CTX-M Genotyping among Cystitis Associated Cefotaxime-Resistant Uropathogenic *Escherichia coli* (CRUPEC)

Ali M. Al-Zuhairy and Hussein O. Al-Dahmoshi

Biology Department, College of Science, University of Babylon, Hilla, Iraq.

http://dx.doi.org/10.22207/JPaM.12.3.56

(Received: 10 August 2018; accepted: 12 September 2018)

Urinary tract infection (UTI) is the second most common bacterial infection and important public health problem of human among all age groups from neonate to geriatric. Uropathogenic *Escherichia coli* (UPEC) accounts for approximately 85% of community acquired UTIs and 50% of hospital acquired UTIs. The current study aimed to investigate dominant CTX-M genotypes among local UPEC isolated from patients with cystitis. Phenotypic detection of Cefotaxime-Resistant Uropathogenic Escherichia coli (CRUPEC) were performed by Kirby-Bauer disk diffusion method (CLSI 2016). The DNA were extracted and investigation of four resistance genes blaCTX-M by a multiplex PCR assay using specific primer pairs. The results of phylogenetic subgrouping of UPEC using multiplex PCR to detect chuA (279bp), yjaA (211bp) and TspE4.C2 (152bp) revealed that among 56 CRUPEC 47 (83.93%) were ExPEC (37 (66.07%) for B2, 6 (10.72%) for B2*, 3 (5.36%) for D1 and 1 (1.78%) for D2). InPEC compile 9 (16.07%). Genotypic investigation of cefotaxime resistance among 56 CRUPEC were performed using specific four primer pairs to detect the genotypes of blaCTX-M clusters (I, II, III and IV). Among 65 CRUPEC, 36 (64.28%) were positive for for blaCTX-MI, 38 (67.85%) for blaCTX-MII, 26 (46.43%) for blaCTX-MIII and 10 (18.14%) for blaCTX-MIV (Figure 9). Concern possessing of isolates for more than one blaCTX-M genotypes were also reported in this study. Our results revealed that blaCTX-M were present in 50 (89.29%) of CRUPEC. Co-existence of more than one genotypes were reported in 38 (67.86%). The results displayed that 40 (80%) of blaCTX-M positive CRUPEC were belong to group B2, 4 (8%) for group D, 3 (6%) for group A and 3 (6%) for group B1. The current study conclude the presence of blaCTX-M clusters (I, II, III, IV) as a main mechanism of cefotaxime resistance among CRUPEC and coexistence of multiple clusters within same isolates that may leads to emergence of new hybrids of blaCTX-M.

**Keywords:** CRUPEC, UTIs , blaCTX-M, Genotypes.

Urinary tract infection (UTI) is the one of the most common bacterial infection in humans and a major cause of morbidity and represent an important public health problem of all ages from neonate to geriatric age group (Mazzariol et al., 2017). Among of the most common infectious diseases, second ranking after respiratory tract infection is urinary tract infection which involve about 250 million people in developing countries annually. (Piranfar et al., 2014). It is classified to bladder infection (cystitis) and kidney infection (pyelonephritis), which can be either symptomatic or asymptomatic (Prakasam et al., 2012). Although diuent causative agents can be responsible for UTIs, bacteria are the major cause being responsible for more than 95% of UTI cases. The *Escherichia coli* (E.coli) accounts for approximately 85% of community acquired UTIs and 50% of hospital acquired UTIs (Ahmad et al., 2015). Clermont and colleagues developed a triplex PCR assay to detect the genes chuA, yjaA, and TspE4.C2 in 2000. Regarding the presence/absence of these three genes, an E. coli strain could be classified into one of the main phylogroups intestinal pathogenic E. coli (InPEC) (include A, B1 group) while extraintestinal pathogenic E. coli (ExPEC) (include B2, or D group) (Clermont et al.,
To increase the discrimination power of *E. coli* population analyses, it has been proposed the use of subgroups A0, A1, B1, B22, B23, D1 and D2, that are determined by the combination of the genetic markers (Escobar-Páramo et al., 2006).

