Molecular Characterisation of ESBL producer E. coli

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Abstract:

Background: Antibiotic resistance emerged as clinical problem challenge the effective treatment of infections. Virulence factor may play an important role in the influence of antimicrobial resistance.

Objective: To determine the frequency of resistance gene in E. coli clinical isolates from women with urinary tract infection.

Materials and Methods: Fifteen E.coli clinical isolates were tested by PCR to determine their molecular characterization.

Results: The bla CTX –M gene was not detected in 6.7% out of the tested 15 E. coli clinical isolates from women with urinary tract infection. However, bla OXA gene was detected in all E. coli tested clinical isolates from pregnant women, female student and diabetic women with urinary tract infection. While bla TEM gene and bla SHV gene were not detected in 33.3% and 40% out of the tested E. coli clinical isolates respectively.

Conclusions: Four types of ESBL genes were detected, and shows new trend of distribution, which indicated the predominance of OXA and CTX-M genes.

Key words: Urinary tract infection, Resistance, PCR, , CTX, OXA, OXA, SHV.

Introduction:

Antibiotic resistance emerged as clinical problem challenge the effective treatment of infections [1-4]. Recurrent urinary tract infections and treatment failure are common in women with urinary tract infections [5,6]. These may be attributed to in proper selection of antibiotics and in proper course which was without consultation to a physicians [6]. However, host factors may play a role in such treatment outcomes [3,7], additionally, E. coli as a common causative agents of urinary tract infections could be with characteristics that influences the treatment failure and recurrent UTI [8-11].

Microbial genetics may play a role in the process of urinary tract infections and harbouring of gene that may attributed to antibiotic resistance and treatment failure is of importance [12]. Thus this study was conducted on ESBL producer E. coli clinical isolates to determine the frequency of resistance genes.
Material and Methods:

Study Population.

A prospective cross-sectional study was conducted during the period from 1st of June 2015 to the end of January 2016. The population included in the study were 563 women, of them 425 (75.5%) were outpatients and 138 (24.5%) were inpatients. Their age range between 18 and 80 years, with a mean age of 33.59±15.29 years. The study proposal was approved by the Ethical Committee of College of Science, Tikrit University and verbal informed consent taken from each woman before enrolment in the study. Fifteen clinical E. coli isolates were included in the study of the molecular characterization of the isolates, of them 5 isolates were collected from students, 5 from diabetic women and 5 from pregnant women. PCR

Biological materials

Enzymes:

Proteinase K: This enzyme was provided by Bio basic Inc.

Taq DNA polymerase: It was provided from Bio labs Company-England.

DNA extraction kit: Bio Basic Inc (Canada)

Molecular DNA Marker (100bp and 10 kb DNA ladder): It was purchased from New England Bio labs Inc.

Primers: These primers were provided by TIB MOLBIOL Company (Synthes labor GmbH Eerburgstra Be -Berlin -Germany). Four primers were used in this study. These primers and their sequences are listed in Table (1).

Table 1. Nucleotide sequences of PCR primers that is used in this study

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>(3’-5’) Primer sequence (5’-3’)</th>
<th>bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>bla TEM</td>
<td>F: CAT TTC CGT GTC GCC CTT ATT C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGT TCA TCC ATA GTT GCC TGA C</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>bla SHV</td>
<td>F: AGC CGC TTG AGC AAA TTA AAC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGT TCA TCC ATA GTT GCC TGA</td>
<td>759</td>
</tr>
<tr>
<td>3</td>
<td>bla CTX-M</td>
<td>F: TTA GGA ART GTG CCG CTG YA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: CGA TAT CGT TGG TGG TRC CAT</td>
<td>600</td>
</tr>
<tr>
<td>4</td>
<td>bla OXA</td>
<td>F: GGC ACC AGA TTC AAC TTT CAA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: GAC CCC AAG TTT CCT GTA AGT</td>
<td>900</td>
</tr>
</tbody>
</table>

