Phenotypic and Genotypic Detection of Extended-Spectrum β-Lactamases (ESBL) among Escherichia coli Isolated from Symptomatic Female's Genital Tract Infection

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Abstract

Clinical E. coli isolates (36) from women (aged 18-45 years) with symptomatic genital tract infection were detected phenotypically and genotypically for ESBL production. Phenotypically, most (30/36: 83.3%) isolates were resistant to cefotaxime (CTX) and more than half of them (26/36: 72.2%) were resistant to ceftazidime (CAZ). All (100%) and 25 (69.4%) of them were ESBL producers by screening and confirmatory tests, respectively. Genotypically, ESBL genotypes were detected in 31/36 (86.1%) of isolates. All four ESBL genotypes were found among these isolates with predominance of CTX-M-type (26/36: 72.2%) followed by SHV-type (22/36: 61.1%), OXA-type (7/36: 19.4%), and TEM-type (1/36: 2.7%). Of These isolates, 25/36 (69.4%) had two types of ESBL genes. Seventeen (17/22: 77.2%) of SHV-type positive isolates were CTX-M positive. Six (6/7: 85.7%) of OXA-type and the TEM-type positives were also CTX-M positive whereas the other OXA-type positive isolate was SHV-type positive. Most (22/25: 88%) isolates with two types of ESBLs were resistant to both CTX and CAZ. It can be concluded that, in female's genital tract infection, ESBL production, especially CTX-M-type, can be added as another factor, in addition to virulence factors, that select for certain strains to survive and cause disease and as vaginal E. coli is a reservoir along the fecal-vaginal-urinary/neonatal course of transmission in extraintestinal E. coli infections, our clinical
microbiology labs and clinicians need to be aware of the presence of these ESBL-producing organisms and should conduct surveillance studies to ascertain this.

Key words: ESBL, E. coli, female's symptomatic genital tract infection.

Introduction

*Escherichia coli* is one of the common organisms in the vaginal microflora of pregnant as well as non-pregnant women (1). Vaginal *E. coli* (VEC) may also cause symptomatic infections such as vaginitis or tubo-ovarian abscess and is associated with life threatening neonatal sepsis and meningitis (2). Recently, *E. coli* is one of the predominant microorganisms in cases of aerobic vaginitis (3, 4, 5). Aerobic vaginitis is a term proposed to describe purulent vaginal discharge with predominance of abnormal aerobic flora (3, 6), and its characteristics are different from those of bacterial vaginosis and elicit an important host response and genital complaints are those of a real vaginitis (5, 6, 7).

β-Lactam antibiotics remain the most commonly used antibacterial agents in the present chemotherapeutic armamentarium, and β-lactamases, the enzymes that hydrolyze β-lactam antibiotics are the major cause of resistance to these compounds (8). ESBL enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams, including third generation cephalosporins, penicillins and monobactams, but have no detectable activity against cephemycines and imipenem (8-10). Until the 2000s, most of the ESBLs were structurally related to the narrow-spectrum TEM- and SHV-type β-lactamases (11). The genetic mutations that give rise to ESBLs broaden the parental resistance pattern to a phenotype that includes resistance to broad-spectrum cephalosporins. Plasmids responsible for ESBL production tend to be large (80 Kb or more in size) and carry resistance to several agents, an important limitation in the design of treatment alternatives (9). Furthermore, in the late 1990s, a novel type of ESBLs, the CTX-M enzymes, emerged worldwide, mostly from *Escherichia coli* (11). The OXA-type enzymes are another growing family of ESBLs and are unique among the ESBLs because they are most often found in *Pseudomonas aeruginosa* rather than in members of the *Enterobacteriaceae* (11, 12).

The incidence of ESBL producing strains of *E. coli* among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options. The resistant organisms are now a worldwide problem (8-10). These organisms pose a therapeutic challenge, since they are frequently resistant to other kinds of antimicrobial drugs, including aminoglycosides, quinolones, and cotrimoxazole (14). We didn’t find (to our knowledge) any study around the world, dealing with ESBL production by vaginal *E. coli*, so that this study was carried out to detect phenotypically and genotypically ESBL production by *E. coli* isolated from non-pregnant women with symptomatic genital tract infection.

Material and Methods

**Bacterial isolates and phenotypic screening for ESBL**

Thirty six isolates of *E. coli* collected from non-pregnant women (aged 18-45 years) over a 2-year period from May 2008 to June 2010 at Obstetrics and Gynecology Clinics in Al-Kut/Wassit Province/Iraq, were included in this study. The isolates were recovered from high vaginal swabs collected from women with symptomatic genital tract infection and were identified by conventional biochemical tests (15). Cefotaxime (CTX) and ceftazidime (CAZ)
were used for screening for reduced susceptibility to oxymino-cephalosporins. The presence of ESBLs was confirmed by the double-disc method as recommended by the Clinical and Laboratory Standards Institute (17).