Cefotaxime is one of third generation cephalosporins with broad spectrum activity and regards one of the WHO Model List of Essential Medicines, contains the medications considered to be most effective and safe to meet the most important needs in a health system (WHO, 2015). It is widely used as post-surgery infection prophylaxis and also used for UTIs. Cefotaxime is one of the extend spectrum cephalosporin that resist hydrolysis by β-lactamase but it is sensitive to one of the extended spectrum β-lactamases (ESBLs). The name ‘CTX’ is an abbreviation for ‘ceftaximase’ and refers to the potent hydrolytic activity of these enzymes against cefotaxime. CTX-M-type β-lactamases constitute a relatively small but growing group of ESBLs. Resistance to cefotaxime conferred by *blaCTX-M* (Bush class A β-lactamases) (Bush and Jacoby, 2010). CTX-M enzyme were common among community acquired and hospital acquired infection with *E. coli* which regards the most important pathogen producing these enzymes (Coque et al., 2008). Chromosome-encoded genes of intrinsic ceftaximases in Kluyvera spp. are proposed to be the progenitors of CTX-M family (Zhao and Hu, 2013). Most of CTX-Ms exhibit powerful activity against cefotaxime and ceftizoxime and some of them for ceftazidime. The family of CTX-M enzymes is grouped on the basis of similarities in amino acid sequences into four major phylogenetic groups: the CTX-M-1 group (CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12, CTX-M-15, CTX-M-22, CTX-M-23, CTX-M-28, CTX-M-29, CTX-M-30

### Table 1. Primer pairs with amplicon size for *blaCTX-M* genotyping

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primer sequence (5’-3’)</th>
<th>Product (bp)</th>
<th>Annealing (°C)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>chuA</em></td>
<td>F GACGAACCAACGGTGCCAGGAT</td>
<td>279</td>
<td>59</td>
<td>(Clermont et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>R TGCCGCCGATACCAAGGACAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>yjaA</em></td>
<td>F TGAGGATGTCAGGAGGCGCTG</td>
<td>211</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R ATGGAGAATGCGTTCCTCAAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSpE4.C2</td>
<td>F GAGTAATGTCGGGGCTATCA</td>
<td>152</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R CCGGCAACAAATGATTACGC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla CTX-M-I</em></td>
<td>F GACGATGTCAGGCCTGCA</td>
<td>499</td>
<td>55°C</td>
<td>(Kiiru et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>R AGCCCGCCTGATACTCAGA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla CTX-M-II</em></td>
<td>F GGCAGTCGGCCAGCAGCAGCA</td>
<td>351</td>
<td>55°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R CGGTGACCGCTGATTACG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla CTX-M-III</em></td>
<td>F CGCTTGGGAGGGAGGACC</td>
<td>305</td>
<td>55°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R GCTCAGTAGGTACGAGGACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla CTX-M-IV</em></td>
<td>F GCTGGAAGGAAAGCAGAGGAG</td>
<td>474</td>
<td>62°C</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Distribution of CRUPEC among phylogenetic subgroups

<table>
<thead>
<tr>
<th>Total</th>
<th>No. (%)</th>
<th><em>chuA</em>/yjaA/TspE4.C2</th>
<th>Phylogenic subgroup</th>
<th>Phylogenic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>9(16.07%)</td>
<td>2(3.57%)</td>
<td>-ve/-ve/-ve</td>
<td>Subgroup A0</td>
<td>Group A</td>
</tr>
<tr>
<td></td>
<td>2(3.57%)</td>
<td>-ve/+ve/-ve</td>
<td>Subgroup A1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5(8.93%)</td>
<td>-ve/-ve/+ve</td>
<td>B1</td>
<td>Group B1</td>
</tr>
<tr>
<td>47(83.93%)</td>
<td>6(10.72%)</td>
<td>+ve/+ve/+ve</td>
<td>Subgroup B2</td>
<td>Group B2</td>
</tr>
<tr>
<td></td>
<td>37(66.07%)</td>
<td>+ve/+ve/+ve</td>
<td>Subgroup B2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3(5.36%)</td>
<td>+ve/-ve/-ve</td>
<td>Subgroup D1</td>
<td>Group D</td>
</tr>
<tr>
<td></td>
<td>1(1.78%)</td>
<td>+ve/-ve/+ve</td>
<td>Subgroup D2</td>
<td></td>
</tr>
</tbody>
</table>
and CTX-M-32), the CTX-M-II group (CTX-M-2, CTX-M-4, CTX-M-5, CTX-M-6, CTX-M-7, CTX-20), the CTX-M-III group (CTX-M-8), and the CTX-M-IV group (CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-18, CTX-M-19, CTX-M-21 and CTX-M-27) (Kiiru et al., 2012). The current study aimed to investigate dominant CTX-M genotypes among local UPEC isolated from patients with cystitis.

**MATERIALS AND METHODS**

**Bacterial Isolates**

Fifty six UPEC isolates with confirmed resistance to cefotaxime (according to CLSI, 2016) were selected to study the phylogenetic subgroups using specific primer pairs to amplify chuA, yjaA and TspE2.C4 genes. Genotyping of blaCTX-M were performed using specific four pairs of primers for blaCTX-M-I, blaCTX-M-II, blaCTX-M-III and blaCTX-M-IV groups.

