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PREVALENCE OF TEM, SHV, AND CTX-M GENES OF EXTENDED SPECTRUM B-LACTAMASE-PRODUCING *ESCHERİCHİA COLİ* STRAİNS İSOLATED FROM URINARY TRACT INFECTIONS IN ADULTS

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ABSTRACT

Urinary tract infections (UTIs) are one of the major sources of widespread infectious diseases in the community as well as in the hospitals which increase the cause of morbidity and mortality. Prevalence of extended spectrum- b-lactamase (ESBL)-producing uropathogenic *E. coli* isolates has been found to be increased rapidly across the world. The present study was undertaken to find out the frequency of blaTEM, blaCTX-M, and blaSHV genes among *E. coli* isolates from UTI and detect their sensitivity pattern. A total of 112 non-repeated *E. coli* isolates obtained from urine samples of UTI diagnosed patients were included in this study. Antibiotic susceptibility test was done by disc diffusion method. Seventy seven (68.75%) isolates were MDR and tested for ESBL. ESBLpositive isolates were screened for blaTEM, blaCTX-M, and blaSHV genes by monoplex PCR (polymerase chain reaction). Among 46 ESBL-producing *E. coli* isolates, 8.69% harboured all the three bla genes. The blaTEM was the predominant (93.47%) gene followed by blaCTX-M (82.6%) and blaSHV (4.34%). It can be concluded that the prevalence of MDR (multidrug resistance) ESBL-producing *E. coli* appears to be high and the highest identified gene was blaTEM. The knowledge of resistance pattern can help physician's select suitable empirical antibiotic regimens, so that antibiotics showing high-resistance pattern can be avoided.

KEYWORDS: Urinary tract infections (UTIs), *E. coli*, blaTEM, blaCTX-M, and blaSHV.

INTRODUCTION

Urinary tract infections (UTIs) are one of the major sources of widespread infectious diseases in the community as well as in the hospitals which increase the cause of morbidity and mortality.[1] Various pathogens are responsible for UTIs,

of which bacteria are the main contributing agents and *E. coli* is the prevalent organismaccountable formore than 80% of infections.[2] *E .coli* is report approximately 50% in health care settings and 85% in community acquired UTIs.[3]

Diagnosis of more than 150 million UTIs every year worldwide, reminds it as one of the most frequent communities acquired as well as nosocomial infections since long time.[4] Overuse and misuse of antibiotics for the treatment of UTIs cause antibiotic selective pressure which results in rapid increase and spread of multidrug resistant bacteria. At present, decreased susceptibility to many antibiotics was observed against uropathogenic *E. coli*. This harshly situation is commonly observed in human medicine and this may lead to the increased hospital cost, prolonged stay at hospital, and also recurrent treatment failure.[5]

Among the array of antibiotics used widely, b-lactams are the most extensively used chemotherapeutic agents because of lack of significant toxicity. They account for over 50% of all systemic antibiotics in use.[6] Production of b-lactamases by several Gram-negative and Gram-positive organisms is possibly the most important single mechanism of resistance to blactam agents.[7]

Extended-spectrum b-lactamases (ESBLs) are enzymes which show resistance to the third generation cephalosporins. As these enzymes are plasmid encoded, spread of bacterial resistance due to these enzymes disseminate rapidly. These plasmids also carry resistance genes which encode for other antibiotics resistance genes such as aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol, and sulfamethoxazole– trimethoprim.[8] Among ESBL-producing uropathogenic bacteria, *E. coli* has been reported to be the major cause of public health issues worldwide. Although ESBL genes are most commonly derived from TEM or SHV parents, since 1998, the prevalence of CTX-M types has been increased significantly in most parts of the World.[9]

Treatment of UTI cases is often started empirically based on the antimicrobial resistance pattern of the urinary pathogens from existing surveillance report. Therefore, the aims of the present study were to determine the prevalence of ESBL-producing E. coli isolated from urine samples of UTI diagnosed patients, to detect their susceptibility to the antibiotics generally used for the treatment of UTI and to detect prevalence of blaSHV, blaTEM, and blaCTX-M genes.

