

# Detection of CTX-M-15 Among Uropathogenic *Escherichia coli* Isolated From Five Major Hospitals in Tripoli, Libya

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## ABSTRACT

**Objectives:** Multidrug resistance (MDR) and emergence of extended-spectrum  $\beta$ -lactamases (ESBLs) among uropathogenic *Escherichia coli* have been reported worldwide, but there was no information on the detection of *bla*<sub>CTX-M-15</sub> in major teaching hospitals in Libya. The aim of the study was to investigate the occurrence of CTX-M-15  $\beta$ -lactamases producers isolated from five teaching hospitals in Tripoli, Libya. **Methods:** A total of 346 urine samples were collected from hospitalized patients in five teaching hospitals with a diagnosis of urinary tract infection (UTI). Phenotypic confirmation of ESBLs was confirmed by E-test strip; all ESBL-producing *E. coli* isolates were screened for the *bla*<sub>CTX-M-15</sub> gene. **Results:** The distribution of ESBL-producing *E. coli* varied among the five hospitals. The highest proportion was identified in Tripoli Medical Centre (67.6%). There were extremely high proportions of isolates resistant to ceftriaxone, cefepime, and ceftazidime (93.0–100.0%) among ESBL producers compared to non-ESBL producers (2.2–4.7%). MDR was detected in 22.2% of isolates. The majority of isolates (85.9%) in which *bla*<sub>CTX-M-15</sub> was identified were ESBL producers. There was a correlation ( $p < 0.001$ ) between expression of CTX-M-15 and resistance to ceftazidime. **Conclusions:** The isolation of MDR ESBL-producing uropathogens expressing the CTX-M-15 gene will limit the choices clinicians have to treat their patients with UTIs. Continued surveillance and implementation of efficient infection control measures are required.

Urinary tract infections (UTIs) are the most prevalent bacterial infections in humans, and *Escherichia coli* is the most important cause of nosocomial UTIs. Narrow- and extended-spectrum cephalosporins have been used to treat such infections.<sup>1</sup> Management of UTIs has become increasingly problematic owing to the increasing production of extended-spectrum  $\beta$ -lactamases (ESBLs).<sup>2</sup> The enzymes TEM and SHV were previously recognized as the main ESBLs; however, CTX-M has recently become more prominent and is considered to be the most prevalent  $\beta$ -lactamases found in clinical isolates of *E. coli* globally.<sup>3</sup> A nationwide survey in the United States during 2009–2010 found 91% of ESBL-producing *E. coli* strains carried CTX-M-type genes.<sup>4</sup> CTX-M-type ESBLs are also dominant in European and Asian countries.<sup>3,5</sup> Similarly, it has been reported

from many African countries like Egypt, Algeria, Tunisia, Tanzania, and Cameroon.<sup>6–10</sup> Within the CTX-M family, CTX-M-15 is currently the most widely disseminated genotype associated with major causes of infections in both community and hospital environments.<sup>11,12</sup> The wide dissemination of CTX-M-15 producing *E. coli* isolates has been explained by many workers. First, it has been proposed that the strain virulence background could be involved in this dissemination process. In fact, many reports have shown that CTX-M-15 is closely associated with the international and pandemic uropathogenic ST131 clone, which have specific virulence factors.<sup>4,13</sup> Second, the association of CTX-M-15 with the IncF plasmids, which are well adapted to *E. coli*, may facilitate the spread of this determinant in the *E. coli* population.<sup>14</sup> In addition to virulence background and IncF plasmids

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bearing CTXM-15, it was recently suggested that the association of various plasmid addiction systems might contribute to plasmid maintenance in their host.<sup>15-17</sup> An addiction system or a toxin-antitoxin system helps maintain plasmids in bacteria during host replication by killing of plasmid-free cells resulting from segregation or replication defects.<sup>18</sup>

To date, few studies have been carried out in Libya to establish the associations between the clinical origin of strains, resistance-encoding phenotypes, and phylogenetic groups among *E. coli* isolates.<sup>19,20</sup> The aim of the present investigation was to determine the prevalence of *E. coli* isolates associated with UTIs in patients attending five hospitals, their susceptibility to antimicrobial agents, and the presence of CTX-M-15 among ESBL producing isolates of *E. coli* by polymerase chain reaction (PCR).

## METHODS

A total of 346 urine samples were collected from hospitalized patients with UTIs from five teaching hospitals in Tripoli: Tripoli Medical Centre (TMC), Tripoli Pediatric Hospital (TPH), Khadra Hospital, Aljala Hospital, and Tripoli Central Hospital (TCH). All urine samples were taken as part of the clinical work-up and these were included in this prospective laboratory-based surveillance study. The study was carried out during 2013 (January–December).

