PCR-Based Molecular Detection of ESBLs Encoding Genes $bla_{TEM}$, $bla_{CTX-M}$ and $bla_{SHV}$ among MDR Escherichia coli Isolates from Diarrhoea Stool Cultures in Cairo, Egypt.

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Abstract: Resistance to β-lactams owing to the production of extended-spectrum β-lactamases (ESBLs) enzymes has become common among diarrhoeagenic E. coli, especially in developing countries. This study aimed to detect ESBLs encoding genes $bla_{TEM}$, $bla_{CTX-M}$ and $bla_{SHV}$ among E. coli isolates from patients with acute infectious diarrhoea in Cairo, Egypt. A total of 60 E. coli isolates were isolated from diarrhoea stool cultures, during the period from March 2014 to December 2015, at three hospitals in Cairo. Isolation and identification of E. coli were performed using standard microbiological methods and confirmed by Vitek 2 automated system. Antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion method against diverse classes of antimicrobial agents. Confirmatory tests for ESBLs production were performed using standard disc diffusion methods according to Clinical and Laboratory Standards Institute (CLSI). The E. coli ESBL-producer phenotypes were investigated for the presence of $bla_{TEM}$, $bla_{CTX-M}$ and $bla_{SHV}$ via polymerase chain reaction (PCR) using each gene-specific primers. Based on CLSI guidelines of E. coli isolates were multi-drug resistant (MDR) ESBLs-producer. All isolates were resistant to penicillins, third- and fourth-generation cephalosporins and aztreonam. E. coli isolates showed high resistance rates to non-β-lactams antimicrobial agents. The combination disc method for detection of ESBLs production showed positive results in 86 % of isolates. The cefotixin-cloxacin double disc synergy test for AmpC production showed positive results in 18 % of isolates. PCR studies revealed that $bla_{TEM}$ was the predominant ESBLs encoding determinant in 85 % of isolates, followed by $bla_{CTX-M}$ in 73,3 % of isolates; $bla_{SHV}$ was not detected in this study. All ESBLs-producing E. coli isolates possessed one or two of the PCR-positive ESBLs determinants. Each $bla_{TEM}$ and $bla_{CTX-M}$ was found alone in 63,3 % and 51,7 % of all isolates, respectively. The $bla_{TEM}$ in combination with $bla_{CTX-M}$ was detected in 21,7 % of E. coli isolates. ESBLs production is a noteworthy increasing among E. coli causing diarrhoeal diseases. The $bla_{TEM}$ is the common genetic mechanism for ESBL production in these pathogens isolated from Egypt. The limited treatment alternatives of infections caused by ESBL-producers E. coli require a public health policy on appropriate prescribing and the rational use of antimicrobial agents.

Keywords: E. coli, diarrhoea, ESBLs, PCR.

1. INTRODUCTION

Diarrheagenic Escherichia coli is one of the most common causes of acute infectious diarrhoea (Alikhani et al., 2013), which is a major cause of morbidity and mortality, particularly among children (Collins, 2007; Sang et al., 2012).3,5 Although the treatment of diarrhoea caused by E. coli has been successful using antimicrobial agents, the rapid development of multi-drug resistance has become an increasingly emerging worldwide problem with serious consequences on public health, particularly in developing countries (Sankaran, 2000; WHO, 2014).5 One of these successful treatments was the β-lactam group of antimicrobial agents. They are killing the microorganism through the irreversible binding to the penicillin binding proteins (PBPs) required for the final transpeptidation step in the biosynthesis of peptidoglycan; the important stage for bacterial cell wall construction. The most clinically important members of this group are the third-generation cephalosporins, β-lactam/β-lactamase inhibitor combinations and carbapenems.5 There are four main mechanisms by which bacteria can overcome β-lactam antimicrobial agents: production of extended spectrum β-lactamases (ESBLs) enzymes, changes in the active site of PBPs, decreased expression of outer membrane...
proteins and efflux pumps. Whilst, the predominant mechanism of resistance to β-lactam antimicrobial agents in *E. coli*, including penicillins, extended-spectrum cephalosporins and carbapenems, is the production of ESBLs enzymes encoded by *bla* genes. These enzymes hydrolyze the β-lactam ring in the molecular structure of β-lactam antimicrobial agents leading to inactivation of the antibacterial activity of these drugs. The most commonly known ESBLs are derived from Ambler class A β-lactamases enzymes which include TEM, SHV and CTX-M. Detection of theses common ESBLs encoding genes in *E. coli* can provide necessary information on their epidemiology helping for more efficient antimicrobial therapy. Therefore, this study aimed to detect ESBLs encoding genes *bla*_TEM, *bla*_CTX-M and *bla*_SHV, using PCR, among MDR diarrheagenic *E. coli* isolates from patients with acute infectious diarrhoea in Cairo, Egypt.