*All primers were purchased from. TIB MOLBIOL Company Berlin -Germany

Bacteria Collection and DNA extraction

Genomic DNA from E.coli was isolated by using Genomic DNA Kit. The number of bacteria was appropriated and spined at 8,000 rpm for 5 minutes at room temperature. Then was removed supernatant completely and discarded then re-suspended in 200 μl cold TE buffer. Four hundred μl Digestion Solution was added to 200 μl sample from (step1) mixed well then added 3 μl proteinase K solution and incubate at 55 °C for 5 minutes to obtained complete listed. Then 260 μl of 100 % ethanol was added to the same solution and mixed well. The mixture applied into the column that is in a 2.0 ml collection tube. Then spined at 8,000 rpm for 1minute. The flow- through discard in the collection tube. Added 500 μl
of Wash solution, and spined at 8,000 rpm for 1 minute. Repeated washing. The flow – through discard and spined at 1,000 rpm for an additional minutes to remove residual amount of wash solution. The EZ-10 column Placed into a clean 1.5 ml microfuge tube. Added 30-50 μl elution buffers into the center part of membrane in the column. Incubated the tube at 55 ºC for 2 minutes. The column spined at 1,000 rpm for 1 minute to elute DNA quantity was Measured by UV absorption at A_{260} (1.0 OD unit is equivalent of 50 μg).

**Spectrophotometric Estimation of DNA**

The DNA samples were diluted 100 times with TE buffer. The optical density (OD) of each sample was read at wave length of (260 & 280 nm) with a spectrophotometer. The DNA concentration in the solutions was calculated according to the following formula:

\[
\text{DNA concentration (μg /μl) } = \frac{[\text{OD}_{260} \times 100 \times 50 \text{ μg/ml}]}{1000}
\]

Since, OD_{260} is optical density of DNA at 260 nm, 100 is the dilution factor, and 50 μg/ml is amounted of double stranded DNA when OD at 260 nm is 1. The purity of DNA was estimated depending on the ratio of OD_{260}/OD_{280}. This ratio for pure DNA is (1.6-1.8) [13].

**Preparation the PCR Master Mix.**

The optimization of parameter for PCR assay was carried out according to Table (1), four primers were used. Table (2) shows the components required for PCR reaction. *Taq* buffer, MgCl_2, dNTPs, primer solution and template DNA were thawed, mixed by vortex, and placed on ice. For optimization of each parameter, a master mix of all the reagents was stated above, and the reagent which assayed individually was added. The PCR master mix was poured into Eppendorf tubes, mixed by vortex and it was completed to 50 μl by adding sterile distilled water, and then placed inside thermal machine. PCR amplification cycles for virulence factors as shown in Table (3).

**Table (2) .The components required for PCR reaction.**

<table>
<thead>
<tr>
<th><em>Reagents</em></th>
<th>Stock conc.</th>
<th>Final conc.</th>
<th>Amount used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard <em>Taq</em> Reaction Buffer</td>
<td>10 X</td>
<td>10 X</td>
<td>5 μl</td>
</tr>
<tr>
<td>MgCl_2</td>
<td>25 mM</td>
<td>2.5 mM</td>
<td>1 μl</td>
</tr>
<tr>
<td>Primers</td>
<td>50 picomoles</td>
<td>3 pmole</td>
<td>2 μl</td>
</tr>
<tr>
<td>Deoxynucleotide Solution Mix</td>
<td>10 mM</td>
<td>200 μM</td>
<td>2 μl</td>
</tr>
<tr>
<td><em>Taq</em> DNA polymerase</td>
<td>(5U/ml)</td>
<td>1.25 units/50 μl PCR</td>
<td>0.3 μl</td>
</tr>
<tr>
<td>DNA Template</td>
<td>25-50 μg</td>
<td>5 μl</td>
<td></td>
</tr>
<tr>
<td>D.D.W</td>
<td>10.25 μl</td>
<td>34.7 μl</td>
<td></td>
</tr>
<tr>
<td>Total volume</td>
<td></td>
<td>50 μl</td>
<td></td>
</tr>
</tbody>
</table>

*All reagents were purchased from New England BioLab Inc*
Table (3). PCR amplification cycles for virulence factor

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>94°C</td>
<td>10 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>20 sec.</td>
<td>25-30</td>
</tr>
<tr>
<td>Annealing</td>
<td>Depending on primers</td>
<td>30 sec.</td>
<td></td>
</tr>
<tr>
<td>Elongation</td>
<td>72°C</td>
<td>20 sec.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>10 min</td>
<td></td>
</tr>
</tbody>
</table>

**Agarose Gel Electrophoresis**

Agarose gels (0.7%) was made by adding 0.7 gm of agarose to 100 ml of 1X TBE buffer solubilized by heating at boiling temperature, then the agarose was left to cool at 55 °C before pouring in a plastic plate to solidify. A comb was placed near one edge of gel, and the gel was left to harden. 1XTBE was poured into gel tank and the gel plate was placed horizontally in electrophoresis tank, 3μl of loading buffer was mixed with 2ml DNA samples, and then samples were added carefully to individual wells. [13] agarose gels were stained with ethidium bromide by immersing them in D.W containing the dye of final concentration of 0.5μg/ml for 30-45 minute.