In this investigation, urine specimens were collected under approved ethical standards and the study was reviewed and approved by the faculty of Pharmacy, University of Tripoli, and participating hospitals. Urine specimens were obtained from all patients with supervision of a medical doctor or senior nurse for patients aged one year or older; midstream urine was collected in a sterile container and processed in the laboratory within two hours of collection. Urine specimens were obtained by suprapubic aspiration from children who were not toilet trained and processed as above. The consent of the patient or their guardian was obtained before urine specimens were collected. Using calibrated loops, urine specimens were inoculated on to MacConkey and blood agar plates. After 18–24 hour incubation at 37 °C, the number of colony forming units (cfu) was counted, and urine samples giving  $\geq 10^5$  cfu/mL of urine were considered significant.

All specimens were cultured on different media by standard bacteriological procedures. Isolated organisms were identified at the species level and tested for their susceptibility to a variety of antimicrobial agents using the BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks Md, USA) according to the manufacturer's instructions. The system uses combination panels for identification (ID) and antimicrobial susceptibility testing (AST) of bacteria. These include the Phoenix NMIC/ID Panels intended for in vitro rapid ID and AST by minimal inhibitory concentration (MIC) of Gram-negative aerobic and facultative anaerobic bacteria from pure culture belonging to the *Enterobacteriaceae* and non-*Enterobacteriaceae* families. Confirmatory tests for ESBL production were performed with all of the isolates initially identified by the Phoenix system. Phenotypic confirmation of ESBLs was done using E-test strips containing ceftazidime, and ceftazidime-clavulanate was used to determine the MIC ratio according to the manufacturer's instructions (epsilometer assay; AB Biodisk Slona, Sweden) performed on Mueller-Hinton agar. All isolates were screened and interpreted for ESBL phenotype according to the criteria of the Clinical and Laboratory Standards Institute.<sup>21</sup> Multidrug-resistant (MDR) bacteria were defined as showing resistance to three or more different classes of antibiotics such as fluoroquinolones, aminoglycosides, and cephalosporins.<sup>22</sup> Reference strain of *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *Klebsiella pneumoniae* ATCC 700603 were used as controls.

ESBL producing *E. coli* isolates were screened for the *bla*<sub>CTX-M-15</sub> gene using previously reported primers.<sup>23</sup> The plasmids were isolated using the QIAGEN Plasmid Mini Kit according to the manufacturer's instructions. The reaction mixture contained a total of 25  $\mu$ l: 5  $\mu$ l of 5X Red Load Taq Mix composed of Taq Polymerase, 0.05 u/ $\mu$ l dNTPs (200  $\mu$ M) (dATP, dCTP, dGTP, dTTP) reaction buffer with KCl and MgCl<sub>2</sub> (1.5 mM) red dye, gel loading buffer, stabilizers (Metabion, Martinsried-Germany); 0.5  $\mu$ l of each primer 10 pmol/ $\mu$ l; and extracted plasmid DNA (2–50 ng). The thermal profile included one cycle of initial denaturation at 95 °C for 2 minutes followed by 35 cycles at 95 °C for 30 seconds, annealing at 52 °C for 30 seconds, and extensions at 72 °C for 45 seconds. The PCR reaction was carried out with a TC-412 thermocycler (Techne, Duxford, Cambridge): 5  $\mu$ l of the PCR amplification

**Table 1:** Antimicrobial resistance patterns of *Escherichia coli* from patients with urinary tract infections.

| Antibiotic     | ESBL,<br>n = 71 (%) | Non-ESBL<br>n = 275 (%) | p-value |
|----------------|---------------------|-------------------------|---------|
| Ampicillin     | 71 (100.0)          | 196 (71.2)              | 0.004   |
| Amikacin       | 2 (2.8)             | 4 (1.4)                 | 0.440   |
| Gentamicin     | 27 (38.0)           | 32 (11.6)               | < 0.001 |
| Cefoxitin      | 27 (38.0)           | 22 (8.0)                | < 0.001 |
| Ceftriaxone    | 69 (97.1)           | 13 (4.7)                | < 0.001 |
| Cefepime       | 71 (100.0)          | 6 (2.2)                 | < 0.001 |
| Ceftazidime    | 66 (93.0)           | 11 (4.0)                | < 0.001 |
| Nitrofurantoin | 12 (16.9)           | 15 (5.4)                | 0.002   |
| Meropenem      | 2 (2.8)             | 0 (0.0)                 | 0.055   |
| Ciprofloxacin  | 48 (67.6)           | 91 (33.1)               | < 0.001 |
| Cefotaxime     | 46 (64.8)           | 112 (40.7)              | 0.0004  |
| Ertapenem      | 2 (2.8)             | 0 (0.0)                 | 0.055   |
| Total MDR*     | 56 (78.9)           | 21 (7.6)                | < 0.001 |

\*n = 77. ESBL: extended-spectrum  $\beta$ -lactamase; MDR: Multidrug resistance.

products were electrophoresed in agarose (2% m/v) containing 0.5  $\mu$ g/mL ethidium bromide. The amplified PCR products were visualized under UV light and electronically documented (MultiDoc-It Digital Imaging System UVP, Cambridge, UK). A 50 bp DNA ladder (Metabion, Martinsried- Germany) was used as a molecular size marker.