2. MATERIALS AND METHODS

2.1. Study Population and *E. coli* Bacterial Isolates

The present study was carried out on 60 non-duplicate *E. coli* isolates recovered from diarrhoea stool cultures of inpatients with acute infectious diarrhoea at three hospitals in Cairo: Kasr El-Aini University hospital, EL-Demerdash University hospital and Abbassia Fever hospital, during the period from March 2014 to December 2015.

2.2. Collection of Specimens and Processing

The diarrhoea stool samples were collected into sterile bottles and transported to laboratory over a period of two hours in Cary-Blair transport medium. All collected specimens were processed in the same day of collection.

2.3. Isolation and Identification of *E. coli* Isolates

Primary isolation was performed following the conventional methods for isolation of enteric bacterial Gram-negative bacilli. On arrival to the laboratory, the stool samples were directly inoculated onto MacConkey and eosin methylene blue (EMB) culture media, and plates were then incubated at 37°C for 24 - 48 hours. Isolates were identified microscopically by Gram-staining and biochemical testing methods.

2.4. Identification and Screening for ESBLs Producers by VITEK® 2 Automated System.

VITEK® 2 automated system (Biomerieux, France) was used to confirm the identification and assay the antimicrobial susceptibility of the tested isolates to screen for ESBLs production using VITEK® 2 GN and VITEK® 2 AST-N204 panels, respectively, according to the manufacturer’s guidelines. Tested isolates were considered as ESBLs producers along with the Clinical and Laboratory Standards Institute (CLSI) recommendations.

2.5. Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities of *E. coli* isolates to different antimicrobial agents were determined manually using Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar (Oxoid, UK) following CLSI guidelines. A number of 15 antimicrobial discs, representing different classes of antimicrobial agents, were included in this study. Discs were the product of Oxoid, UK: ampicillin, piperacillin, amoxicillin-clavulanate, cefoxitin, cefuroxime sodium, ceftaxime, ceftriaxone, aztreonam, kanamycin, tetracycline, ciprofloxacin, levofloxacin, norfloxacin, trimethoprim-sulfamethoxazole and chloramphenicol. Discs were stored at 4°C and allowed to reach room temperature before being used. The inhibition zones developed around the discs measured in millimetre (mm) were interpreted as susceptible (S), intermediate (I) or resistant (R) to a particular antimicrobial agent according to CLSI. Isolates that showed resistance to at least three different classes of antimicrobial agents were considered as MDR.

2.6. Detection of ESBL Phenotypes

Phenotypic confirmation of ESBL production was performed according to CLSI guidelines. The antimicrobial discs used for the following ESBLs phenotypic confirmatory tests were the product of Bio-Rad, France: ceftazidime (30 μg), ceftazidime-clavulanate (30 μg/10 μg), ceftotaxime (30 μg) and cefotaxime-clavulanate (30 μg/10 μg).

2.6.1. Combination Disc Method for Detection of ESBLs

This test was performed according to Garrec et al. An overnight culture of the test isolate was suspended to the turbidity of 0.5 McFarland and used to swab a Muller-Hinton agar plate. Disks of ceftazidime, ceftazidime-clavulanate, cefotaxime and cefotaxime-clavulanate were placed on MH
PCR-Based Molecular Detection of ESBLs Encoding Genes \( bla_{\text{TEM}} \), \( bla_{\text{CTX-M}} \) and \( bla_{\text{SHV}} \) among MDR *Escherichia coli* Isolates from Diarrhoea Stool Cultures in Cairo, Egypt

agar. Isolates were considered ESBL-producer if the inhibition zone measured around one of the combination disks after overnight incubation was at least 5 mm larger than that of the corresponding cephalosporin disc alone as recommended by the manufacturer and CLSI.

2.6.2. Cefoxitin-cloxacillin double disc synergy test (CC-DDS)

This test is used for the detection of the production of AmpC β-lactamase enzyme. The test was performed according to Polsfuss et al., using 0.5-McFarland of test isolate prepared from overnight culture. The suspension was used to swab a Muller-Hinton agar plate, and then discs containing 30 µg of cefoxitin and containing 30 µg of cefoxitin plus 200 µg of cloxacillin (inhibitors of the AmpC enzyme) were added. Plates were incubated at 35°C for 16 hours. A difference in the cefoxitin-cloxacillin inhibition zones minus the cefoxitin alone zones of ≥ 4 mm was considered indicative for AmpC production.

