Emergecne of CTX-M Gene Among ESBL Positive E. Coli Isolates from UTI Outpatients at Benghazi

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Abstract—Background & Objective: The detection of clinical Escherichia coli producing extended-spectrum b-lactamases (ESBLs) are increasingly important cause of community acquired infection worldwide. The aim of this study was to investigating the presence of bla OXA, bla TEM, bla SHV and bla CTX-M genes among ESBL-producing E. coli isolated from Urinary Tract Infection (UTI) out-patients in Benghazi Center of Infectious Disease and Immunity (BCIDI).

Methodology: 40 E. coli isolates were collected from UTI outpatients at the Benghazi Center of Infectious Disease and Immunity during the period of 2011 to 2012. The antibiotic susceptibility of E. coli isolates were determined by disc-diffusion method as accordance with British society for antimicrobial chemotherapy (BSAC) [5] and by using the Phoenix Automated Microbiology System (Becton Dickinson, USA). ESBL production was screened by using the double disk synergy test. ESBLs coding genes (bla OXA, bla TEM, bla SHV and bla CTX-M (CTX-M universal, CTX-M-3G)) were identified by PCR.

Results: 16 ESBL-producing E. coli were identified among the E. coli isolates. Molecular investigated isolates (17) were negative for bla TEM, bla SHV, bla OXA-1 genes, three strains were positive for bla CTX-M-3G gene, six strains were carrying each of bla CTX-M-U and bla CTX-M-3G genes, three strains was positive for bla CTX-M-U gene and five strains were negative for CTX-M-U and CTX-M-3G type b-lactamases.

Conclusions: The emergence of CTX-M gene among ESBL positive E. coli isolates from UTI outpatients at Benghazi were documented; however, further studies are required to study the epidemiology and genetic characterization of other CTX-M types of ESBLs.

Key words: urinary tract infection; ESBL-producing E. coli; CTX-M-gene.

INTRODUCTION

B-lactamase is enzyme produced by micro-organism that hydrolyse b-lactam molecules. It is the most common B-lactam resistance mechanisms that contribute wide spread resistance among gram negative bacteria [6].

B-lactamase differ from each other in substrate profile and inhibitor profile. They also differ in a composition of hydrolytic parts of these enzymes [7]. The evolution of ESBL OXA-type -lactamases from parent enzymes with narrower spectra has many parallels with the evolution of SHV- and TEM-type ESBLs (Paterson & Bonomo, 2005).
ESBLs were initially derived from TEM and SHV and were essentially restricted to health care facilities that have evolved from parent enzymes, such as TEM-1, TEM-2, and SHV-1 [19 - 4]

B- lactamases resistance is expressed chromosomally or plasmid borne. The ability of E. coli to acquire and transfer anti- microbial resistance genes is considered. ESBLs-producing E. coli isolates where detected in hospitals as well as the community [20-19]

CTX-M enzymes were reported in Germany and Argentina in 1989 and since the increase of these strains have been reported in different geographical areas and they have become the most prevalent ESBLs [8]. Detection of CTX-M by molecular methods in ESBL-producing bacteria and their pattern of antimicrobial resistance is important for infection control. It can provide useful information about its epidemiology and help in rational antimicrobial therapy [14 -12].

So far 4 groups of B-lactamase have been identified based on substrate specification pencillinase, Amp c- type cephalosporinase, ESBLs and carbapenemase. In the present study, we investigate the occurrence of ESBLs genes in UTI patients using phenotype and molecular methods.

MATERIALS AND METHODS

**Bacterial strains:**

40 E. coli samples were isolated from uropathogens of UTI outpatient at BCIDI during the period of 2011 to 2012. They were identified with Phoenix Automated Microbiology System (Becton Dickinson, USA) (BD). All identified isolates were stored in brain heart infusion (BHI) broth containing 20% glycerol at -20ºC.

**Antimicrobial susceptibility testing:**

Antimicrobial susceptibility testing for E. coli isolates were determined by using the Phoenix Automated Microbiology System. The panel (NMIC/IC-94) for gram negative bacteria was containing amikacin, amoxicillin/clavulanate, ampicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, cefuroxime, cefoxitin, cephalothin, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, piperacillin/tazobactam, trimethoprim/sulfamethoxazole. The results are also interpreted through a computerised system.

**Phenotype method for ESBL:**

Phenotypic detection of ESBL production in isolated samples was performed by their susceptibility to the third generation cephalosporin’s [ceftazidime (30 g), cefotaxime (30 g) and ceftriaxone (30 g)] and aztreonam (30 g) (Oxoid Ltd., Cambridge, UK) by using Kirby Bauer disk diffusion method. The diameters of the zones of inhibition were measured according to the British society for antimicrobial chemotherapy [5] recommendations. The isolates that showed resistance to at least one of the four antibiotics were tested for ESBL production by the double-disk synergy test (DDST method) as described by [15].