All statistical analyses were performed with the SPSS Statistics (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.). Statistical analyses were performed using the chi-square test. The statistical significance was set at  $p = 0.050$ .

## RESULTS

A total of 346 isolates were collected and confirmed as uropathogenic *E. coli* by the Phoenix automated system. The age of the patients with UTI ranged from < 1–90 years old. The distribution of ESBL-producing *E. coli* varied among the different hospitals of Tripoli. The majority were isolated from patients at TMC (67.6%) compared with other hospitals; TPH (9.9%), Khadra Hospital (9.9%), Aljala Hospital (8.4), and TCH (4.2%). The majority of the isolates expressing ESBL were obtained from female patients 51/71 (71.8%) and 20/71 (28.2%) were obtained from males.

The results of antimicrobial susceptibility tests for ESBL-producing 71/346 (20.5%) strains and non-ESBL producers 275/346 (79.5%) are summarized in Table 1. Extremely high resistance rates ( $p <$

0.001) were observed for ceftriaxone, cefepime, and ceftazidime (93.0–100.0%) among ESBL producers compared to non-ESBL producers (2.2–4.7%). High rates of resistance were also demonstrated to ciprofloxacin (67.6%) and cefotaxime (64.8%). Fewer isolates (2.8%) were resistant to amikacin, meropenem, and ertapenem. ESBL producers were more often resistant to major classes of antibiotics compared with non-ESBL producers. MDR was documented for 77/346 (22.2%) of isolates, and was significantly higher ( $p < 0.001$ ) among ESBLs compared with non-ESBL producer uropathogenic *E. coli*.

All 71 ESBL-positive *E. coli* isolates were confirmed by E-test. PCR analysis found 61/346 (17.6%) of isolates contained *bla*<sub>CTX-M-15</sub>, the



**Figure 1:** Amplification of CTX-M-15 gene in extended-spectrum  $\beta$ -lactamase producing *Escherichia coli* isolates by single polymerase chain reaction. Lane M 50 bp ladder; Lanes 1 to 5 CTX-M-15-producing isolates. The amplified amplicon size is 481 bp.

majority of these isolates 61/71 (85.9%) that harbored CTX-M-15 gene were found to be ESBL producers [Figure 1]. There was no significant difference between gender and expression of CTX-M-15 gene; however, there was a correlation between expression of CTX-M-15 and resistance to ceftazidime ( $p < 0.001$ , odd ratio 179.142 [59.2–541.9]). CTX-M-15-positive isolates were highly sensitive to ertapenem, meropenem, and amikacin (97.2%). It was observed that the majority of isolates 44/71 (61.9%) defined as MDR organisms also had *bla*<sub>CTX-M-15</sub> gene and classified as ESBL positive.

## DISCUSSION

This study provides molecular-epidemiological data on ESBL *E. coli* isolated from patients in the five university hospitals of Tripoli in Libya during 2013. The distribution of ESBL-producing *E. coli* varied (4.2–67.6%) among the different hospitals of Tripoli; the highest proportions (67.6%) was observed among isolates obtained from the largest tertiary teaching hospital, TMC. In this study, ESBL was detected in a higher proportion of isolates (20.5%) compared to a previous study that found ESBL in 6.7% of uropathogenic *E. coli*.<sup>11</sup> These results were consistent with the results of reports from the Middle East.<sup>24,25</sup>

Identification of ESBL among isolates varied from 66.7% in India,<sup>26</sup> 54.7–61% in Turkey, and 72.1% in Iran.<sup>26–28</sup> These figures were lower in America and Europe.<sup>3,27</sup> SMART data have shown that the Middle East has the second highest ESBL prevalence after Asia (around 37% for UTI isolates).<sup>29</sup> The Middle East is the only region in the world where the prevalence of ESBLs has been increasing significantly for UTIs,<sup>29</sup> ESBL in UTI-associated *E. coli* isolates from Lebanese patients, increased consistently from 2.3% in 2000 to 16.8% in 2009.<sup>30</sup> Three studies carried out in Saudi Arabia revealed that an increasing prevalence of ESBL *E. coli* isolated from UTI over two years, 2013 = 20.4%, 2014 = 33.3%, and 2014 = 35.8%.<sup>24,31,32</sup> Although the prevalence of ESBLs-producing bacteria is a worldwide problem, identification of these isolates can vary by countries and institutions within a country.<sup>33</sup>