2.7. Molecular detection of ESBLs encoding genes by PCR

*E. coli* ESBLs phenotypes, confirmed by the CLSI selection criteria, were PCR examined for the presence of ESBLs encoding genes: \( bla_{\text{TEM}} \), \( bla_{\text{SHV}} \) and \( bla_{\text{CTX-M}} \).

2.7.1. PCR oligonucleotide primers and DNA extraction

PCR primers used in this study are listed in Table 1 and synthesized by Invitrogen, UK. The lyophilized powder was reconstituted using nuclease free water (Promega, USA) and the concentration of each primer was adjusted to 10 pmol/µl. Total crude DNA was extracted from all isolates by heating bacterial cells suspension in sterile distilled water at 95°C for 10 min, followed by removal of cellular debris by centrifugation at 14,000 rpm for 1 min. The supernatant was collected and used template DNA for PCR amplification.

Table 1. Nucleotide sequences of PCR oligonucleotide primers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5’ – 3’)</th>
<th>PCR product size (bp)</th>
<th>Source</th>
</tr>
</thead>
</table>
| \( bla_{\text{TEM}} \) | F: 5’ TCCGCTCATGAGACAATAACC 3’  
R: 5’ TGGTCTGACAGTACAAATGC 3’ | 931                   | 1      |
| \( bla_{\text{CTX-M}} \) | F: 5’ TCTTCCAGAATAAGGAATCCC 3’  
R: 5’ CCGTTTCCGCTATTACAAAC 3’ | 909                   |        |
| \( bla_{\text{SHV}} \) | F: 5’ TGTTATAGCGTTATATTGCC 3’  
R: 5’ GGTTAGCGTTCGAGTCT 3’ | 868                   |        |

1. Kiratisin et al.14

2.7.2. PCR amplification of ESBLs encoding genes

PCR reactions were performed in total volumes of 20 µl containing 10 µl of GoTaq® Green Master 2x Ready Mix (Promega, USA), 1 µl (10 pmol concentration) of each primer, 2 µl of DNA template and the volume was completed to 20 µl by adding 6 µl of nuclease free water. The PCR program for amplification for \( bla_{\text{TEM}} \), \( bla_{\text{CTX-M}} \) and \( bla_{\text{SHV}} \) was as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 30 seconds and extension at 72°C for 1 minute; and a final extension at 72°C for 7 minutes.

2.7.3. TAE (Tris-acetate-EDTA)-agarose gel electrophoresis

Reaction aliquots of 10 µl were resolved through TAE agarose gel electrophoresis prepared in a concentration of 1 % of molecular biology grade agarose (Bioline, UK) in 1x TAE buffer. DNA fragments, stained with ethidium bromide, were visualized under UV light using UV illumination and directly photographed. DNA molecular size marker 100bp – 1500 bp (New England Biolabs, UK) was used to assess PCR product size.

3. Results

In this study, based on the results of VITEK® 2 automated system, phenotypic identification and antimicrobial susceptibility testing, a total of 60 *E. coli* isolates recovered from acute diarrhoea stool samples were identified as MDR *E. coli* as they were resistant to three or more different classes of antimicrobial agents. The antimicrobial susceptibility studies of these isolates showed that all isolates (100 %) were resistant to ampicillin, piperacillin cefuroxime, cefotaxim, ceftriaxone, aztreonam and tetracycline. While there were high resistance rates to trimethoprim/sulfamethoxazole (91.7 %),

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ciprofloxacin (85 %) amoxicillin/clavulanic acid (83.3 %), each levofloxacin and norfloxacin (81.7 %), kanamycin (71.7 %), cefoxitin (65 %) and finally chloramphenicol (43.3 %) (Table 2).

The 60 E. coli isolates were confirmed as ESBLs producers according to CLSI guidelines of being resistant to all penicillins, third- and fourth-generation cephalosporins and aztreonam (Table 2). Based on CLSI guidelines of cefotaxime and ceftazidine susceptibility with or without clavulanate, these isolates showed positive results of combination disk method for detection of ESBLs test in 52/60 (86 %) isolates and AmpC production with the positive results of cefoxitin-cloxacillin double disc synergy test (CC-DDS) in 11/60 (18 %) isolates (Figure 1).

Table 2. Antimicrobial susceptibility percentages of MDR ESBLs producing E. coli isolates included in this study.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>E. coli (60 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant No. (%*)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>50 (83.3)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>39 (65)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>43 (71.7)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>60 (100)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>51 (85)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>49 (81.7)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>49 (81.7)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>55 (91.7)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>26 (43.3)</td>
</tr>
</tbody>
</table>

*Percentage correlated to the total number of isolates.

