flagella. Most *E. coli* strains have the adhesive organs fimbriae (pili) that outspread from the bacterial surface into the surrounding medium (5,6).

It is employ several mechanisms of resistance agents different groups of antibiotics including; target modification, alteration of cell membrane permeability , production of enzymes, alteration of metabolic pathways and have the efflux pumps(7).

One of the most common mechanisms of resistance is the production of extended-release beta-lactamases (ESBL) and carbapmase that degrade all penicillins, cephalosporins, ximino, cephalosporins, and monomers (8).

Carbapenemase genes, mediated by mobile genetic elements carrying additional resistance elements, confer resistance to various groups of antibiotics, resulting in multidrug resistance (MDR). *OXA-48*, a class D carbapenemase, is of major concern owing to its difficulty in detection and its association with treatment failure. Moreover, *OXA-48* like enzyme variants are plasmid coded and hence associated with rapid dissemination in community settings (9).

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. The enzyme responsible for this particular ESBL phenotype not affecting ceftazidime was named as *CTX-M 1* in reference to its preferential hydrolytic activity against cefotaxime (*CTX -M*) (10).

## Materials and methods

### Collection of samples

One hundred and eighty midstream urine samples (MSU) were collected from patients with urinary tract infection (UTI) of all ages and both sexes. Samples were collected from Al-Karama Teaching Hospital and Al-Yarmouk Teaching Hospital from the beginning. Identification of *E.coli* manual biochemical tests that were used Api20 E test. For final embedded in VITEK2 compact system.

### Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors in study parameters. Least significant difference – LSD and (Analysis of Variation-ANOVA) was used to significantly compare between means. The Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

### Antimicrobial susceptibility testing

The *E. coli* isolates (50) were tested for antibiotic sensitivity by using against 12 different antibiotics. Amikacin, Imipenem, Azetrenam, Ampicillin, Cefepime, Cephalothin, Cefixime, Ceftriaxone, Ceftazidime, Cefotaxime, Cephale-xin and Ciprofloxacin (11).

### Extraction and measured concentration and purity of DNA

## Bacterial DNA extraction

DNA Extracted has been performed using (Qubit) NEB® (England) for all viteck-2 diagnosed *E.coli* isolates.

## Detection of *E. coli 16SrRNA* gene using conventional PCR

Genes encoding the cephalosporin resistance determinants, *OXA-48* and *CTX_ M_ 1*, were investigated by PCR using specific primers in table (1) of each primer reaction the following steps: an initial denaturation step at 94°C for 5 min; followed by 30 cycles of 94°C for 30 sec, 50°C for 45 sec and 72°C for 7 mins.Table (2) component of PCR master mix reaction. Where is the gel electrophoresis concentration 2% and voltage use 90 volt.

**Table (1): Primer sequences used of PCR in this study**

| Primer | Primer sequence (5—3) | Product size bp | Reference |
|---|---|---|---|
| ***16SrRNA*** | **F-** CTTAATCGACCATACGCTTTG | 400 | (12) |
| | **R-** GTATTGTGTCTGCCATTAACC | | |
| ***OXA-48*** | **F** –GCTGGTTCGCCCGTTTAA | 159 | (13) |
| | **R-** ATTATCGGAATGCCAGCGGT | | |
| ***CTX-M-1*** | **F-**GCTAAGCTCAGCCAGTGACA | 733 | (14) |
| | **R-** CGCGGTGCTGAAGAAAAGTG | | |

**Table (2): Component of PCR Master Mix Reaction**

| PCR Master mix reaction components | | Volume |
|---|---|---|
| PCR Premix | | 12.5Ml |
| DNA template | | 5Ml |
| Primers | Forward | 1.5Ml |
| | Reverse | 1.5µL |
| nuclease-free $H_2O$ | | 4.5µL |
| Total volume | | 25µL |

