Evaluation of Multidrug Resistance and TEM and SHV Broad-Spectrum Beta-Lactamase Genes in Escherichia coli Obtained from Patients with Urinary Tract Infections in Alborz Province, Iran

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ABSTRACT

Background & Objective: Investigating multidrug resistance and TEM and SHV broad-spectrum beta-lactamase genes in Escherichia coli bacteria isolated from patients with urinary tract infections is very useful to improve the treatment of infection and prevent the failure of treatment of urinary tract infections.The aim of this study was to investigate multidrug resistance and TEM and SHV broad-spectrum beta-lactamase genes in Escherichia coli bacteria isolated from patients with urinary tract infections.

Materials & Methods: In this study, 188 strains of Escherichia coli, which cause urinary tract infections in Alborz province, were studied. Urine samples were cultured on EMB and Blood Agar media. Differential tests were performed for final identification. ESBL-producing strains were identified, PCR was performed to survey the abundance of ESBL-producing genes.

Results: Based on the results of the disk diffusion and Double-disk synergy testS, 82 (43.6%) strains were determined as the final producer of ESBL. out of these isolates, the frequency of SHV, TEM, and CTX genes measured 64.3%, 55.9%, and 21.4%, respectively. These results showed that 12 (14.28%) of Escherichia coli isolated have all genes, 26 (30.95%) had 2 genes and 36 (42.85%) had one gene.

Conclusion: According to the results, it was found that imipenem with the lowest resistance is the best drug in the treatment, and carbapenems are the best drug for treating diseases caused by Escherichia coli. The results of the current study may be useful in replacing ESBL enzyme resistance screening with more modern sensitivity measurement methods such as MIC and Etest.

Keywords: Resveratrol, Stilbenes derivatives, *Leishmania*, Leishmaniasis, Meta-analysis

Introduction

Escherichia coli is a common gram-negative bacterium that can cause a variety of infections, including urinary tract infections but some serotypes cause food poisoning and diarrhea (1, 2) .E. coli can become resistant to beta-lactam antibiotics through the production of beta-lactamase enzymes.Extended-spectrum beta-lactamases (ESBLs) are a type of beta-lactamase that can hydrolyze broad-spectrum The bacterium is passed from person to person through oral-feces and is the most frequent cause of urinary tract infections (3).ESBL-producing bacteria are a major public health concern, as they can make infections difficult to treat.The TEM type is a type of beta-lactamase that is commonly found in E. coli.The

resistance rate of E. coli to cephalosporins has been increasing in recent years, and this is thought to be due in part to the spread of TEM-type beta-lactamase enzymes(4).

The mechanisms of bacterial resistance to antibiotics are variouse. However, one of these resistance mechanisms, which has become very problematic for us, is the production of beta-lactamase enzymes in bacteria. Hydrolysis of the central nucleus of beta-lactam antibiotics causes their inactivation. There are diffrent categories of beta-lactamases, such as SHV and TEM

enzymes (5, 6). The genes that produce these two enzymes are among the genes located on the plasmid. With the widespread use of antibiotics, including cephalosporin antibiotics, another class of beta-lactamase genes emerged that had a broader spectrum of activity than primary beta-lactamases. These enzymes were able to hydrolyze penicillin, cephalosporins, and aztreonam antibiotics. The occurrence of point mutations in the amino acid sequence of primary beta-lactamases such as TEM-1, TEM-2, and SHV-1, led to the derivation and emergence of these new and broad-spectrum enzymes which are now known as ESBLs (Extended-Spectrum Beta-Lactamase) (7). ESBLs are a particular type of drug resistance that includes several enzymes, which allow the hydrolysis of broad-spectrum beta-lactam antibiotics (8). In recent years, broad-spectrum beta-lactamase-producing bacteria have become so prevalent worldwide because of the use of antimicrobial drugs. This phenomenon can be considered a significant problem in the field of medicine (9). This is so important that we must always seek to produce new drugs with higher potency.

The TEM type is a type of beta-lactamase enzyme that has been identified in most members of the Enterobacteriaceae family, both in animals and humans. The resistance rate of all E. coli isolates in their area have decreased in recent decades to various antibiotics, especially cephalosporins, which have been found to contain the TEM type of beta-lactamase enzymes (10, 11). Therefore, the present study endeavored to explore the phenomenon of multidrug resistance as well as the presence of TEM, SHV, and CTX genes encoding broad-spectrum beta-lactamases in Escherichia coli strains that were isolated from individuals suffering from urinary tract infections in the Alborz province of Iran.