**Detection of beta-lactamase genes:**

Molecular characterization of uropathogenic E.coli was investigated only on 17 of 40 UTI samples. Total DNA extraction was performed Laboratory of Microorganisms and Active Biomolecules, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunisia.
Molecular detection of gene coding for the TEM, SHV, OXA-1, CTX-M (CTX-M universal) and CTX-M3G (CTX-M-1 group) type beta-lactamases was performed by using polymerase chain reaction (PCR) described previously in [16-11]. The oligonucleotide primer sets specific for the β-lactamase genes and conditions of amplification of these genes in isolated genomic DNA samples used in PCR assays are listed in Table 1. PCR products were analysed by 1% agarose gel electrophoresis and photo-documented after stained with ethidium bromide.

**Table 1:** primers sequences and condition used for the polymerase chain reaction (PCR) amplification of different B-lactamase genes.

<table>
<thead>
<tr>
<th>PCR Target</th>
<th>Primer name</th>
<th>Primer sequence (5′–3′)</th>
<th>amplicon size</th>
<th>Reference</th>
<th>Conditions amplification</th>
</tr>
</thead>
<tbody>
<tr>
<td>BlaTEM</td>
<td>TEM-F</td>
<td>F: TTCTTGAAGACGAAAAGGC R: CGCTCAGTGGAACGAAAAC</td>
<td>1150pb</td>
<td>[16]</td>
<td>94°C 3min 1 cycle 94°C 1min 60°C 1min 30cycle 72°C 1min 72°C 5min 1 cycle</td>
</tr>
<tr>
<td></td>
<td>TEM-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bla SHV</td>
<td>SHV-F</td>
<td>F: CACTCAAGGATGTATTGTG R: TTAGCGTTGCCAGTGCTCG</td>
<td>885pb</td>
<td>[16]</td>
<td>96°C 15s 1 cycle 96°C 15s 52°C 15s 24cycle 72°C 2min 72°C 3min 1 cycle</td>
</tr>
<tr>
<td></td>
<td>SHV-R</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>bla OXA-1</td>
<td>OXA-1 F</td>
<td>F: ACACAATACATATCAACTTCGC R: TGTGTATGGAATGGTGATC</td>
<td>813pb</td>
<td>[16]</td>
<td>96°C 5min 1 cycle 96°C 1min 61°C 1min 35cycle 72°C 2min 72°C 10min 1 cycle</td>
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<tr>
<td></td>
<td>OXA-1 R</td>
<td></td>
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<tr>
<td>BlaCTX-M (CTX-M universal)</td>
<td>CTX-M-U-F</td>
<td>F: CGATGTCAGTCACCTAACAGTA R: TTAGTGACCAGAATCGCGG</td>
<td>454 pb</td>
<td>[11]</td>
<td>94°C 5min 1 cycle 94°C 30s 52°C 30s 35cycle 72°C 1min 72°C 5min 1 cycle</td>
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<tr>
<td></td>
<td>CTX-M-U-R</td>
<td></td>
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<tr>
<td>bla CTX-M3G (CTX-M-1 group)</td>
<td>CTX-M3G-F</td>
<td>F: GTTACAATGTTGAGAAGCAG R: CGTTTCCGCTATTACAAAC</td>
<td>1041pb</td>
<td>[16]</td>
<td>94°C 7min 1 cycle 94°C 50s 50°C 40s 35cycle 68°C 1min 68°C 5min 1 cycle</td>
</tr>
<tr>
<td></td>
<td>CTX-M3G-R</td>
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</table>

**RESULTS**

Antimicrobial susceptibility of clinical isolates:
ESBL-producing E. coli were high resistant to ampicillin (94.1%), amoxicillin / clavulanate (82.4%), ciprofloxacin, ceftriaxone, levofloxacin, (76.5%), cefotaxime, ceftazidime, aztreonam, cefuroxime (70.6%), trimethoprim/sulfamethoxazole, cefepime (64.7%). All ESBL-producing E. coli were high sensitive to imipenem, meropenem and amikacin (100%), nitrofurantoin (94.1%), ertapenem (94.1%), %), cefoxitin (88.2%), piperacillin-tazobactam (82.4%) and gentamycin (76.5%), Figure 1.

Characterization of beta lactamases:

Molecular detection of 16 ESBL-producing E. coli were isolated from UTI outpatient were confirmed by phenotype methods using the double disk synergy test (Fig. 2). Only one strain was non-ESBL strain (Table 2).

All isolates were negative for bla TEM, bla SHV, bla OXA-1 genes, three strains were positive for blaCTX-M-3G gene, six strains were carrying each of bla CTX-M-U and bla CTX-M-3G genes, three strains was positive for bla CTX-M-U gene and five strains were negative for CTX-M-U and CTX-M-3G type b-lactamases. Table 2 shows also presence of GTX-M-3G in sample 15 which was ESBL negative by phenotype methods, (fig.3 and fig.4).