**Statistical Analysis.**

The data presented as percentages and means according to their appropriateness. Statistical analysis was performed using SPSS package [version 20]. Chi square was used to calculate significance for frequency, while t test used to determine significance in mean difference. Multiple antibiotic resistance index [MARI] calculated by dividing the number of resistance isolates antibiotic to the total tested isolates [14]. While multidrug resistance value was considered when the isolates were resistant to more/ equal to 3 antibiotics belong to different antimicrobial agents group.

**Results:**

The *bla* CTX–M gene was not detected in 1 (6.7%) out of the tested 15 *E. coli* clinical isolates from women with urinary tract infection. This *bla* CTX-M negative *E. coli* isolate was from diabetic women with UTI. All the positive isolates demonstrated a single band with 600 bp. All *E. coli* isolates from female student and pregnant women were *bla* CTX-M positive, Fig. (1) and Table (4).
Figure (1). Molecular detection of bla CTX-M antibiotic resistance genes. PCR amplification of bla CTX-M gene in E. coli

The bla OXA gene was detected in all E. coli tested clinical isolates from pregnant women, female student and diabetic women with urinary tract infection. The isolates show 1 to 3 band, 60% [9/15] show single band, 13.3% [2/15] show double band, and 20% [3/15] show 3 band, with 170 to 900 bp, Fig(2) and Table (1).

Figure(2). Molecular detection of bla -OXA antibiotic resistance genes. PCR amplification of bla-Oxa gene in E. coli

The bla TEM gene was not detected in 5 (33.3%) out of 15 tested E. coli clinical isolates. The bla TEM gene negative isolates was 1 (6.7%) from female student, 2 (13.4%) from diabetic women, and 2 (13.4%) from pregnant women. The bla TEM gene positive isolates were 9 (60%) with single band and 1 (6.7%) with double band, (500-800 bp), Fig(3) and Table(1).
Figure (3). Molecular detection of \textit{bla} TEM antibiotic resistance genes. PCR amplification of \textit{bla}-TEM gene in \textit{E. coli}

The \textit{bla} SHV gene was not detected in 40% [6/15] of the \textit{E. coli} tested isolates. Of the total 6 negative isolates, 2 [13.4%] were from female student, 3 [20%] from diabetic women and 1 [6.7%] from pregnant women. All the \textit{bla} SHV gene were with single band [759 bp], Fig (4) and Table (1).

Figure (4). Molecular detection of \textit{bla} SHV antibiotic resistance genes. PCR amplification of \textit{bla}-HSV gene in \textit{E. coli}

The frequency of combination was 6.67% for CTX-M with OXA; TEM with OXA, and OXA, TEM with SHV. While combination of CTX-M, OXA with TEM forms 26.67%, however, CTX-M, OA with SHV also forms 26.67%. The four gene combination accounted for 26.67% and this finding demonstrated 6 trends of gene combination, Table (2).