Materials and Methods

Sample collection and characterization of Escherichia coli isolates

In this descriptive study, 400 urine samples were collected from hospitalized and outpatient adult patients with urinary tract infections referred to university hospitals in Alborz province, Iran, for 6 months, from January 2016 to June 2017. The collected urine samples were inoculated on selective Eosin Methylene Blue Agar (EMB) medium and incubated at 37 °C for 48 hours. After the incubation period, the preliminary identification of each suspected colony was done using gram stain, catalase, and oxidase. Subsequently, biochemical tests such as TSI (Triple Sugar Iron), Simon citrate, urease, MR / VP (Methyl Red/ Voges-Proskauer), SIM (Sulfide Indole Motility), and lysine decarboxylase were used to characterize as Escherichia coli.

Antimicrobial Susceptibility Testing

After confirming the existence of E.coli, the antimicrobial susceptibility testing and the initial screening of ESBL-producing isolates were performed using the Kirby-Bauer disk diffusion method, as suggested by the guidelines of the Clinical Laboratory Standards Institute (CLSI)(12). The following antibiotic disks were used, gentamicin (10 μg), cotrimoxazole (1.25 μg), nalidixic acid (30 μg), imipenem (10 μg), ciprofloxacin (5 μg), 30 μg), amoxicillin (30 μg), ceftazidime (30 μg), chloramphenicol (30 μg) and streptomycin (10 μg).

Phenotypic screening of ESBL

According to the zones given above, if there were any suspicions of a strain being an ESBL producer, then confirmatory tests of a phenotypic nature would be conducted by cephalosporin/clavulanate combination disks were done as recommended by CLSI guidelines. According to CLSI, if the growth inhibition zone around the ceftazidime-clavulanic disc is ≥5 mm compared to ceftazidime alone, or if the zone of inhibition around the cefotaxime-clavulanic acid disc is ≥5 mm compared to cefotaxime alone, it indicates that the isolate is an ESBL producer (Figure 1) (13).
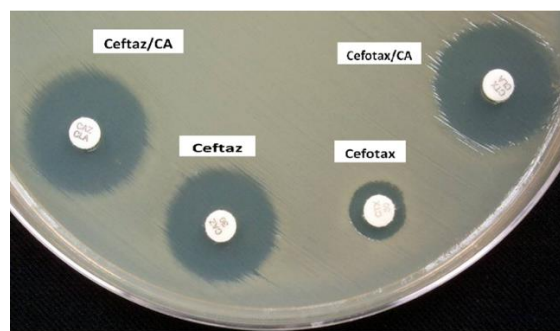

Figure 1. The Phenotypic Confirmatory Disc Diffusion Test is used to confirm the production of ESBL. This confirmation is based on the observation of an increase in the zone of inhibition by at least 5 mm for ceftazidime (Ceftaz) and ceftazidime/clavulanic acid (Ceftaz/CA), as well as for cefotaxime (Cefotax) and cefotaxime/clavulanic acid (Cefotax/CA).

Genome extraction and Polymerase chain reaction (PCR)

To perform the PCR test, we must first extract the DNA of the bacteria. The isolates stored in the enriched TSB were cultured in a McConkey medium. After 24 hours of incubation, these strains could be used for DNA extraction. For this purpose, the genomic DNA of harvested colonies was extracted using a kit (Bioneer, Korea), The extracted DNA concentration and its quality were measured by quantitative (Nano Drop™ One Microvolume UV-Vis Spectrophotometers) method and electrophoresis on 0.8% agarose gel.

