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# Antibiotic Susceptibility Profile, ESBL Production and $bla_{CTX-M1}$, $bla_{SHV}$ and $bla_{TEM}$ Types Among *Escherichia coli* Blood Isolates

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**Abstract**

**Background:** Plasmid and chromosomal extended-spectrum beta-lactamases (ESBLs) have been increasingly spread everywhere and $bla_{CTX-M1}$ is one predominant beta-lactamase.

**Objectives:** This study was fulfilled to determine the production of ESBL and prevalence of $bla_{CTX-M1}$, $bla_{SHV}$, and $bla_{TEM}$ among *Escherichia coli* blood isolates in Tehran.

**Patients and Methods:** Twenty-three isolates were adopted to be studied during 2015-2016. The antibiotic susceptibility testing was performed using Kirby–Bauer method. The combined disk method was used for the detection of phenotypic ESBL production. The most effective antibiotics were piperacillin, amikacin, and ofloxacin. The minimum inhibitory concentration (MIC) of ceftazidime was determined using micro-broth dilution method. Polymerase chain reaction (PCR) was used for detecting the $bla_{CTX-M1}$, $bla_{SHV}$, and $bla_{TEM}$ genes.

**Results:** In the broth dilution test, 19 (82%) isolates showed MIC≥1, and 18 (78.3%) isolates were ceftazidime resistant. In the combined disk test, 19 (82%) isolates were ESBL producers. The results of the MIC and ceftazidime resistance were the same for ESBL selection. The results of MIC, in fact confirmed the disk diffusion in determining the phenotypic ESBL production. The frequency of $bla_{CTX-M1}$, $bla_{SHV}$, and $bla_{TEM}$ genes among blood ESBL producing isolates was 26% (n=6), 8.6% (n=2), and 0%, respectively. Isolates that showed higher MIC were positive for these genes.

**Conclusion:** The prevalence of multidrug-resistant blood isolates and ESBL phenotype was high in military hospitals. A low number of blood strains amplified $bla_{CTXM1}$ and $bla_{SHV}$ type beta–lactamases. There was a relationship between the MIC and the presence of beta-lactamase genes.

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## Background

*Escherichia coli* Blood isolates can initiate from gastrointestinal or urinary tracts and cause fatal infections. In addition, multidrug-resistant isolates harbor plasmids such as Inc FII/IncI1 and so on, leading to the resistance to several classes of antibiotics besides cephalosporins. The genetic location of extended-spectrum beta-lactamases (ESBLs) is the mobile elements and the chromosome of Enterobacteriaceae.[1] Recent data have shown that $bla_{CTX-M1}$ clones are mostly widespread at an endemic status worldwide and in Iran.[2] The ESBLs are increasing everywhere.[3] These ESBLs are inhibited by clavulanic acid, sulbactam, and tazobactam that help their detection.[4] On the other hand, resistance due to ESBLs is often accompanied with resistance to other antibiotics, including fluoroquinolones, aminoglycosides, and sulfamethoxazole /trimethoprim.[5] The pandemic *E. coli* clone of ST131 with a high virulent potential encoding CTX-M-15 was characterized by the multidrug resistance (MDR) through the co-production of OXA-1 or TEM-1b as well as aac(6')-Ibeta-cr. This clone produces $bla_{CTX-M-}$15 beta-lactamase.[6-8] CTX-M-type ESBLs are complex and heterogeneous families 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 are the most ESBLs detected in Enterobacteriaceae. They are not only found in hospitals, but also have been detected in the community, thus changing the epidemiology of ESBLs.[11] The $bla_{CTX-M}$ and $bla_{TEM}$ ESBLs can hydrolyze third and fourth generation cephalosporins. Several studies have demonstrated a relationship between ESBL enzymes and minimum inhibitory concentration (MIC) to third and fourth generation cephalospo-

rins, including ceftazidime, cefepime, and cefotaxime.[12]

## Objectives

The aim of this study was to determine the ESBL positive blood *E. coli* strains and the prevalence of $bla_{CTX-M}$, $bla_{SHV}$, and $bla_{TEM}$ types among ESBL positive blood isolates among 3 military hospitals in Tehran, Iran.