The higher frequency rate of \textit{E. coli} antibiotic resistance gene was demonstrated by \textit{bla}-OXA gene (100%; 15/15), followed by \textit{bla} CTX-M gene [86.7%, 13/15], and \textit{bla}-TEM gene (66.7%, 10/15), while the lowest one was \textit{bla} SHV gene which detected in 60% (9/15) of the isolates. In addition, higher number of band were demonstrated by \textit{bla} OXA gene, which was 21 band in 15 positive isolate (ratio =1.4), 13 band in 13 positive \textit{bla} CTX-M gene positive isolate (ratio=1), 10 band in 10 \textit{bla} TEM positive isolate (ratio=1), and 9 band in 9 \textit{bla} SHV positive isolate (ratio=1), Table (1).
Multiple antibiotic resistance rate ranged from 9 to 17 from 23 tested antibiotics (39.1%-73.9%). The \textit{E. coli} isolates that demonstrated resistance to 17 antibiotics was positive for \textit{bla} CTX-M, \textit{bla} OXA and \textit{bla} TEM genes and negative for \textit{bla} SHV gene. While the isolates that demonstrated resistance to 16 antibiotics, 2 of them were positive for \textit{bla} CTX-M, \textit{bla} OXA and \textit{bla} TEM genes and negative for \textit{bla} SHV gene. However, the 3rd isolate that was resistant to 16 antibiotics was positive for the 4 genes. Thus the higher rate of multiple antibiotic resistance were demonstrated in \textit{E. coli} isolated that were positive for \textit{bla} CTX-M and \textit{bla} OXA genes, however, there was no specific trend of positivity association with multiple antibiotic resistance, Table (3).

### Table (1). Resistance gene in \textit{E. coli} clinical isolates from women with urinary tract infection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Students</th>
<th>Pregnant Women</th>
<th>Diabetes Women</th>
<th>Total number of bands</th>
<th>Mean of MDR ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>\textit{bla} CTX-M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MDR</td>
<td>-</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>No. bands</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>bla OXA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MDR</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>No. bands</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>\textit{bla} TEM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MDR</td>
<td>7</td>
<td>-</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>No. bands</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Multiple antibiotic resistance</td>
<td>11</td>
<td>9</td>
<td>17</td>
<td>12</td>
<td>9</td>
</tr>
</tbody>
</table>

### Table (2). Resistance Gene Combination Frequency

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Gene combination</th>
<th>Number [%]</th>
<th>Antibiotics Number</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>CTX-M, OXA</td>
<td>1 [6.67]</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>OXA, TEM</td>
<td>1 [6.67]</td>
<td>13</td>
<td>7.5</td>
</tr>
<tr>
<td>1</td>
<td>OXA, TEM, SHV</td>
<td>1 [6.67]</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>2,3,13,14</td>
<td>CTX-M, OXA, TEM</td>
<td>4 [26.67]</td>
<td>15±3.7</td>
<td>6.7±0.51</td>
</tr>
<tr>
<td>5,7,11,15</td>
<td>CTX-M, OXA, SHV</td>
<td>4 [26.67]</td>
<td>12±1.89</td>
<td>5.2±1.09</td>
</tr>
<tr>
<td>4,6,8,10</td>
<td>CTX-M, OXA, TEM, SHV</td>
<td>4 [26.67]</td>
<td>12±2.63</td>
<td>5.2±0.52</td>
</tr>
</tbody>
</table>
Table (3). Comparison of MDR between CTX-M, TEM and SHV positive and negative isolates

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mean ± SD</th>
<th>T Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>TEM</td>
<td>4.80 ± 1.09</td>
<td>6.30 ± 1.56</td>
<td>1.91</td>
</tr>
<tr>
<td>CTX-M</td>
<td>6.50 ± 0.71</td>
<td>5.61 ± 1.12</td>
<td>1.07</td>
</tr>
<tr>
<td>SHV</td>
<td>6.33 ± 0.52</td>
<td>5.38 ± 1.19</td>
<td>1.82</td>
</tr>
</tbody>
</table>

Discussion

The *bla* CTX-M gene was not detected in 14 [93.3%] out of the tested 15 *E. coli* clinical isolates from women with urinary tract infection. This *bla* CTX-M negative *E. coli* isolate was from diabetic women with UTI. All the positive isolates demonstrated a single band with 600 bp. All *E. coli* isolates from female student and pregnant women were *bla* CTX-M positive. [15], Japan, CTX-M9 was detected in 55.9% of the *E. coli* clinical isolates and forms the predominantly detected ESBL genotype. However, other studies indicated variation in the frequency of CTX-M genotypes, demonstrated different trend of frequency in Japan, Pakistan and India [16,17]. Numerous studies, CTX-M ESBL producing *E. coli* emerged as an etiology of community acquired UTI in India, Hong Kong, Europe, USA and Japan [15,18-21]. Lartigue et al. [22], France, found that 1/3 of ESBL positive EC were CTX-M genotype [CTX-M-3, CTX-M-10, CTX-M-14 and CTX-M-15] in clinical isolates from hospitals located in Paris, however, during the same period, other studies reported that 90% of ESBL positive were CTX-M-3 CTX-M-24 in isolates from hospital outside Paris [23,24]. Other two studies reported that CTX-M-15 was the predominant ESBL genotype in other hospital in France and UK [19,25].