To evaluate and screening of ESBL, Primer pairs for genetic loci including blaCTXM, blaTEM, and blaSHV as genetic markers for resistance against beta-lactam antibiotics were selected (14-16) and analyzed using the blastN program (National Center for

Biotechnology Information (NCBI) available at (http//ncbi, nlm, nih.gov/BLAST). These primers were synthesized by Genfanavaran Ltd. Co. (Iran). The nucleotide sequence of these primers is shown in Table 1. The DNA that was obtained was employed in the process of polymerase chain reaction (PCR), which took place in a final reaction mixture measuring 25 µl. This mixture comprised of 12.5 µl of PCR master mix, 0.5 µl of each primer, 1 µl of template DNA, and 10.5 µl of distilled water. The PCR reaction was executed using an amplification thermal cycler (Peqlab, Germany) as follows: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s (35 cycles), annealing at the suggested temperature for each primer for 30 s, extension at 72 °C for 30 s, and then final extension at 72 °C for 10 min. Finally, the PCR products were subjected to electrophoresis on 1.5% agarose gel, stained with DNA Green Viewer® (Pars Tous, Iran), and visualized on a UV transilluminator. DNA extracts of standard strains (ATCC 9290, 7881, and 8740) are used as positive controls and their band formation pattern is compared with PCR products related to the DNA of the samples and distilled water is used as negative control.

Table 1. Nucleotide sequences of primers

Gene	Primers	References
SHV	F: GCCTGTGTATTATCTCCCTGTTAGC R: CAGATAAATCACCACAATGCGC	(14)
TEM	F: AGA TCA GTT GGG TGC ACG AG R: CG GTA TCA TTG CA G CAC TG	(15)
CTX-M-1	F: CGTGGCGATGAATAAGCTG R: GGTGGTATTGCCTTTCATCC	(16)

Results

Out of 400 isolated samples 188(47%) were identified as e.coli. . The results of the disc agar diffusion assay for 10 selected antibiotics are presented in Figure 2. Based on the results of antibiogram tests on isolated bacteria, the sensitivity to studied antibiotics was as follows: chloramphenicol 85.1%, imipenem 100%, nalidixic acid 65.21, cotrimoxazole 35.41%, streptomycin 47.77%, amoxicillin 14.27 %, cefotaxime 51.91%, ceftazidime 50.53%, ciprofloxacin 41.6% and gentamicin 68.92%. As a result, it was observed that all strains were sensitive to imipenem and showed the highest resistance to Amoxicillin antibiotic 81.98%.
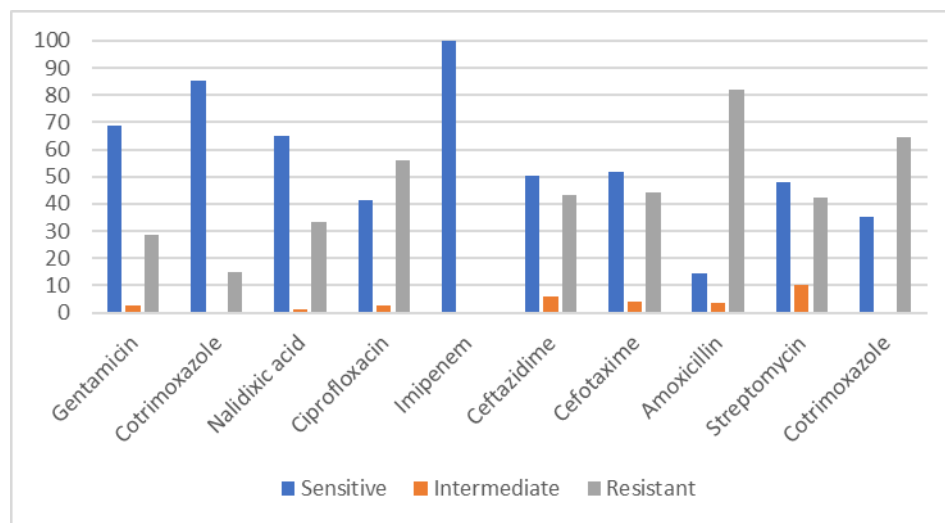

Figure 2. Antibiogram results of Escherichia coli isolates in Alborz province in terms of percentage.

According to the CLSI, any decrease in susceptibility of the studied strains to ceftazidime and cefotaxime could indicate the presence of beta-lactamase (ESBL) resistance. In this study, 128 (64%) isolates resistant to at least one of the antibiotics ceftazidime and cefotaxime were selected for confirmatory testing. Based on the agar diffusion disk screening test results, 84 (44.7%) strains were introduced as ESBL producers. During the double synergism test, 82 (43.6%) strains were identified as the final producer of ESBL.