Figure 1. Positive results of A, combination disc method for detection of ESBLs production; B, CC-DDS for AmpC \( \beta \)-lactamase production.

The PCR-based investigation of the ESBLs encoding genes among these ESBLs-producing E. coli confirmed phenotypes revealed the presence of TEM and/or CTX-M Ambler class A ESBLs encoding genes in all isolates. The \( \text{bla}_{\text{TEM}} \) type ESBL was the most frequent as found in 51 (85 %), followed by \( \text{bla}_{\text{CTX-M}} \) gene in 44 (73.3 %) of E. coli isolates. The \( \text{bla}_{\text{SHV}} \) gene was not detected in the isolates included in this study (Table 3, Figure 2). All isolates harboured the ESBLs encoding genes; either one only or two genes co-existed together. The genes \( \text{bla}_{\text{CTX-M}} \) and \( \text{bla}_{\text{TEM}} \) were found together in 71.6 % of E. coli isolates (Table 4).
PCR-Based Molecular Detection of ESBLs Encoding Genes \( \text{bla}_{\text{TEM}} \), \( \text{bla}_{\text{CTX-M}} \) and \( \text{bla}_{\text{SHV}} \) among MDR Escherichia coli Isolates from Diarrhoea Stool Cultures in Cairo, Egypt

Table 3. Prevalence of CTX-M, SHV and TEM encoding genes among E. coli ESBLs phenotypes.

<table>
<thead>
<tr>
<th>ESBLs phenotypes</th>
<th>ESBLs encoding genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{bla}_{\text{TEM}} ) No. (%) *</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>51 (85)</td>
</tr>
<tr>
<td>(55 isolates)</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage correlated to the total number of isolates.

Table 4. Genotypic profiles of E. coli ESBLs phenotypes.

<table>
<thead>
<tr>
<th>ESBLs encoding genes</th>
<th>No. of E. coli ESBLs phenotypes (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{bla}_{\text{TEM}} ) alone</td>
<td>38 (63.3)</td>
</tr>
<tr>
<td>( \text{bla}_{\text{CTX-M}} ) alone</td>
<td>31 (51.7)</td>
</tr>
<tr>
<td>( \text{bla}<em>{\text{CTX-M}} ) + ( \text{bla}</em>{\text{TEM}} )</td>
<td>13 (21.7)</td>
</tr>
</tbody>
</table>

*Percentage correlated to the total number of isolates.

Figure 2. Representative agarose gel (1 %) electrophoresis of PCR products of ESBLs encoding genes \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{CTX-M}} \). M, DNA molecular weight marker; Lanes 1 – 10 in each panel, E. coli isolates.

4. DISCUSSION AND CONCLUSION

Diarrheagenic E. coli is the most common cause of infectious diarrhoea worldwide, especially in developing countries. It is occupying the second most important cause of mortality among childhood. Seriously, the treatment of the E. coli caused diarrhoeal diseases and/or infections are increasingly becoming difficult because of the development of multiple-resistance to diverse antimicrobial agents. Resistance to \( \beta \)-lactam antimicrobial agents in E. coli is most commonly occurred through the production of ESBLs enzymes. ESBLs exhibit a high degree of diversity and encoded by diverse determinants distributed in different geographic regions of the world (Livermore et al., 2007). ESBL testing may still be useful for epidemiological and infection control purposes. Thus, in this study, a total of 60 E. coli isolated from faecal specimens of patients with acute infectious diarrhoea were investigated for the most common ESBLs encoding genes \( \text{bla}_{\text{TEM}} \), \( \text{bla}_{\text{CTX-M}} \) and \( \text{bla}_{\text{SHV}} \).