Dallenne et al [26], In France, it was found that CTX-M-15 genotype of ESBL *E. coli* was the predominant; however, CTX-M-1 and CTX-M-9 were also detected. [27], China, reported that ESBL CTX-M genotype form 42.34% of the total isolates and 33.3% were CTX-M-14 and 10.8% were CTX-M-15 type. Yazdi et al., [28], Turkey, found that 68.8% of ESBL producer *E. coli* isolates were carrying CTX-M genotype. Harada et al., [29], Japan reported that ESBL positive *E. coli* carrying CTX-M genotype in 25% of their tested isolates, in particular CTX-M-9 group. Kang et al., [30], Korea, study shows that CTX-M was predominant and form 76.7% in ESBL producer *E. coli*. Ludden et al., [31], Ireland, found that 97% of ESBL producer *E. coli* isolates harboured CTX-M genotype. Another study from Korea [32] showed that 90% of ESBL producing *E. coli* were of CTX-M genotype. In Iraq, previous studies indicated that CTX-M gene was the most frequently detected in ESBL *E. coli* isolates [33-35].
The \textit{bla} OXA gene was detected in all \textit{E coli} tested clinical isolates from pregnant women, female student and diabetic women with urinary tract infection. The isolates show 1 to 3 bands, 60\% (9/15) show single band, 13.3\% (2/15) show double band, and 20\% (3/15) show 3 bands, with 170 to 900 bp. In addition, the \textit{bla} TEM gene was detected in 10 (66.7\%) out of 15 tested \textit{Ecoli} clinical isolates. The \textit{bla} TEM gene negative isolates was 1 (6.7\%) from female student, 2 (13.4\%) from diabetic women, and 2 (13.4\%) from pregnant women. The \textit{bla} TEM gene positive isolates were 9 [60\%] with single band and 1 (6.7\%) with double band, (500-800 bp). Furthermore, the \textit{bla} SHV gene was not detected in 40\% (6/15) of the EC tested isolates. Of the total 5 negative isolates, 2 (13.4\%) were from female student, 3 (20\%) from diabetic women and 1 (6.7\%) from pregnant women. The entire \textit{bla} SHV gene was with single band (759 bp).

Thus present study data indicated that OXA gene demonstrated in 100\% of ESBL \textit{E. coli} uropathogenic isolates tested in this study, while TEM gene presented in 66.7\% of the isolates and 60\% were harbouring SHV gene. Previous studies reported variable gene frequencies, 76\% for OXA in Ireland [31], while TEM gene reported in 86.24\% of \textit{E. coli} ESBL isolates in Turkey [28], 40\% in Japan [29], 34\% in Ireland [31], 16.81\% in Iran [36], 10\% in Iraq [33], 5\% in Kenya [37], and 0.9\% in Japan [15]. But TEM genotype was found in 80\% of \textit{E. coli} ESBL isolates in Japan [29], 72.23\% in Turkey [28], 12.1\% in Iran [36], 3.33\% south Korea [32], 3\% in Ireland [31], 3\% in Kenya [37] and 2.8\% in Japan [15].