In the polymerase chain reaction test, out of 84 isolates, the frequency of SHV, TEM, and CTX genes measured 64.3%, 55.9%, and 21.4%, respectively. These results showed that among E. coli isolates, 12 isolates (14.28%) had all 3 genes, 26 isolates (30.95%) had 2 genes, and 36 isolates (42.85%) had one gene.
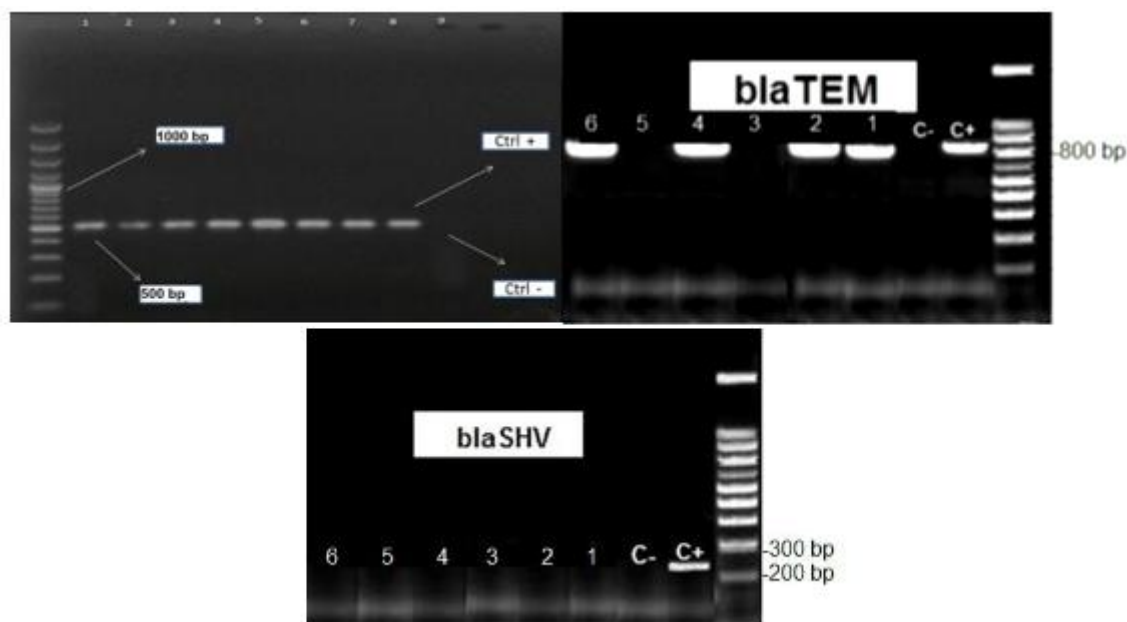
Figure 3. PCR amplification of CTX-M-I, TEM and SHV genes. M: Lader 100 bp; C-: Negative control; C+: Positive control. A, Lines 1-7: Isolates of E. coli containing CTX-M-I gene. B, Lines 1,2,4 and 6: Isolates of E. coli containing TEM gene. C, Line 1-5, and 6: Isolates of E. coli without SHV gene.

Discussion

Escherichia coli is one of the most important causative agents of the urinary tract and nosocomial infections (17). over the past few decades, beta-lactam antibiotics have been used in the treatment of these infections due to their potency, broad-spectrum and selective toxicity against prokaryotic cells, and this has caused an increase in resistance to the aforementioned drugs. The presence of beta-lactamase genes in bacteria, particularly ESBL genes, serves as influential determinants in the augmentation of resistance towards beta-lactam antibiotics, encompassing broad-spectrum cephalosporins. Organisms carrying these genes cause the spread of disease and in some cases cause death in infected people, and the continuation of the growing trend in the occurrence of such resistances may pose a serious danger to society (18-20).

In the present study, it was observed that all strains were sensitive to imipenem and showed the highest resistance to amoxicillin (81.98%). These results are consistent with Alisha Akya et al. (2019), Sahebnasagh et al. (2015), and Miraalami et al. (2015) findings in Iran (21-23). Another study by Sheikhi et al. (2018) in the western region of Iran indicated the highest rates of resistance were related to amoxicillin (91%), cefoxitin (45%), and piperacillin (45%), and the lowest rates of resistance were related to amikacin (24%) and imipene (0%) (24). The results of antibiotic sensitivity and resistance of this research are similar to our research, with the difference that the resistance to amoxicillin (85.8%) is less reported in our research. Our results are in agreement with different regions of Europe and North America findings which showed resistance to amoxicillin has often reached higher than 30% (25).