**Extraction of Genomic DNA**

Favor Prep™ Genomic DNA Mini Kit (Favorgen/Taiwan) was used to extract genomic DNA from *E. coli* isolates following the manufacturer’s protocol.

**Polymerase Chain Reaction**

Fifty-six isolates were screened for the resistance genes CTX-M by a multiplex PCR assay using specific primer pair (Table 1). PCR amplification reactions were performed in a volume of 20 µl containing. The cycling parameters were as follows: an initial denaturation at 94°C for 2 min; followed by 30 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 30s; and with a final extension at 72°C for 5 min (Clermont et al., 2000; Kiiru et al., 2012). The amplified PCR products were subjected to electrophoresis at 1.5 % agarose gel in 0.5X TBE buffer.

---

**Fig. 1.** 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to blaCTX-M-I amplicon (499bp) and blaCTX-M-III amplicon (305bp); lane M represent DNA marker size(100bp) while E1-E88 represent the isolates

**Fig. 2.** 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to blaCTX-M-I amplicon (499bp) and blaCTX-M-III amplicon (305bp); lane M represent DNA marker size(100bp) while E89-E118 represent the isolates

**Fig. 3.** 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to blaCTX-M-II amplicon (351bp); lane M represent DNA marker size(100bp) while E1-E39 represent the isolates
RESULTS

The results of phylogenetic subgrouping of UPEC using multiplex PCR to detect chuA (279bp), yjaA (211bp) and TspE4.C2 (152bp) revealed that among 56 CRUPEC 47(83.93%) were ExPEC (37(66.07%) for B2, 6(10.72%) for B2', 3(5.36%) for D1 and 1(1.78%) for D2). InPEC compile 9(16.07%) (Table 2). The results in accordance with many similar studies, ExPEC compile 80%-94.82% (Lee et al., 2016; Ochoa et al., 2016; Lara et al., 2017; Al-Khaqani et al., 2017; Salehzadeh and Zamani, 2018). Predominance of B2 subgroup were stated by many studies (Alizade et al., 2014; Merza and Jubrael, 2015; Al-Khafaji and Al-Thahab, 2017).

Genotypic investigation of cefotaxime resistance among 56 CRUPEC were performed using specific four primer pairs to detect the genotypes of blaCTX-M groups. Multiplex-PCR were used to detect blaCTX-M-I (499bp) and blaCTX-M-III (305bp) while monoplex-PCR for blaCTX-M-II (351bp) and blaCTX-M-IV(474bp) (Figures 1-8). The results revealed existence of all blaCTX-M genotypes in different percentage: 36 (64.28%) for blaCTX-MI, 38(67.85%) for blaCTX-MII 26(46.43%) for blaCTX-MIII and 18(32.14%) for blaCTX-MIV (Figure 9). Concern

Fig. 4. 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to blaCTX-M-II amplicon (351bp); lane M represent DNA marker size(100bp) while E40-E87 represent the isolates

Fig. 5. 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to blaCTX-M-II amplicon (351bp); lane M represent DNA marker size(100bp) while E88-E120 represent the isolates

Fig. 6. 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to blaCTX-M-IV amplicon (474bp) lane M represent DNA marker size(100bp) while E1-E40 represent the isolates
Fig. 7. 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to *blaCTX-M-IV* amplicon (474bp) lane M represent DNA marker size(100bp) while E41-E88 represent the isolates

Fig. 8. 1.5% Agarose gel electrophoresis at 72 volt for 90 minutes of PCR to *blaCTX-M-IV* amplicon (474bp) lane M represent DNA marker size(100bp) while E89-E120 represent the isolates

Fig. 9. Distribution of *blaCTX-M* genotypes among CRUPEC

possessing of isolates for more than one *blaCTX-M* genotypes were also reported in this study. Our results revealed that *blaCTX-M* were present in 50 (89.29%) of CRUPEC. Co-existence of more than one genotypes were reported in 38 (67.86%) (Table 3). The results displayed that 40 (80%) of *blaCTX-M* positive CRUPEC were belong to group B2, 4 (8%) for group D, 3 (6%) for group A and 3 (6%) for group B1. Our results were roughly similar to those stated by other researcher. Mohajeri et al., (2014) found that UPEC that have *blaCTX-M* compile (93.3%). Occurrence of *blaCTX-M* ranged from 70-90% (Hernandez et al., 2014; Micenková et al., 2014; Poovendran and Ramanathan, 2015; Al-Mayahie and Al Kuriashy, 2016; Nojoomi et al., 2016; Padmavathy et al., 2016).
Table 3. Distribution of cefotaxime resistance genotypes among UPEC

