Prevalence of ESBL phenotype, \( \text{bla}_{\text{CTX-M-1}}, \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{TEM}} \) genes among uropathogenic \textit{Escherichia coli} isolates from 3 military hospitals of Tehran, Iran

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ABSTRACT

Objective: To determine the extended-spectrum beta-lactamase (ESBL) production and prevalence of \( \text{bla}_{\text{CTX-M-1}}, \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{TEM}} \) genes among uropathogenic \textit{Escherichia coli} (UPEC) isolates from 3 military hospitals of Tehran 2015–2016.

Methods: One-hundred and eleven isolates were adopted. The antibiotic susceptibility testing was conducted according to Clinical and Laboratory Standards Institute guidelines. The combine disk was used for phenotypic ESBL production. The ceftazidime MIC was conducted with the micro-broth dilution test. The PCR assay was used to detect the \( \text{bla}_{\text{CTX-M-1}}, \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{TEM}} \) genes.

Results: In the broth microdilution method, 103 (92.7%) isolates showed minimal inhibitory concentration (MIC) ≥ 1 µg/mL, and also in the combined disk method, 89 (80.1% of all) were ESBL positive. On the other hand, among 91 ceftazidime resistant isolates, 86 (77.4% of all) were ESBL positive. The difference between the two methods for ESBL confirmation was not significant. The result of MIC was similar to the disk diffusion method in the detection of phenotypic ESBL production. Among ESBL producer isolates, the prevalence of \( \text{bla}_{\text{CTX-M-1}}, \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{TEM}} \) was 77.4% (n = 86), 47.4% (n = 53) and 2.4% (n = 2), respectively. These genes were amplified in a wide range MIC of ceftazidime.

Conclusions: The prevalence of multi-drug resistant UPEC and ESBL positive isolates was high in military hospitals. The majority of UPEC isolates amplified \( \text{bla}_{\text{CTX-M-1}} \) and \( \text{bla}_{\text{SHV}} \) type \( \beta \)-lactamase genes. One-third of isolates were positive in presence of both these genes. There was no relation between ceftazidime MIC and presence of \( \beta \)-lactamase genes.

1. Introduction

Uropathogenic \textit{Escherichia coli} (UPEC) isolates can persist in urothelial cells and cause recurrent infections. Furthermore, multi-drug resistant isolates carry plasmids (Inc FII/IncI1, etc.) that confer the resistance to multiple classes of antibiotics in addition to cephalosporins. The genetic location of extended-spectrum beta-lactamases (ESBLs) include the mobile elements and chromosome of Enterobacteriaceae[1]. Recent data have shown that \( \text{bla}_{\text{CTX-M-1}} \) clones are mostly widespread at an endemic status worldwide similar to results from Iran[2]. The frequency of ESBLs is increasing everywhere[3]. These ESBLs are inhibited by clavulanic acid, sulbactam, and tazobactam. This phenomena can help to detect these \( \beta \)-lactamases in the phenotypic confirmatory test[4]. On the other hand, ESBLs are often associated with resistance to other antibiotics, including fluoroquinolones, aminoglycosides and sulfamethoxazole/trimethoprim[5]. The pandemic \textit{Escherichia coli} ST131 clone encoding CTX-M-15 with a high virulent potential was characterized by a multidrug resistance result and co-production of OXA-1 or TEM-1b as well as aac(6')-Ib-cr. This clone produces \( \text{bla}_{\text{CTX-M-15}} \) beta lactamase worldwide[6-8]. CTX-M-type ESBLs are a complex and heterogeneous family and may be subdivided into 5 major groups (CTX-M-1, 2, 8, 9 and CTX-M-25)[9,10]. These enzymes have spread worldwide and include most ESBLs detected in Enterobacteriaceae. They are not only found in hospitals, but also in the community settings, thus changing the epidemiology of ESBLs[11]. The \( \text{bla}_{\text{CTX-M}} \) and \( \text{bla}_{\text{TEM}} \) ESBLs can hydrolyze third and fourth generation cephalosporins. Several studies have demonstrated a relationship between ESBL enzymes and minimal inhibitory concentration (MIC) of 3rd and 4th generation cephalosporins, including ceftazidime, cefepime and cefotaxim[12]. The aim of this study was determination of ESBL positive UPEC strains and prevalence of \( \text{bla}_{\text{CTX-M}}, \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{TEM}} \) types among ESBL positive UPEC strains among 3 military hospitals in Tehran.
2. Materials and methods

2.1. Clinical isolates

One-hundred and eleven UPEC isolates were collected during 2015–2016, from three hospitals in Tehran. These isolates were obtained from different urine cultures of patients with ages ranging from 5 to 73 (mean = 46.0 ± 1.3) years old. Furthermore, seventy patients were female (mean age of 46.63) and 41 (mean age of 34.21) were male. The isolates were identified by both conventional biochemical and molecular tests advised for UPEC strains.

2.2. Susceptibility tests and phenotypic ESBL detection

Susceptibility testing was performed by the disk diffusion method following the guidelines of Clinical and Laboratory Standards Institute (CLSI). Seventeen antimicrobial disks were used including aztreonam (30 µg), piperacillin (100 µg), augmentin (30 µg), cefotaxime (30 µg), cefpodoxime (10 µg), ceftriaxone (30 µg), meropenem (10 µg), piperacillin-tazobactam (110 µg), imipenem (10 µg), cefazidime (30 µg) and cefepime (30 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), amikacin (30 µg) and tobramycin (10 µg), gentamicin (120 µg).