## Results and discussion

One hundred and eighty midstream urine samples (MSU) were collected from patients with urinary tract infection (UTI) of all ages and both sexes. Samples were collected from Al- From 180 urine samples; preliminary results showed growth in 112 (62.3%) specimens and 68 samples no growth in the other (37.7) % E. *coli* had the highest percentage, representing 44.7%, followed by *Klebsiella pneumonia* with 16% and *Pseudomonas aeruginosa*. 8% and *Proteus mirabilis* 2.7%, *Staphylococcus aureus* 14.3% *Enterococcus spp* 6.3% and fungi 8%, represents 8.0 % in table (3). Recent study shown similar result in Iraq by Al-Quraishi (2021) (15), the results were similar, as *E.coli* 68%, *Klebsiella pneumonia* 15%, *Pseudomonas aeruginosa* 10%, *Proteus mirabilis* 6%, *Staphylococcus* 65% and *Enterococcus* 35%. In Iraq a study by Khalil *et al.* (2016) (16) found that *E. coli* was the most frequent cause of UTIs in patients (28.6%), led by *Klebsiella spp*. (21.7%), and *Staphylococcus spp*. (16.7%), out of a total of 143 urine samples.

**Table (3): Numbers and percentage of total specimens (N=180)**

| Bacteria | No. | Percentage % |
|---|---|---|
| ***E.coli*** | 50 | 44.7 |
| ***Klebsiella pneumonia*** | 18 | 16 |
| ***Pseudomonas aeruginosa*** | 9 | 8 |
| ***Proteus mirabilis*** | 3 | 2.7 |
| ***Staphylococcus aureus*** | 16 | 14.3 |
| ***Enterococcus spp*** | 7 | 6.3 |
| ***Fungi*** | 9 | 8.0 |
| **Total** | **112** | **100** |
| **Chi Square** | **-** | **85** |
| **p-value** | **-** | **<0.001** |

As for age, the age groups of (11-20) years had the highest rate of infection with 29.5%, followed by (21-30) years with a rate of 16.1% in table(4). While the age groups over 60 and (1-10) were at 12.5% and ( 51-60) and (31-40) were 10.7%, and the lowest percentage of infection was (31-40).

**Table (4): Percentages of infection by age groups**

| Age group | Number infection | Percentage % |
|---|---|---|
| 1-10 | 14 | 12.5 |
| 11-20 | 33 | 29.5 |
| 21-30 | 18 | 16.1 |
| 31-40 | 12 | 10.7 |
| 41-50 | 9 | 8.0 |
| 51-60 | 12 | 10.7 |
| Over 60 | 14 | 12.5 |
| **Total** | **112** | **100** |
| **Chi Square** | **-** | **21.5** |
| **P –value** | **-** | ****0.001** |

**Table (5): Percentages of infection by gender**

| Gender | Number | Percentage % |
|---|---|---|
| **Female** | 65 | 58 |
| **Men** | 47 | 42 |
| **Total** | 112 | 100 |
| **Chi Square** | - | 2.5 |
| **P –value** | - | 0.11 |

Where it was found that the rate of infection in females was 58 % and in men 47% which represent. This study is similar to Saeed *et al*., 2015 (17) study in Iraq, Erbil city, where the infection rate was (53.2% females, 46.8 % males) in table (5).

The antibiotic susceptibility test was carried out for *Escherichia coli* by Kirby Bauer disk diffusion method on a Mueller-Hinton agar according to. All identified *E.coli* were exposed to antimicrobial agents in table (6).

Similar findings were reported in a study carried out in Iraq by AL-Taie(18) which showed that out of 80 isolates of *E. coli* were resistant at different rates Ciprofloxacin (47%), Ceftazidime (97%), Cefepime 72%, Ceftriaxone (72%), Ampicillin (100%), and Gentamycin (67%) Tetracycline (23%) and Imipenem (7%).