## Patients and Methods

### Bacterial Isolates

Twenty-three *E. coli* blood isolates were collected during 2015-2016, from 3 hospitals of Tehran, Iran. These isolates were obtained from the patients 2-23 years of age. Fourteen patients were female (mean age of 14.62) and 9 were male (mean age of 12.33). The isolates were identified by both confirmatory biochemical and molecular tests advised for *E. coli*.

### Susceptibility Tests and Detection of ESBLs

Susceptibility testing was performed using the disk diffusion method as per the guidelines of Clinical and Laboratory Standards Institute (CLSI). Seventeen antimicrobial disks were used as indicated in Table 1.

The standard ATCC25922 *E. coli* was used as the quality control of antimicrobial susceptibility testing. The ESBL phenotype determination was done using combined disk and synergy test methods employing cefotaxime and ceftazidime with and without clavulanic acid disk. The MICs of blood isolates were determined using broth microdilution method against ceftazidime in the range of 0.25–128 ug/mL (CLSI 2014). Isolates with MIC ≥1 were further tested for ESBL production in addition to ceftazidime resistant strains.

### DNA Extraction

Total DNA was isolated with the boiling Method. Briefly, 200 µL of bacterial suspension was prepared in distilled water. After boiling in 95°C for 10 minutes, the centrifugation of tubes was performed and the supernatant was preserved at -20°C for later use.

### Amplification of ESBL Genes

The Polymerase chain reaction (PCR) detection of CTX-M, SHV, and TEM ESBLs was done with the specific primers shown in Table 2.

### Statistical Analysis

The relations were compared by *t* test using SPSS version 20.0. The value of $P < 0.05$ was considered to be significant.

## Results

### The Susceptibility Testing and ESBL Production

The susceptibility testing profile between ESBL positive and negative blood strains is depicted in Table 3.

In the broth microdilution procedure, 21 (92.7%) isolates showed MIC ≥1, and also in the combined disk test, 19 (80.1% of all) isolates were ESBL producers. On the other hand, there was a correlation between ceftazidime resistance and MIC results for ESBL production. The results of ceftazidime MIC confirmed the disk diffusion for guidance in the phenotypic production of ESBL by the isolates.

**Table 1.** The Antimicrobial Susceptibility Disks

| Antibiotic family | Disks and concentrations |
|---|---|
| Beta-lactams | 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), ceftazidime (30 µg) and cefepime (30 µg) |
| Fluoroquinolones | Ofloxacin (5 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) |
| Aminoglycosides | Amikacin (30 µg), tobramycin (10 µg), gentamicin (120 µg), |

**Table 2.** The Specific Primers Used in the Present Study

| Primer | Sequence (5′ to 3′) | Target(s) | |
|---|---|---|---|
| CTXM1-F3 | GAC GAT GTC ACT GGC TGA GC | CTX-M group I | CTX-M-1, -3, -10 to -12, -15 (UOE-1), -22, -23, -28 to -30 |
| CTXM1-R2 | AGC CGC CGA CGC TAA TAC A | | |
| TOHO1-2F | GCG ACC TGG TTA ACT ACA ATC C | CTX-M group II | CTX-M-2, -4 to -7, and -20 and Toho-1 |
| TOHO1-1R | CGG TAG TAT TGC CCT TAA GCC | | |
| CTXM825F | CGC TTT GCC ATG TGC AGC ACC | CTX-M group III | CTX-M-8 and -25 |
| CTXM825R | GCT CAG TAC GAT CGA GCC | | |
| | GCT GGA GAA AAG CAG CGG AG | CTX-M group IV | CTX-M-9, -13, -14, -16 to -19, -21, and -27 and Toho-2 |
| | GTA AGC TGA CGC AAC GTC TG | | |
| **Primer** | **Sequence (5′ to 3′)** | **Nucleotide Numbers** | **Amplicon** |
| TEM-F2 | TCG GGG AAA TGT GCG CG | 90 to 105 | 971 bp |
| TEM-R2 | TGC TTA ATC AGT GAG GCA CC | 1062 - 1042 | |