**PCR amplification for detection of β-lactamase genes**

All isolates were screened for the resistance genes TEM, SHV, CTX-M, and OXA by a multiplex PCR assay using universal primers (Bioneer, Korea) (Table 1, 18, 19, 20). Each isolate was subcultured on trypticase soy agar plates for 24 h at 37°C. From the agar plate, 5 colonies were picked and suspended in 100 µl sterile distilled water. Bacterial suspensions were run for 10 min at 94°C (21) in a DNA thermocycler (MultiGene, Labnet International, Inc., USA) and cell debris were removed by centrifugation (12,000 rpm for 1 min). Five µl of supernatant was used as a template in PCR. PCR amplification reactions were performed in a volume of 50 µl containing 25 µl of KapaTaq 2x Ready Mix (KAPA Biosystems, USA), 25 pmol concentrations of each primer, and 5 µl of DNA template. The cycling parameters were as follows: an initial denaturation at 94°C for 3 min; followed by 35 cycles of 94°C for 30 s, 45°C for 1 min, and 72°C for 1 min; and with a final extension at 72°C for 10 min. The amplified PCR products were subjected to electrophoresis at a 2% agarose gel in 0.5X TBE buffer.

**Statistical analysis**

The $\chi^2$ test was used for statistical comparison of groups; values < 0.05 were regarded as significant (22).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
<th>Reference (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaTEM</td>
<td>F AAACGCTGGTGAAGAATA</td>
<td>822</td>
<td>18, 19</td>
</tr>
<tr>
<td></td>
<td>R AGCGATCTGTCTAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaSHV</td>
<td>F ATGCGTTATATTCCGCTGTA</td>
<td>753</td>
<td>18, 19</td>
</tr>
<tr>
<td></td>
<td>R TGCTTTGTATTTCCCGGCAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaCTX-M</td>
<td>F CGCTTTTGCATGTGCGAG</td>
<td>550</td>
<td>18, 19</td>
</tr>
<tr>
<td></td>
<td>R ACCCGATATCGTTGAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaOXA</td>
<td>F ATATCTCTACTTGTGCGATCT</td>
<td>619</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>R AAACCTTCAAAACCATCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results**

Thirty six *E. coli* isolates from females with symptomatic genital tract infection, were surveyed phenotypically and genotypically for ESBL production. Phenotypically, most (30/36: 83.3%) isolates were resistant to CTX and more than half of them (26/36: 72.2%) were resistant to CAZ. All (100%) and 25 (69.4%) of them were ESBLs producers when tested by screening and confirmatory tests, respectively (Table 2).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%) of positive <em>E. coli</em></th>
</tr>
</thead>
</table>
Genotypically, ESBL genotypes were detected in 31/36 (86.1%) of isolates. All four ESBL genotypes were found among these isolates (Fig. 2) with predominance of CTX-M-type (26/36: 72.2%) followed by SHV-type (22/36: 61.1%), OXA-type (7/36: 19.4%), and TEM-type (1/36: 2.7%). Of these isolates, 25/36 (69.4%) had two types of ESBL genes. Seventeen (17/22: 77.2%) of SHV-type positive isolates were CTX-M positive. Six (6/7: 85.7%) of OXA-type and the TEM-type positives were also CTX-M-positive whereas the other OXA-type positive isolate was SHV-type positive. Most (22/25: 88%) isolates with two types of ESBLs were resistant to both CTX and CAZ except three isolates: one with genotype of CTX-M and TEM which was resistant to CTX and sensitive to CAZ, the second with genotype of SHV and OXA, which had intermediate resistance to CTX and was sensitive to CAZ, and the third isolate with CTX-M and SHV, which had intermediate resistance to both antibiotics. Among ESBL-positive isolates, 26/31 (83.8%), had CTX-M-type and 22/31 (70.9%) had SHV-type ESBL.

Fig. 2: Percent distribution of four ESBL genotypes among *E. coli* isolated from women with symptomatic genital tract infection.