**Table (6): Percentages of antimicrobial susceptibility test for 50 *E.coli* isolates**

| Antibiotic (A.B) | Resistant | | Intermediate | | Sensitive | | Chi Square | P –value |
|---|---|---|---|---|---|---|---|---|
| | Number Sample | % | Number Sample | % | Number Sample | % | | |
| **Amikacin** | 12 | 24 | 21 | 42 | 17 | 34 | 4.88 | 0.08 |
| **Ampicillin** | 37 | 74 | 12 | 24 | 1 | 2 | 81.68 | <0.0001 |
| **Imipenem** | 8 | 16 | 12 | 24 | 30 | 60 | 32.96 | <0.0001 |
| **Azetrenam** | 20 | 40 | 22 | 44 | 8 | 16 | 13.76 | 0.001 |
| **Cephalothin** | 25 | 50 | 19 | 38 | 6 | 12 | 22.64 | <0.0001 |
| **Cefepime** | 34 | 68 | 16 | 32 | 0 | 0 | 12.76 | <0.0001 |
| **Cefixime** | 38 | 76 | 7 | 14 | 5 | 10 | 82.16 | <0.0001 |
| **Ceftriaxone** | 41 | 82 | 5 | 10 | 4 | 8 | 106.64 | <0.0001 |
| **Ceftazidime** | 37 | 74 | 12 | 24 | 1 | 2 | 81.68 | <0.0001 |
| **Cefotaxime** | 39 | 78 | 8 | 16 | 3 | 6 | 91.28 | <0.0001 |
| **Cephalexin** | 20 | 40 | 22 | 44 | 8 | 16 | 13.76 | <0.0001 |
| **Ciprofloxacin** | 14 | 28 | 10 | 20 | 26 | 52 | 16.64 | <0.0001 |
| **Gentamycin** | 26 | 52 | 19 | 38 | 5 | 10 | 27.44 | <0.0001 |
| **Tetracyclic** | 39 | 78 | 4 | 8 | 7 | 14 | 90.32 | <0.0001 |

**Molecular identification of *E.coli* by*16S rRNA* gene, *OXA-48* and *CTX-M-1* gene**

All Phenotypically positive isolates of *E.coli* strains were subjected to molecular identification using the specific initiator of the *16S rRNA* gene to confirm its diagnosis by PCR. The results showed that all bacterial isolates belong to *E.coli* (Figure 1).

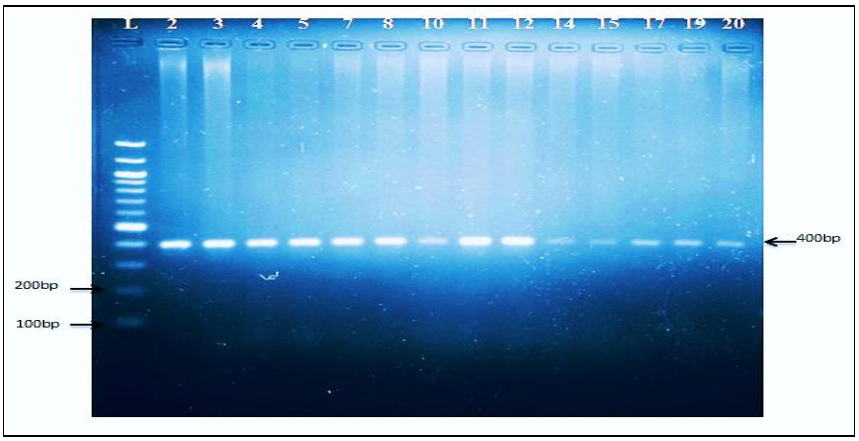


**Figure (1): The gel electrophoresis of PCR product of *16SrRNA gene* (400 bp) of *E.coli,* 100bp DNA ladder; (1% Agarose, 90 V for 70 mint).**

The positive isolates identified by the Vitek 2 system assay gave a positive result in the detection of 16SrRNA gene by polymerase chain reaction amplification. All isolates were confirmed to be *E. coli* using 16SrRNA-PCR. Included 30 (60%) *E. coli* were positive (100%).

The results of the current study agree with the results of the local study conducted by the researcher Ibrahim (2), where the percentage of bacterial isolates possessing 16 is 100%. The

current study also agrees with the study reached by the researcher Maleki *et al*. (19) in Iran.

The current results were similar to the study performed by Khalaf, *et al* (20) in Baghdad / Iraqi all 10 samples were identified as *E. coli* and it depends on the 16SrRNA gene 100%.

**Detection of *E. coli CTX-M-1* gene using conventional PCR**

To analyze the composition and molecular mechanisms of antibiotic resistance of Gram-negative bacteria - pathogens of urinary tract infections (UTIs) All the isolates were confirmed as *E. coli* using *CTX-M -1* PCR. Total 30 isolates included were positive (100%). The positive result isolates are shown in figure (2) .AL -Nassyriah city in Iraq by Lhwak and Abbas (21). This study was conducted for detection of extended spectrum β lactamases enzyme and *CTX-M-1* gene among *E.coli* isolates that causes urinary tract infections for pregnant women at gene *CTX-M-1* was found in 17 isolates from 57 (29.8%)in *E. Coli*. Results were reported in a study by Ahmed *et al*. (22) the results of bacterial examination showed successful confirmation of E. coli which has been isolated from samples of, detection of *CTX-M-1* gene was confirmed in 53.7% (36/67) of *E. coli* isolates.