**Table 3.** The Results Of Disk Diffusion Test Compared Between ESBL Negative and Positive Isolates

| Antibiotic Family | Disks and Resistance (ESBL Negative and ESBL Positive) |
|---|---|
| Beta-lactams | Piperacillin (4.4% and 6.3%), aztreonam (31.2% and 97.3%), augmentin (87.3% and 23.5%), cefpodoxime (36.3% and 91.4%), cefotaxime (18.9% and 89.6%), ceftriaxone (67.7% and 89.3%), piperacillin-tazobactam (4.6% and 7.4%), imipenem (7.3% and 13.5%), meropenem (6.1% and 13.4%), ceftazidime (23.1% and 82%) and cefepime (17.2% and 67.6%) |
| Fluoroquinolones | Ofloxacin (31.1% and 78.2%), ciprofloxacin (33.4% and 77.7%), levofloxacin (12.4% and 73.3%) |
| Aminoglycosides | Amikacin (5.3% and 54.8%), tobramycin (8.9% and 57%), gentamicin (9.3% and 60.1%), |

Abbreviation: ESBL, extended-spectrum beta-lactamase.

**Table 4.** Some Demographic Data, MIC Results, and $bla_{CTX\text{-}M1}$ and $bla_{SHV}$ Genes Among ESBL Positive Isolates

| Isolate | MIC | ESBL DDST | Genus | Age | $bla_{CTXM\text{-}I}$ | $bla_{SHV}$ | $bla_{TEM}$ | No. of Non-susceptible Antibiotics |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | + | F | 3 | | | ND | 6 |
| 2 | 4 | + | F | 12 | | | ND | 7 |
| 3 | 4 | + | M | 4 | | | ND | 5 |
| 4 | 4 | + | F | 16 | + | | ND | 6 |
| 5 | 4 | + | F | 22 | | | ND | 4 |
| 6 | 8 | + | M | 13 | + | | ND | 11 |
| 7 | 16 | + | M | 15 | + | | ND | 11 |
| 8 | 16 | + | F | 12 | + | + | ND | 10 |
| 9 | 32 | + | M | 19 | + | + | ND | 13 |
| 10 | 32 | + | M | 18 | + | | ND | 14 |

Abbreviation: ESBL, extended-spectrum beta-lactamase; DDST, double disk susceptibility test; F, female; M, male; ND, not detected.

## Genotypic Detection of ESBL Enzymes

The prevalence of $bla_{CTX\text{-}M1}$, $bla_{SHV}$, and $bla_{TEM}$ genes among ESBL producing isolates was 26% (n = 6), 8% (n = 2) and 0%, respectively (Figure 1). The $bla_{CTXM1}$ and $bla_{SHV}$ genes were associated with higher MIC against ceftazidime. The relationship among the MIC, characteristics of isolates, and presence of beta-lactamase genes for 10 adopted isolates is indicated in Table 4.

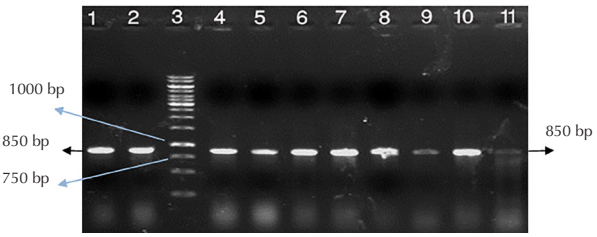


**Figure 1.** PCR Product of $bla_{SHV}$ Gene With 850 bp Size. Columns 1, 2, and 4-9: positive samples, column 3: DNA ladder, columns 10 and 11: positive and negative controls, respectively.