The above frequencies varies with geographical areas of the performed studies, however, the global trends indicated an increase of CTX-M gene in ESBL \textit{E. coli} isolates and became responsible for outbreak of community and hospital acquired UTI infection [15,22,38-43]. The higher frequency rate of \textit{E. coli} antibiotic resistance gene was demonstrated by \textit{bla}-OXA gene (100\%; 15/15), followed by \textit{bla} CTX-M gene (86.7\%, 13/15), and \textit{bla}-TEM gene (66.7\%, 10/15), while the lowest one was \textit{bla} SHV gene which was detected in 60\% (9/15) of the isolates. In addition, higher number of band were demonstrated by \textit{bla} OXA gene, which was 21 band in 15 positive isolate [ratio =1.4], 13 bands in 13 positive \textit{bla} CTX-M gene positive isolate (ratio=1), 10 bands in 10 \textit{bla} TEM positive isolate (ratio=1), and 9 band in 9 \textit{bla} SHV positive isolate (ratio=1). Multiple antibiotic resistance rates ranged from 9 to 17 antibiotics from 22 tested panels (40.9\%-77.27\%). The EC isolates that demonstrated resistance to 17 antibiotics was positive for \textit{bla} CTX-M, \textit{bla} OXA and \textit{bla} TEM genes and negative for \textit{bla} SHV gene. While the isolates that demonstrated resistance to 16 antibiotics, 2 of them were positive for \textit{bla} CTX-M, \textit{bla} OXA and \textit{bla} TEM genes and negative for \textit{bla} SHV gene. However, the 3rd isolate that was resistant to 16 antibiotics was positive for the 4 genes. Thus the higher rate of multiple antibiotic resistance were demonstrated in \textit{E. coli} isolates that were positive for \textit{bla} CTX-M and \textit{bla} OXA genes, however, there was no specific trend of positivity association with multiple antibiotic resistance.
ESBL producer *E. coli* isolates harbouring different resistance genes showed variable MDR mean values, isolates with TEM gene show higher MDR [6.30±1.06] and with wider range between the tested isolates. While isolates harbouring SHV showed the lower MDR mean value [5.38±0.98], however, OXA and CTX-M harbouring isolates showed MDR mean value of 5.67 and 5.61 respectively. Thus, the tested isolates were with MDR to antimicrobials from more than 5 antibiotics groups who harbour CTX-M, OXA and SHV, while those with TEM genes were with multidrug resistance to antibiotics belong to more than 6 antimicrobial groups. The mean MDR of TEM, CTX-M and SHV positive *E. coli* isolates were significantly higher than those TEM, CTX-M and SHV negative isolates. In addition, when the analysis performed on individual antibiotic strata it indicated that the frequency of resistance rate to antibiotics between *bla* CTX-M, *bla* TEM and *bla* SHV genes positive *E. coli* and those negative isolates indicated differences. Thus the present study findings illustrated an association between frequency, MARI and MDR and carrying of the isolates for TEM, CTX-M and SHV as compared to non-harbouring isolates.

The frequency of combination was 6.67% for CTX-M + OXA; TEM + OXA, and OXA + TEM + SHV. While combination of CTX-M + OXA + TEM form 26.67%, and CTX-M + OXA + SHV also form 26.67%, the four gene combination accounted for 26.67%. Thus this study finding demonstrated a 6 trends of gene combination and none of the 15 tested *E. coli* isolates harbouring a single gene. Harada et al., [29], Japan, found that the predominant genotype was CTX-M + TEM which forms 48.3% for *E. coli*. Single TEM or SHV or CTX-M accounted for 8.1%, 3.1% and 28.2% respectively, while TEM+SHV genotypes form 1.3% and CMX-M + SHV genotypes form 0.6%. Presence of more than gene in the same isolates was reported in literature and previous studies reported that co-existence of CTX-M and TEM is the predominant combination in studies performed in Iran, India and Saudi Arabia.[44] which contrasted with the present study finding.

In the present study four types of ESBL genes were detected, and shows new trend of distribution, which indicated the predominance of OXA and CTX-M genes. The high rate of CTX-M genotype was consistent with that reported for Iran (74%) [44], Lithuania (96%) [45], Portugal (76%) [46], and Turkey (83.18%) [47]. However, other studies reported the predominance of TEM genotype of ESBL in Turkey (72.7%) [48], Sweden (63%) [49], Italy (45.4%) [50] and Portugal (40.9%) [51], which lower to the present study finding (66.7%). SHV genotype detected in 60% of *E. coli* isolates tested in this study, which was higher to the rate reported for India (57.5%) [52], Iran (45%) [44], Saudi Arabia (23.1%) [53], Turkey (9.09%) [48], Sweden (6%) [49] and Thailand (3.8%) [54], which contracted with the present study findings.
In a recent study in Iraq [55], reported that 93.3% of their patients with recurrent UTI were with multidrug resistance, however, their interpretation represent multiple antibiotic resistance rate rather than multidrug resistance rate. Our MDR rate was 55.95% in ESBL producer *E. coli* and was 62.1% in ESBL negative *E. coli* isolates. Daoud et al., [56], In Lebanon, it was found that ESBL CTX-M gene present in 100% of the tested isolates, while TEM present in 68.2%, SHV in 30.7% and OXA in 29.6%. In contrast, our study indicated that OXA harbored by *E. coli* in 100% of the isolates, followed by CTX-M in 93.3%, TEM in 66.7% and SHV in 60%, a trend which was different from that for Lebanon. In addition, 26.67% of the *E. coli* isolates carrying 4 genes, which was higher to that reported by others as the corresponding value was 15.9% [56].