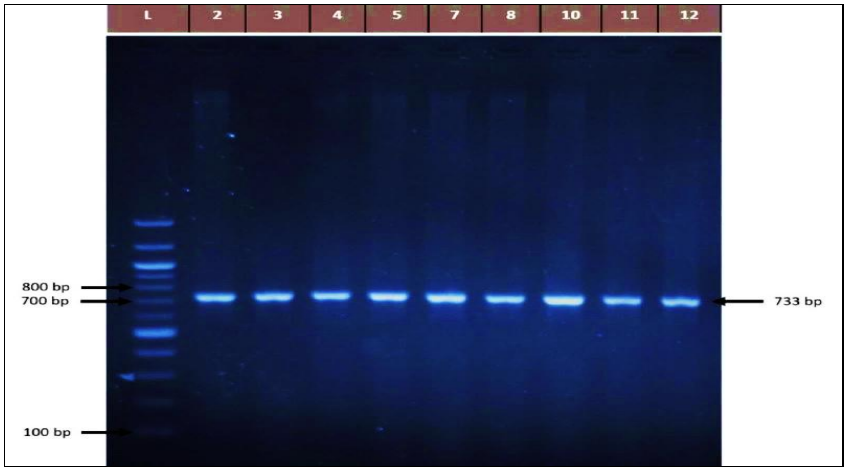


**Figure (2): The gel electrophoresis of PCR product of *CTX-M-1* gene (733 bp) of *E.coli*, 100bp DNA ladder; (1% Agarose, 90 V for 70 mints).**

**Detection of *E. coli OXA-48* genes using conventional PCR**

The results showed in our current study, among the 30 isolates that were entered into the PCR machine, only isolates were found, which represent about 23.3%, containing the *OXA-48* gene. The positive result isolates are shown in figure (3). The current results were similar to the study performed in Algeria by Loucif *et al.*(23) The genotyping results of carbapenemase among the obtained isolates are shown in of the 35 isolates, seven were carbapenemase producers, and four isolates were positive for were positive. *bla OXA-48*. However, a study conducted another study in Napal by Muktan *et at* (24). It has found that out of a total of 11 samples clinical that produce the enzyme carbapenemase, an isolate contains the *OXA-48* gene.

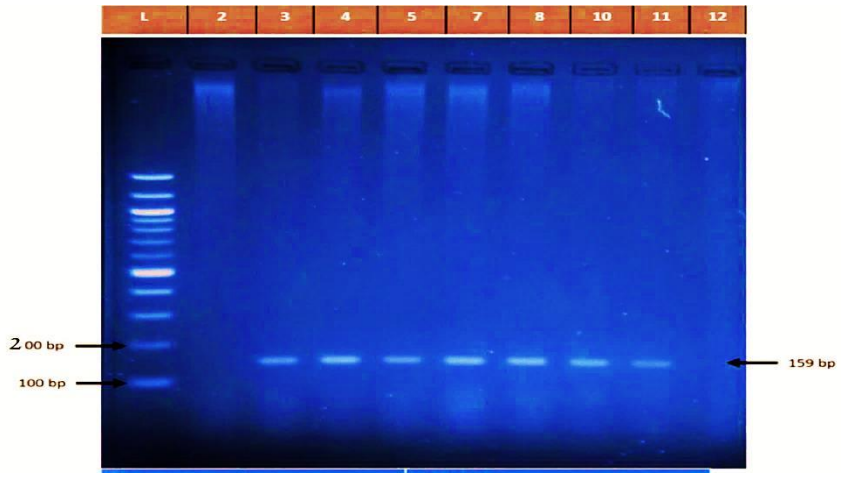


**Figure (3): The gel electrophoresis of PCR product of *OXA-48 gene* (159 bp) of *E.coli*, 100bp DNA ladder; (1% Agarose, 90 V for 70 mints).**

## Conclusions

The current study showed that most *E. coli* isolates have a high rate of antibiotic resistance. Ceftazidime is an effective cephalosporin antibiotic used in the treatment of urinary tract infection caused by *Escherichia coli.* Identification of isolates containing *OXA-48* and *CTX-M-1* genes using conventional PCR. These samples are resistant to cephalosporin groups when compared to the molecular and phonotypical susceptibility test.

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