## Discussion

In the current study, the level of ESBL production and MDR phenotype among *E. coli* blood isolates was high. Analysis of susceptibility of the strains to antibiotics demonstrated that among beta-lactam classes, the most effective drugs were meropenem (67%) and piperacillin (85%). Most patients were aged between 2-22 years old. In addition, among non-beta-lactam antibiotics, amikacin exhibited the highest activity against the isolates (63%). Similar to these results, in some previous studies conducted in Tehran and other areas of Iran, a high rate of ESBL among Enterobacteriaceae was revealed.[13-15] Recent publications have uncovered the predominant interference of $bla_{CTXM1}$ in ESBL positive isolates all over the world. In the present study, $bla_{CTXM1}$ group was accounted for 26% of blood ESBL production. This was the first study detecting the ESBL production in blood *E. coli* as well as molecular detection of related beta-lactamases in 3 military hospitals of Tehran. Similarly, results from previous molecular studies have demonstrated that $bla_{CTX\text{-}M1}$ is endemic and is present among ST131 or non ST131 clones in healthcare and community settings.[16-19] The $bla_{CTXM1}$ was detected in the range of 4-32 ug/mL ceftazidime, although higher MIC was associated with MDR and presence of both $bla_{CTXM1}$ and $bla_{SHV}$ genes. It has been demonstrated that $bla_{CTXM1/14}$ and $bla_{CTXM\text{-}15}$ genes cause high level of resistance to cefepime/ceftriaxone and ceftazidime, respectively.[12] On the other, despite most of these studies, blood isolates exhibited a low frequency of ESBL encoding genes, supposed that other beta-lactamase classes interfere in the resistance to third generation cephalosporins. Our results were different from those of a study by Mohajeri et al in the West of Iran in which $bla_{CTXM1}$, $bla_{SHV}$, and $bla_{TEM}$ were detected in 93.3%, 68.3%, and 43.2% of isolates, respectively.[20] In another study by Mehrgan et al, of 130 isolates producing ESBL, 22 (16.3%) were $bla_{CTXM}$ type positive. In this study, 2 blood isolates harbored both $bla_{CTX\text{-}M1}$ and

*bla*$_{SHV}$ types and none of them could amplify *bla*$_{TEM}$ gene. Moreover, this study showed a correlation between higher MIC and co-resistance to multiple antibiotics, except for ciprofloxacin. The MIC, ESBL phenotype, and related genes were not significantly in relation with the age or genus of patients ($P = 0.14$ and 0.31, respectively).

Although the prevalence of MDR rate and ESBL production were high, *bla* genes were not highly detected. The isolates with higher MIC amplified both *bla*$_{CTXM-I}$ and *bla*$_{SHV}$ type beta–lactamases. Ceftazidime MIC was in accordance with the disk resistance for detecting phenotypic ESBL production. There may be multiple mechanisms for resistance to the third generation cephalosporin drugs.

## Authors' Contributions

AG and FN performed and advised the work, and meanwhile ME and SK helped during the work.

## Conflict of Interest Disclosures

The authors have declared that no conflict of interests exists.

## Ethical Approval

This study was approved by AJA University of Medical Sciences, Tehran, Iran.