The findings of the present study identified CTX-M-15 among *E. coli* isolated from UTI in Tripoli, Libya. The results indicated that overall 17.3% of clinical *E. coli* isolated carried

CTX-M-15 gene, the majority (85.9%) of ESBL-carrying *E. coli* isolates CTX-M-15 gene. The results of this study are consistent with the global distribution of CTX-M-15.<sup>5,34</sup> It is worth noting that although some of the CTX-M enzymes have been associated with specific countries, such as CTX-M-9 and CTX-M-14 in Spain, CTX-M-1 in Italy, and CTX-M-2 in Israel, Japan, and most South American countries.<sup>35,36</sup> Others, such as CTX-M-15, have been detected worldwide.<sup>5,12,35,36</sup> In Arab countries, the first description of CTX-M was in Egypt and then in the United Arab Emirates and Kuwait.<sup>37–39</sup> In these countries, the predominant ESBL was CTX-M-15. After 2000, the prevalence of CTX-M increased steadily in the Tunisian healthcare setting especially CTX-M-15, which in many studies was most prevalent among ESBLs.<sup>40,41</sup> Mnif et al,<sup>42</sup> reported that 72% of ESBL-positive *E. coli* isolates were CTX-M producers. High rates were demonstrated in Saudi Arabia; CTX-M-type enzymes accounted for 100% of all ESBLs, and the predominant ESBL was CTX-M-15 (92.1%). A similar trend (98%) was observed in Iran.<sup>43</sup> SMART study (2011–2013) conducted in Lebanon and Jordan showed that CTX-M-15 was the most prevalent ESBL produced.<sup>44</sup> This results are similar to those for Lebanon; the majority of the strains (83%) express CTX-M-15 ESBL.<sup>45</sup> Previously, Abujnah and colleagues in Libya reported that 6/13 of *E. coli* isolates expressed *bla*<sub>CTX-M</sub> gene, but they failed to determine the prevalence of CTX-M-15.<sup>19</sup>

Xia et al,<sup>46</sup> reported low rates (18.2%) of *bla*<sub>CTX-M-15</sub> ESBL-producing *E. coli* isolated from patients with community-onset or hospital-onset infections in China. Detection of CTX-M-15 among all studied isolates (17.3% overall) was lower than Argentina where the occurrence of CTX-M-15 was 45.2% among *E. coli* isolates.<sup>47</sup> Similarly, in Lithuania the most common CTX-M  $\beta$ -lactamase among the *E. coli* isolates, collected during a period of five years, was CTX-M-15 followed by CTX-M-14 and CTX-M-92 (56.7%, 13.3%, and 11% respectively).<sup>48</sup> These results reflect the global trend toward a pandemic spread of CTX-M-type ESBLs in *E. coli*.<sup>34</sup>

The prevalence and spread of antibiotic resistance and MDR *E. coli* isolates was previously determined.<sup>19</sup> Extremely high resistance rates were observed to ceftriaxone, cefepime, and ceftazidime among ESBL producers (93.0–100.0%) compared to non-ESBL producers (2.2–4.7%). High rates of

resistance were also demonstrated to ciprofloxacin (67.7%) and cefotaxime (64.8%). It is very important to emphasize that carbapenems maintained excellent activity (97.8%) against ESBL-producing *E. coli* isolates during the study period. Overall, MDR were detected in 22.2% of isolates lower than previously detected in 32.2% of *E. coli* isolated from patients with UTIs attending Zawia Hospital, in Libya.<sup>24</sup> The majority (62%) of isolates simultaneously defined to be MDR organisms were also found to express the *bla*<sub>CTX-M-15</sub> gene and were designated ESBL positive. Alzohairy and Khadri reported higher rates of MDR among *E. coli* (53.6%) isolated from community and hospital-acquired UTIs in Qassim province, Saudi Arabia.<sup>49</sup> Generally, most CTX-M  $\beta$ -lactamases are known to hydrolyze cefotaxime more efficiently than ceftazidime, but CTX-M-15 also hydrolyzes ceftazidime efficiently.<sup>50</sup> This study showed that there was a significant correlation between expression of the CTX-M-15 gene and resistance to ceftazidime.

The risk factors associated with acquisition of these organisms were not determined in this study that includes: underlying illness, presence of a catheter, prior exposure to beta-lactam and aminoglycosides/fluoroquinolones antibiotic, length of hospitalization, and admission to the intensive care unit.

## CONCLUSION

This study stresses the importance of specific surveillance to optimize empiric treatment of uropathogenic *E. coli*. Further studies should continue to monitor ESBL-producing bacteria and explore the mechanisms contributing to the spread of CTX-M genotypes in these bacteria to provide reference data to enable relevant infection control.

### Disclosure

The authors declared no conflicts of interest. No funding was received for this study.

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