E. coli isolates were identified based on conventional cultural characteristics and biochemical testing, in addition to confirming the identification using the fully automated VITEK® 2 system. Based on the records of the antimicrobial susceptibility testing performed in this study, all E. coli isolates were considered as MDR and demonstrated high resistance rates to the tested antimicrobial agents. Previous reports revealed good antibacterial activity of ampicillin, chloramphenicol, tetracycline and aminoglycoside against enteric bacterial pathogens including E. coli. The antimicrobial susceptibility patterns in this study showed that all isolates were resistant to ampicillin and tetracycline. In addition, isolates were highly resistant to kanamycin (71.7 %) and gentamicin (55.9 %), and chloramphenicol (43.3 %). In addition, there were higher resistance percentages of E. coli isolates of 100 % to piperacillin cefuroxime, cefotaxim, ceftriaxone and aztreonam, 66.7 % to trimethoprim-sulfamethoxazole. In addition, there were high resistance rates to trimethoprim/sulfamethoxazole (91.7 %), ciprofloxacin (85 %) amoxicillin/clavulanic acid (83.3 %) and levofloxacin or norfloxacin (81.7 %). These records indicated that these antimicrobial agents are not fit for initiation of empirical antimicrobial therapy of diarrhoea in the study hospitals when infection with an ESBL-producing E. coli is suspected. This high resistance rates may be explained by the misuse and/or overuse of these antimicrobial agents in Egypt and/or this region of the country.
All isolates in this study were described as MDR E. coli based on the definition of being resistant to three or more different classes of antimicrobial agents. This could be attributed to the extensive use of antimicrobial agents in developing countries, like Egypt, which has led to the emergence of MDR bacteria causing diseases. Thus, regular antimicrobial susceptibility examination is necessity before treating infectious diarrhoea in a country like Egypt.

E. coli isolates included in this study were subjected to screening for ESBLs production by the fully automated VITEK® 2 system, antimicrobial susceptibility testing and confirmatory phenotypic assays for ESBLs production. The E. coli isolates included in this study showed positive results in 86 % of isolates. Moreover, the AmpC production test using cefoxitin-clavulanic acid double disc synergy test showed positive results in 18 % of isolates. This might well reflect the high rates of ESBLs production in E. coli and faecal colonization in a country like Egypt. AmpC β-lactamases, are mainly chromosomally encoded in Enterobacteriaceae and they confer resistance to cephalothin, cefazolin, cefoxitin, most penicillins and to β-lactam inhibit like clavulanic acid.

Notably, the finding of this study that ESBLs-producers E. coli being MDR is consistent with the fact that ESBLs-producing E. coli often display resistance to non-β-lactam antimicrobial agents. Indeed, a high rate of co-resistance to potentially active antimicrobial agents of divers classes is a common feature of ESBL-producing E. coli strains, which was also found in the current study. This is explained by that genes coding for ESBLs and those conferring resistance to other antimicrobial agents often reside within the same conjugative plasmids. The conjugal transfer of plasmid-mediated ESBLs occurs efficiently in the intestinal tract, where enteric rods, in particular E. coli, often act as a reservoir of self transmissible resistance markers that can be exchanged between different species and/or strains of the Enterobacteriaceae family (Bonnet, 2004; Franiczek et al., 2012). In one study, it was found that 39 % of children had commensally gut flora strains, including E. coli, which were resistant to all antimicrobial agents tested and 70 % of the children had MDR resistant strains. These bacteria could act as reservoirs for transmission of resistance markers to other enteric pathogens.

CTX-M-type β-lactamases are the most prevalent ESBLs in Enterobacteriaceae in many geographical areas. However, the PCR investigation of Ambler class A ESBLs (TEM, CTX-M, SHV) encoding genes revealed that blatem was the predominant ESBLs encoding determinant in 85 % of isolates, followed by blaCTX-M in 73.3 % of isolates, however blaSHV was not detected in this study. All ESBLs-producing E. coli isolates in this study possessed one or two of the PCR-positive ESBLs determinants. Each blatem and blaCTX-M was found alone in 63.3 % and 51.7 % of all isolates, respectively. The blatem in combination with blaCTX-M was detected in 21.7 % of E. coli isolates. The results of this study are consistent with the study of Zaki et al., which was carried out on diarrheagenic E. coli isolated from acute diarrhoea patients in Mansura Governorate, Egypt. They reported that the commonest gene among diarrheagenic E. coli was blatem, however they detected blaSHV with low percentage. This may indicate the most common carriage of TEM-type ESBL among diarrhoea causing E. coli in Egypt. Although, the study of Fernandes et al. in Portugal reported also that that TEM-type ESBL was the most prevalent type followed by CTX-M. Moreover, a study from Iran by Ghorbani-Dalini et al. reported clearly that blatem was the most common gene in diarrheagenic ESBLs-producer E. coli. The origin of SHV is the chromosome of Klebsiella spp. and has only a narrow β-lactam hydrolyzing activity conferring resistance to penicillin and ampicillin. In addition, SHVs are more prevalent in Europe, which may explain the absence of blaSHV in this study.

In conclusion, ESBLs production is a noteworthy increasing among E. coli causing diarrheal diseases. The blatem is the common genetic mechanism for ESBL production in these pathogens isolated from Egypt, followed by blaCTX-M Egypt. The limited treatment alternatives of infections caused by ESBL-producers E. coli require a public health policy on appropriate prescribing and the rational use of antimicrobial agents.

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REFERENCES