In researches conducted by Kifferr et al. in Brazil and Ling et al. in China, the prevalence of resistant strains of E. coli isolated to cefotaxime were reported to be 14.6% and 2.7%, respectively, while, in our research, the rate of resistance of strains to cefotaxime was 48.1% (26, 27). On the other hand, in the recent studies conducted in Iraq (2022) and Pakistan (2021), E. coli isolates showed high resistance to ceftriaxone (89% and 80%, respectively) (28, 29). It can therefore be assumed that the differences observed in the rate of antibiotic resistance may be related to the difference in the pattern of antibiotic consumption and the difference in the pattern of antibiotic resistance and in addition to the indiscriminate use of antibiotics in our country.

Based on our findings, the detection rate of ESBL-Producing E. coli was 43.6%. out of these isolates, the frequency of SHV, TEM, and CTX genes measured 64.3%, 55.9%, and 21.4%, respectively. Our results are almost in line with Soltan Dallal's (2010) findings in Tehran, Tabriz, and Khoy which showed the rate of ESBL-production among E. coli isolates was 79.5% 56.7%, and 52.1%, respectively, and 57.8%, 7.9%, and 12.5% of them contained the TEM gene, respectively (15, 30). The results of our research are consistent with this study in terms of the frequency of ESBL producing isolates, but the frequency of the TEM gene was higher (55.9%) in our study.

However, in the study of Miraalam et al. (2015) in Tehran (Iran), the highest rates of sensitivity among the E.coli isolates obtained from urinary tract infections were to Ciprofloxacin and Imipenem, but the rate of the presence of TEM and CTX genes was 47.27% and 74.54%., and the SHV gene was not detected in any of

the isolates (22). Compared to our research, although there is agreement with the rate of sensitivity to imipenem, the most identified gene in our research was the SHV gene with a frequency of 64.3%, followed by the TEM gene with a frequency of 55.9%.

In studies conducted in Karaj (Iran), Ghane et al (2020) showed that rate of ESBL production among E. coli isolates was 36%, and among the ESBL-positive isolates, blaTEM gene was detected in 44.72% of the isolates, which had lower rates than the results of our study (31). Also, in a recent study in north Iran, 46% of E. coli isolates were detected as ESBLs, and blaSHV, blaTEM, and blaCTX-M were detected in 34.7%, 36.4%, and 38.8% cases, respectively (32).

In other parts of the world including Korea, India, Turkey, Colombia, Nigeria, and Bosnia and Herzegovina, the frequency of ESBL-Producing E. coli isolates was reported 16%, 29.1%, 17%, 3.3%-4.7%, 37%, and 3.36%, respectively (33-38).These results are less than the amount obtained in our study. On the other hand, Zhou's study in Shanghai (China) and Jayanti Jena's study in India, was shown that 47.4% and 41% of E.coli isolated from patients produced ESBL, respectively which was similar to the results of our study (39, 40).

Overall, these results indicate that the frequency of ESBL-Producing E. coli isolated from different countries and various hospitals of the same country is distinct, which depends on the infection control system and the treatment method of that hospital.

Conclusion

The current study showed that the majority of studied E. coli isolated from urinary tract infections were MDR strains and show resistance to several antibiotics simultaneously. Examination of the Double-disk synergy test to display the ESBL phenotype among E. coli isolates showed that 43.6% of isolates were ESBL-Producing E. coli. From the data obtained from molecular analysis for the detection of three genes mentioned in this study, we concluded that only 44.7% of the isolates were genetically positive for ESBL genes coding ESBL enzymes, and the most abundant gene for ESBLs among isolates was the SHV gene. Taken together, our results suggest that imipenem with the lowest resistance is the best drug in the treatment, and carbapenems are the best drug for treating diseases caused by E. coli and careful monitoring of their use for UTI treatment is necessary. Also, the results of the present study may be useful in replacing ESBL enzyme resistance screening with more modern sensitivity measurement methods such as MIC and Etest.

Authors' Contribution

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval and consent to participate

The ethical approval was not necessary for the reason that our study was a meta-analysis belonging Availability of data and materials.

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