### Table 1: Phenotypic and Genotypic Detection of ESBLs

<table>
<thead>
<tr>
<th>Resistance to:</th>
<th>CTX</th>
<th>CAZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic detection of ESBLs:</td>
<td>30 (83.3): R = 26; I = 4</td>
<td>26 (72.2): R = 25; I = 1</td>
</tr>
<tr>
<td>Genotypic detection of ESBLs (bla genotype):</td>
<td>ESBL screening test</td>
<td>36 (100)</td>
</tr>
<tr>
<td></td>
<td>ESBL confirmatory test (DDST)</td>
<td>25 (69.4)</td>
</tr>
<tr>
<td>CTX-M</td>
<td>26 (72.2)</td>
<td></td>
</tr>
<tr>
<td>SHV</td>
<td>22 (61.1)</td>
<td></td>
</tr>
<tr>
<td>TEM</td>
<td>1 (2.7)</td>
<td></td>
</tr>
<tr>
<td>OXA</td>
<td>7 (19.4)</td>
<td></td>
</tr>
</tbody>
</table>

CTX: cefotaxime; CAZ: ceftazidime; R: resistant; I: intermediate resistant; DDST: double disc synergy test; ESBL: extended spectrum β-lactamase.

Discussion

This study was designed to detect phenotypically and genotypically ESBLs’ distribution among clinical *E. coli* isolated from non-pregnant women with symptomatic genital tract infection. Most isolates were resistant to CTX...
and about half of them were resistant to CAZ. All of CAZ resistant isolates were also resistant to CTX. This indicated the widespread resistance to broad spectrum cephalosporins in our community as a result of extensive use of these antibiotics for treatment. Concentrated use of third-generation cephalosporins in Iraq may be the most prominent risk factor for emergence of ESBL-producing pathogens (23). High rate of ESBL production by E. coli may be due to the selective pressure imposed by extensive use of antimicrobials (24). The over-reliance on antibiotics, and insufficient application of infection control measures and improved hygiene, has eroded the effectiveness of older, inexpensive agents and threatens the efficacy of recently introduced ones (8).

The members of Enterobacteriaceae possess many mechanisms of resistance to β-lactam antibiotics such as loss of porin, efflux pumps, etc. However, β-lactamases are the most common and clinically significant mechanism of resistance to β-lactam antibiotics among this bacterial group (8). Four types of ESBLs were detected genotypically in this study, namely: TEM-, SHV-, CTX-M-, and OXA-type. Genotypically, 86.1% of this study included isolates were ESBL producers. CTX-M-type ESBL was the most common, followed by SHV- and OXA-type while TEM-type was rare. This result is consistent with the present situation in most parts of the world. During the last 2 decades, most of the ESBL found in E. coli and, in general, in gram-negative bacilli, has been of TEM or SHV lineage. Recently TEM and SHV types have been replaced by CTX-M-type ESBL (11, 25). CTX-M β-lactamases have spread among Enterobacteriaceae in most parts of the world (26-28). In the Middle East area, reports pointed out that CTX-M is the predominant ESBL in E. coli (23, 29-31). Among the different ESBLs, particular attention should be paid to the worldwide increasing prevalence of the CTX-M types. These enzymes are prevalent not only in nosocomial environment, but also in the community setting (32-34). Antibiotic selective pressure probably contributes to the increasing prevalence of cefotaxime and ceftriaxone hydrolyzing CTX-M β-lactamases in clinical setting (35-36). This high distribution of CTX-M-type ESBLs among this study's isolates explains the high rate of resistance to CTX in comparison to CAZ since CTX-M β-lactamases, in contrast to most TEM and SHV ESBLs, preferentially hydrolyze cefotaxime over ceftazidime (37-38), but point mutations around the active site of some enzymes belonging to the CTX-M-1 and CTX-M-9 groups have increased their ability to hydrolyze ceftazidime significantly (39-40).

ESBL confirmatory test results were not correlated with genotyping results (Table 2), as 80.6% (25/31) of ESBL genotype positive isolates by PCR, were positive by confirmatory test. This can be explained by several ways each of which needs further investigation. Extended-spectrum β-lactamases (ESBLs) are generally sensitive to inhibition by clavulanic acid, though resistant variants have been reported. Combinations of β-lactamase inhibitors and penicillins have led to selection of the phenotype that resists inhibition of β-lactamase (8). The effectiveness of inhibitor may be reduced in the presence of multiple ESBLs in the bacteria (41). ESBL testing in AmpC-produing species of Enterobacteriaceae is an unresolved issue in the field of ESBL testing (42). Another possibility is the possession of more than one resistance mechanism (8).

It can be concluded that, in female's genital tract infection, ESBL production, especially CTX-M-type, can be added as another factor, in addition to virulence factors, that select for certain strains to survive and cause disease and as vaginal Escherichia coli is a reservoir along the fecal-vaginal-urinary/neonatal course of transmission in extraintestinal E. coli infections, our clinical microbiology labs and clinicians need to be aware of the presence of ESBL-producing organisms and should conduct surveillance studies to ascertain this.
References