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## References

1. Rodríguez I, Thomas K, Van Essen A, et al. Chromosomal location of *bla*$_{CTX-M}$ genes in clinical isolates of Escherichia coli from Germany, The Netherlands and the UK. Int J Antimicrob Agents. 2014;43(6):553-557. doi:10.1016/j.ijantimicag.2014.02.019.
2. Dahmen S, Métayer V, Gay E, Madec J-Y, Haenni M. Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. Vet Microbiol. 2013;162(2):793-799. doi:10.1016/j.vetmic.2012.10.015.
3. Chen LF, Freeman JT, Nicholson B, et al. Widespread dissemination of CTX-M-15 genotype extended-spectrum-β-lactamase-producing Enterobacteriaceae among patients presenting to community hospitals in the southeastern United States. Antimicrob Agents Chemother. 2014;58(2):1200-1202. doi:10.1128/AAC.01099-13.
4. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol. 2010;13(5):558-564. doi:10.1016/j.mib.2010.09.006.
5. Coque T, Baquero F, Canton R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill. 2008;13(47):5437-5453.
6. Park SH, Byun J-H, Choi S-M, et al. Molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* in the community and hospital in Korea: emergence of ST131 producing CTX-M-15. BMC Infect Dis. 2012;12:149. doi:10.1186/1471-2334-12-149.
7. Peirano G, van der Bij AK, Gregson DB, Pitout JD. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J Clin Microbiol. 2012;50(2):294-299. doi:10.1128/JCM.06025-11.
8. Pitout JD, Gregson DB, Campbell L, Laupland KB. Molecular characteristics of extended-spectrum-β-lactamase-producing *Escherichia coli* isolates causing bacteremia in the Calgary Health Region from 2000 to 2007: emergence of clone ST131 as a cause of community-acquired infections. Antimicrob Agents Chemother. 2009;53(7):2846-2851. doi:10.1128/AAC.00247-09.
9. Madec J-Y, Poirel L, Saras E, et al. Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*$_{CTX-M-15}$-carrying plasmids. J Antimicrob Chemother. 2012;67(3):578-581. doi:10.1093/jac/dkr542.
10. Literacka E, Bedenic B, Baraniak A, et al. *bla*CTX-M genes in Escherichia coli strains from Croatian hospitals are located in new (*bla*$_{CTX-M-3a}$) and widely spread (*bla*$_{CTX-M-3a}$ and *bla*$_{CTX-M-15}$) genetic structures. Antimicrob Agents Chemother. 2009;53(4):1630-1635. doi:10.1128/AAC.01431-08.
11. Woerther P-L, Burdet C, Chachaty E, Andremont A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev. 2013;26(4):744-758.
12. Ramos JMC, Stein C, Pfeifer Y, Brandt C, Pletz MW, Makarewicz O. Mutagenesis of the CTX-M-type ESBL—is MIC-guided treatment according to the new EUCAST recommendations a safe approach? J Antimicrob Chemother. 2015;70(9):2528-2535. doi:10.1093/jac/dkv153.
13. Behrooozi A, Rahbar M, Jalil V. Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *Klebseilla pneumonia* isolated from urine in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res. 2010;4(9):881-884.
14. Mehrgan H, Rahbar M. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* in a tertiary care hospital in Tehran, Iran. Int J Antimicrob Agents. 2008;31(2):147-151.
15. Noori M, Karimi A, Fallah F, et al. High prevalence of metallo-beta-lactamase producing acinetobacter baumannii isolated from two hospitals of Tehran, Iran. Archives of Pediatric Infectious Diseases. 2014;2(3):e15439. doi:10.5812/pedinfect.15439.
16. Woodford N, Ward M, Kaufmann M, et al. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. J Antimicrob Chemother. 2004;54(4):735-743.
17. Mansour W, Grami R, Khalifa ABH, et al. Dissemination of multidrug-resistant bla CTX-M-15/IncFIIk plasmids in *Klebsiella pneumoniae* isolates from hospital-and community-acquired human infections in Tunisia. Diagn Microbiol Infect Dis. 2015;83(3):298-304. doi:10.1016/j.diagmicrobio.2015.07.023.
18. Xia S, Fan X, Huang Z, et al. Dominance of CTX-M-type extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from patients with community-onset and hospital-onset infection in China. 2014.
19. Peerayeh SN, Eslami M, Memariani M, Siadat SD. high prevalence of *bla*$_{CTX-M-1}$ Group extended-spectrum β-lactamase genes in *Escherichia coli* isolates from Tehran. Jundishapur J Microbiol. 2013;6(7):e6863. doi:10.5812/jjm.6863.
20. Mohajeri P, Rostami Z, Farahani A, Norozi B. Distribution of ESBL producing Uropathogenic *Escherichia coli* and carriage of selected β-lactamase genes in Hospital and community isolates in west of Iran. Annals of Tropical Medicine and Public Health. 2014;7(5):219-222.