**TABLE 2.** Antimicrobial susceptibility profiles and bla genes detected by PCR in sixteen phenotypic positive ESBL-producing E. coli and one phenotypically negative sample Cefotaxime (CTX), ceftazidime (CAZ), cefuroxime (CXM), ceftriaxone (CRO), aztreonam (ATM), amoxicillin/clavulanate (AMC), cefepime (FEP), imipenem (IPM), cefoxitin (FOX), gentamicin (CN), ampicillin (AMP), ciprofloxacin (CIP), ertapenem (ETP), levofloxacin (LEV), nitrofurantoin (F), trimethoprim/sulfamethoxazole (STX).

<table>
<thead>
<tr>
<th>No of E.coli isolates</th>
<th>Antibiotics</th>
<th>Detected lactamase b-3G</th>
<th>Resistance phenotype to non b-lactam antibiotics</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CTX</td>
<td>CAZ</td>
<td>CXM</td>
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<tr>
<td>1</td>
<td>F</td>
<td>R</td>
<td>R</td>
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<tr>
<td>2</td>
<td>F</td>
<td>R</td>
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<td>9</td>
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<td>11</td>
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<td>17</td>
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<td>S</td>
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</table>
Figure 1: Susceptibility of ESBL-producing E. coli isolates.

Figure 2: ESBL-producing E. coli were confirmed by using the double disk synergy test on Muller Hinton agar.

Figure 3: Result of the PCR screening test of E. coli for bal genes using CTX-M-U primer. Line 1: positive control for CTX-M-U gene, Line 2 – 17 PCR product of ESBL-producing E. coli isolated samples, Line 18: negative control for CTX-M-U gene.
The aim of this study was to determine the prevalence and characterize ESBL producers among uropathogens E. coli collected from UTI outpatients in BCIDI. The indicate confirm the high prevalence of CTX-M-3G, CTX-M-U gene (35.3%), CTX-M-U gene (17.6%) and CTX-M-3G gene (17.6%), while the prevalence of other SHV, TEM and OXA-1 genes was absent (Table 2). Bourjilat et al. [3] in Morocco and Ahmed [1] have studied Seven strains of E. coli producers of ESBLs among the 535 E. coli isolates, he found that (6/7) of the strains were carriers of the gene CTX-M, Two strains were high prevalence was 6/7 observed by --- and Sudan (Coque et al, 2008) carrying TEM gene in combination with CTX-M-15 or SHV-5. In Khartoum, Sudan, the prevalence of E. coli producers of ESBL has also increased with a predominance of CTX-M (71.4%), TEM and SHV were 55.1%, 6.1% respectively [1]. Recently, the predominant ESBL genotype in E. coli in Europe has changed from TEM / SHV to CTX-M [26].

Yumuk [26] reported that CTX-M is common among the ESBL producing E. coli isolates obtained from community acquired UTIs in Izmir, Turkey. In Norway the CTX-M is the ESBL enzyme most frequently found in E. coli [23]. Vidhya and Sudha [24] and Jouini et al. [16] showed that there was a high prevalence of the bla CTX-M gene (80%) among ESBL positive E. coli isolates from the UTI patients using CTX-M primer in Coimbatore, South India.

The prevalence and distribution of CTX-M enzymes data are limited in Benghazi, there is no data comparing CTX-M, TEM and SHV type ESBLs in hospitalized and outpatients. In this study, the rate of CTX-M type ESBL producing E. coli was found to be quite high as 66.7%. Although, we do not know the exact prevalence of these enzymes and factors influencing their high rate of ESBL producing strain in Benghazi, the current study suggests that CTX-M-producers have already begun to disseminate throughout Benghazi.
The presence of ESBL enzyme type M was in arrangement with synergy test results. In some cases such as isolates 17 PCR product indicate the presence of CTX-M3G gene but this isolates was sensitive to most studied antibiotic. The negative phenotype results of this isolates maybe due to site directed mutagenesis [21]. This data shows that molecular detection of ELSB is more sensitive than using phenotype method.

In recent years, the general susceptibility pattern of ESBL producer strains to antibiotics has decreased in many countries [13–10]. The ESBL producers in this study were high resistance to ampicillin, ciprofloxacin, levofloxacin, amoxicillin/ clavulanic acid, trimethoprim/ sulfamethoxazole which is of great concern because these the drugs of choice for first-line empiric treatment of both community and hospital acquired UTIs. Among oral agents licensed for treatment of urinary tract infection (UTI), only nitrofurantoin was generally active. The occurrence of CTX-M enzymes represents treatment problems in the community [25].

CONCLUSION

In our study, CTX-M type of ELSB was clearly the most prevalent enzyme among the community acquired E. coli causing UTIs in Benghazi. The results of antibiotic susceptibility revealed a high rates of resistance against the fluoroquinolones which are widely used in treatment of the urinary tract infections in Benghazi. The usage of carbapenems will still be suitable for a successful treatment of infections by E. coli. After carbapenems, aminoglycosides can be considered as the second most effective agent.

The emergence of CTXM from Benghazi is alarming; however, further studies are required to be conducted with the purpose of studying the epidemiology and genetic characterization of CTX-M types of ESBLs.

REFERENCES