Escherichia coli ATCC 25922 was used for the quality control of susceptibility testing. The ESBL positive phenotype was detected by combined disk method using ceftazidime and cefotaxime disks with and without clavulanic acid. The MICs of 3rd generation cephalosporin resistant isolates were determined by broth micro dilution method using ceftazidime and cefotaxime disks with range of 0.25–128.00 µg/mL (CLSI 2014). Each isolate with MIC ≥ 1 µg/mL was further tested for ESBL production in addition to the results of disk diffusion.

2.3. PCR amplification of ESBL genes

The CTX-M, SHV and TEM type ESBLs were amplified with specific primers shown in Table 1. The PCR amplification of the genes was performed with 2 µL of template DNA that was added to a final 50 µL master mix containing: 50 mmol/L KCl, 0.1% Triton X-100, 10 mmol/L Tris-HCl (pH 9), 2 mmol/L MgCl2, 200 µmol/L dNTPs, 1 µL of each primer, 1.25 IU of Taq DNA polymerase and 32.25 µL distilled water. For blaCTX-M, 25 cycles with annealing temperature of 55 °C (1 min) was used. For blaSHV and blaTEM types, 25 cycles with annealing temperatures of 54 °C (30 s) and 56 °C (30 s) were used respectively. The primers of TEM included: F 5'-TCG

Sequence (5' to 3')

| CTXM-1, -3, -10 | CTXM-1-1, -3, -10 |
|----------------|------------------|
| CTT-AC TAT-GCC TCG GCA-CTT TAC AA | TTG-ATC ACT-TGC GCA TCG |
| TOHO-2F | TOHO-2F |
| GAG GAC TAC GG-CC-CA-CC-CA--TAC | AAT-ACAT-CAT-GC-GC-CG-CG |
| TOHO-1R | TOHO-1R |
| CCC GGC TAT-TGC CCC GTC CTT TAC GGC | GGC TAT-TGC CCC GTC CTT TAC GGC |
| CTXM825F | CTXM825F |
| GCT TTT GCC ATG TGC AGC ACC | GCT TTT GCC ATG TGC AGC ACC |
| CTXM825R | CTXM825R |
| GGG ATG TGT GCG CGT-3' | TGC TTA ATC AGT GAG |

2.4. Statistical analysis

Comparisons of variants were conducted using the student unpaired t-test. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. The susceptibility testing and ESBL production

The antibiotic susceptibility testing of ESBL positive and negative UPEC isolates have been depicted in Table 2.

| Antimicrobial | ESBL negative | ESBL positive |
|---------------|---------------|---------------|
| Aztreonam     | 31.2          | 97.3          |
| Piperacillin  | 4.4           | 6.3           |
| Augmentin     | 87.3          | 23.5          |
| Cefotaxime    | 18.9          | 89.6          |
| Cepodoxime    | 36.3          | 91.4          |
| Ceftriaxone   | 67.7          | 89.3          |
| Meropenem     | 12.1          | 13.4          |
| Piperacillin-tazobactam | 4.6      | 5.4           |
| Imipenem      | 11.3          | 13.5          |
| Cefazidime    | 23.1          | 82.0          |
| Cefepine      | 27.2          | 87.6          |
| Ofloxacin     | 31.1          | 78.2          |
| Ciprofloxacin | 78.2          | 77.7          |
| Levofloxacin  | 32.4          | 73.3          |
| Amikacin      | 53.3          | 54.8          |
| Tobramycin    | 9.9           | 57.0          |
| Gentamicin    | 10.3          | 60.1          |

In the broth microdilution method, 103 (92.7%) isolates showed MIC ≥ 1 µg/mL, and in the combined disk method, 89 (80.1% of all) were ESBL producer strains. On the other hand, among 91 cefazidime resistant isolates, 86 (77.4% of all) were ESBL positive. The results of these two methods in the ESBL confirmation were similar. The result of MIC was approximately similar to the disk diffusion for isolates in phenotypic ESBL production test.

3.2 Genotypic detection of ESBLs

The prevalence of blaCTX-M, blaSHV and blaTEM genes among ESBL producer strains was 77.4% (n = 86), 47.4% (n = 53) and 2.4% (n = 2) respectively. The blaCTX-M was related to higher MIC to cefazidime. The relation between the MIC of isolates and presence of blaCTX-M, blaSHV and blaTEM genes for 28 community isolates has been depicted in Table 3.

| Isolate | MIC (µg/mL) | DDST | Gender | Age (years) | blaCTX-M | blaSHV | blaTEM | Number of non-susceptible antibiotics |
|---------|-------------|------|--------|------------|----------|--------|--------|-----------------------------------|
| 1       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 2       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 3       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 4       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 5       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 6       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 7       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 8       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 9       | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 10      | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 11      | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 12      | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 13      | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 14      | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |
| 15      | 31.2        | +    | Male   | 32         | +        | +      |        | 6                                 |

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There was no relation between MIC of 64 and 128 µg/mL for both of the two genes. There was no relation between MIC of 64 and 128 µg/mL for both of the two genes. There was no relation between MIC of 64 and 128 µg/mL for both of the two genes. There was no relation between MIC of 64 and 128 µg/mL for both of the two genes. There was no relation between MIC of 64 and 128 µg/mL for both of the two genes.