<table>
<thead>
<tr>
<th>Cefotaxime Resistance Genotypes</th>
<th>Phylogenetic Subgroups</th>
<th>A0</th>
<th>A1</th>
<th>B1</th>
<th>B2</th>
<th>B22</th>
<th>B23</th>
<th>D1</th>
<th>D2</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>A0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>38 (67.86)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>A1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>38 (67.86)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>B1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>14 (21.43)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>B2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6.4 (10.71)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>B22</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>(67.86)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>B23</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>(67.86)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>D1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6.4 (10.71)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>D2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>(67.86)</td>
</tr>
<tr>
<td>CTX-MI/CTX-MIII/CTX-MIV</td>
<td>Total</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>35</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>(100)</td>
</tr>
</tbody>
</table>

DISCUSSION

The dominance of B23 phylogenetic subgroup can be attributed to that most of ExPEC belong to group B2 and D while presence of isolated belong to intestinal phylogroups (A and B1) may be due to contamination and autoinoculation from feces in person with low personal hygiene (Cao et al., 2011; Luo et al., 2011; Merza and Jubrael, 2015; Abdullah and Lakshmidevi, 2016). BlaCTX-M is Extend spectrum beta-lactamase (ESBLs) with hydrolytic activity against cefotaxime and ceftiraxone with high susceptibility to tazobactam. The high prevalence of BlaCTX-M may attributed to the quick spread worldwide among both hospital and community acquired infections especially UTIs and so it called pandemic-BlaCTX-M (Cantón and Coque, 2006). The global spread of this enzyme may be due to high usage of third generation especially cefotaxime which leads to explosive of resistance. Also due to high spread of plasmid, transposon, integron and insertion sequence which ac a carrier for blaCTX-M may responsible for their high prevalence (Cantón et al., 2012). The spread of blaCTX-M may be via international traveler (Arcilla et al., 2017). There are more than 25 variant of BlaCTX-M and emergence of these types may be resulted from recombination as a result of presence of the two different blaCTX-M types (Sun et al., 2017). Dominance of blaCTX-MI were reported in many studies and blaCTX-M15 is the commonly documented variant among UPEC (Park et al., 2012; Castanheira et al., 2014; Hasan et al., 2015; Bonger et al., 2016; Mshana et al., 2016; Abrar et al., 2017; Hashemizadeh et al., 2018). The low prevalence of blaCTX-M-III and blaCTX-M-IV were also reported in many studies from different geographical region (Tekiner and Özpınar, 2016; Nairoukh et al., 2018). Concern co-existence of multiple genotypes of blaCTX-M clusters, it is very risky and may leads to new variant of blaCTX-M. Our results revealed 38/56 CRUPEC isolates with multiple blaCTX-M clusters. He et al., 2016 found that as a results of recombination of blaCTX-MI cluster (blaCTX-M-15) and blaCTX-MIV cluster (blaCTX-M-14), the new variants with high stability and catalytic activity will results like blaCTX-M-64. Several stable and highly active hybrid including blaCTX-M-123, blaCTX-M-137, and blaCTX-M-132 were also resulted from recombination of blaCTX-M-15 and blaCTX-M-14 (Nagano et al., 2009; He et al., 2013; Tian et al., 2014; He et al., 2015; Liu et al., 2015).
CONCLUSION

The current study conclude the presence of blaCTX-M clusters (I, II, III, IV) as a main one mechanism or cefotaxime resistance among CRUPEC and coexistence of multiple cluters within same isolates that may lead to emergence of new hybrides of blaCTX-M.

ACKNOWLEDGEMENT

I am warmly thanks Dr. Noor S.K. Al-Khafaji for kind cooperation and many thanks for Dr. Naeem R. Al-Jebori for assistance in isolate collection.

REFERENCES

3. Ahmad, W., Jamshed, F. and Ahmad, W., Frequency of escherichia coli in patients with community acquired urinary tract infection and their resistance pattern against some commonly used anti bacterials. Journal of Ayub Medical College Abbottabad, 2015; 27(2), pp.333-337.


34. Mohajeri, P., Rostami, Z., Farahani, A. and Norozi, B., Distribution of ESBL producing Uropathogenic Escherichia coli and carriage of selected β-lactamase genes in Hospital and community isolates in west of Iran. Annals of