Another study performed in Libya showed that OXA was found in 76.9% of *E. coli* urinary isolates, TEM in 53.8%, and CTX-M in 46.2% [57]. While, Bourjilat et al.,[58], Casablanca, Morocco, found that CTX-M genes in 85.7% of isolates collected during 2004 - 2007. Shi *et al* [59], China, found that TEM ESBL genotype was with a range of 63.2% to 96.7% in 5 hospitals, while OXA with a range of 10.5% - 33.3% and overall rate of 21.5% in the five hospitals, but SHV was with a range of 0% - 30% and overall rate of 17.7%. In Sweden, OXA ESBL gene accounted for 59%, while SHV was detected in only 6% of ESBL producer *E. coli* isolates [49].

Potron *et al*[60], studied the spread of OXA ESBL *Enterobacteriaceae* and concluded that this gene spread through single plasmid and identified multiple cases of importation of OXA-48 ESBL in Europe and gene endemicity in France. North African and Middle East countries are reservoirs of OXA-48 ESBL gene [61]. OXA-48 ESBL gene firstly in *K. pneumonia* in Turkey in 2001 and later in *E. coli* in Turkey in 2010 [62,63] and detected for several years in Turkey only and in hospital setting [63]. However, in the present study *E. coli* harbouring OXA ESBL was from community and hospital acquired infections and not confined mainly to inpatients as mentioned above.

ESBL producers *E. coli* isolates have become public health problems due to its role as a cause of UTI with the emergence of new trends of ESBL genes such as CTX-M and OXA in both hospital and community acquired infections [64,65]. In Lebanon, [66], found that CTX-M, OXA, TEM, and SHV ESBL genes were detected in rate of 98.6%, 45.2%, 21.9% and 4.1% respectively in *E. coli* ESBL producers isolates. In addition, the co-existence of CTX-M+TEM form 8.2%, CTX-M + OXA form 30.14%, CTX-M+SHV form 2.74%, CTX-M+OXA+TEM form 12.33%, and CTX-M+OXA+TEM+SHV form 1.37%, while CTX-M alone form 43.8% and OXA form 1.37%. However, the present study shows only 3 comparable combination, CTX-M +OXA form 6.67%, while in Sana et al [66] study form 30.14%; while CTX-M+OXA+TEM form 26.67% in our cohort and 12.33% in Sana study, and the combination of 4 genes from 26.67% in our study and 1.37% in Sana study.
Almayahie and Alkuraishy [55] in a recent study in Iraq, reported that CTX-M ESBL gene was detected in 70.3% of *E. coli* isolates, while OXA gene present in 32.9%, SHV in 10.9% and TEM was not detected in any isolate. Co-existence of genes was found in two forms, the SHV+CTX-M (6.5%) and OXA+CTX-M (32.9%). In the present study, the CTX-M +OXA gene combination with rate of 6.67%, which is much lower to that of Almayahie and Alkuraishy, [55], but others co-existence pattern in our study was not reported by them.

The *E. coli* bla TEM producer isolates were detected in 66.7% of the tested clinical isolates from women with urinary tract infection, and was higher to that reported in Sweden (63%) [49]. The EC bla TEM positive isolates were 60% from each of pregnant and diabetic women groups, while 80% from female student group.

The global distribution of *E. coli* ESBL genes varied between different studies, however, in general the CTX-M gene emerged after SHV and TEM, but now was the predominant worldwide [22,39,40,49,65,67-73]. In the present study CTX-M accounted for 93.3% of *E. coli* ESBL producer isolates, while OXA gene was the predominant gene. The predominance of OXA gene harbouring is firstly reported in this study.

In conclusion, the OXA ESBL gene was detected in all tested *E. coli* isolates followed by CTX-M gene [93.3%], TEM [66.7%] and SHV [60%] and the presence of these genes significantly influenced the emergence of antibiotic resistance of the tested isolates.

References