MATERİALS AND METHODS

Isolation and biochemical identification

Urine samples were collected, from patients of age more than 18 years, in sterile containers by the following aseptic techniques from patients suspected to have urinary tract infection from outpatient department (OPD), wards, cabins, intensive care unit (ICU), and neonatal intensive care unit (NICU). Urine samples were also examined microscopically especially for pus cell to confirm urinary tract infection. Urine (1 ll) was inoculated onto cysteine lactose electrolyte deficient (CLED, Hi-Media Laboratories, Mumbai, India) medium. Organisms grown in pure culture and in significant numbers ([105cfu/ml for midstream urine samples) were identified by the standard biochemical tests. Of the total 2560 urine samples obtained over a period of 1 year (March 2023–March 2024), only 290 samples were selected basing on the above criteria and processed further. Flow chart for sample selection for this study is given. NationalCollection of Type Cultures (NCTC) strain number 10,418 (b-lactamase negative) was used as the reference strain for all experiments.

Antibiotic susceptibility tests

Antibiotic sensitivity of the isolates was done by Kirby Bauer's Method using antibiotic disks from Himedia, Mumbai. Antibiotics used were ceftazidime (30 µg) (CAZ), amikacin (30 µg) (AK), amoxyclav (30 µg) (AMC), ofloxacin (5 µg) (OF), norfloxacin (5 µg) (NX), ceftriaxone (30 µg) (CTR), piperacilin/tazobactun (100/ 10 µg) (PIT), co-trimoxazole (25 µg) (COT), gentamicin (10 µg) (GEN), nitrofurantoin (300 µg) (NIT), cefoperazone/ sulbactum (75/30 µg) (CFS), cefepime (30 µg) (CPM), netilimicin (30 µg) (NET), imipenem (10 µg) (IPM), and colistin (10 µg) (CL).

Test for ESBL production

Those isolates which show resistance or with decreased susceptibility (intermediate by CLSI criteria) to the third generation cephalosporins were tested for ESBL production by NCCLS confirmatory test as described previously.[10]

Isolation and quantification of plasmid DNA

Plasmid DNA was isolated from ESBL-producing bacterial cells by alkaline-lysis method.[11] The plasmid DNA was stored at minus 20°C for further use. The samples were run on 0.8% agarose gel and stained with ethidium bromide. The stained gel was examined under UV light to find the presence of plasmid bands. To evaluate the molecular weight, k DNA double digested with hind III was used as marker.

PCR amplification for blaSHV, blaTEM, and blaCTX-M genes

ESBL-positive isolates were taken for PCR assay to ensure the presence of the blaSHV, blaTEM, and blaCTX-M genes. The PCR master mix was as follows: 2.5 µl of PCR buffer, 2.5 µl of 25 mm $MgCl_2$, 0.2 µl of 2mm dNTPs, 0.17 µl of Taq polymerase (Merck, Germany), 16.63 µl double distilled sterile water, and 1 µl of each of the forward and the reverse primers. After giving out 24 µl of the master mix in the individual PCR reaction tubes, 1 µl of the extracted plasmid DNA was added in the corresponding tubes to make up the total volume to 25 µl.

The reaction mixture was initially denatured for 5 min at 94°C, subjected to 30 cycles of denaturation at 94 °C for 1 min, annealed at 55 °C for 1 min, extended at 72 °C for 1 min, finally extended at 72 °C for 10 min, and soaked to 4 °C. The amplified PCR products were analyzed using 1.5% agarose gel electrophoresis. Gels were stained with ethidium bromide and visualized by UV trans-illuminator.

RESULTS

Of the 290 positive cultures, 112 (38.6%) isolates were identified to be E. coli that was predominant organism followed by Staphylococcus aureus 49 (38.62%), Klebsiella spp. 34 (11.72%), Enterobacter spp. 23 (7.93%), Acinetobacter spp. 18 (6.2%), Pseudomonas spp. 11 (3.79%), Citrobacter spp. 10 (3.44%), Proteus spp. 8 (2.75%), Providencia 2 (0.68%), Coagulase Negative Staphylococci (CoNS) 14 (4.82%), and Candida albicans 4 (1.37%). Seventy seven (68.75%) E. coli isolates were considered MDR as these isolates showed resistant to two or more unrelated classes of antibiotics.

Antibiotic sensitivity pattern

The drug resistance patterns of 77 MDR *E. coli* uropathogens were observed that ceftazidime shows the highest percentage (100%) of resistance among all other antibiotics by disc diffusion method, followed by ceftriaxone (96.1%), cefepime (96.1%), norfloxacin (94.8%), ofloxacin (93.5%), and amoxyclav (90.9%). The lowest rate of resistance was observed in colistin (3.89%).

Prevalence of ESBL by phenotypic method

Seventy seven consecutive MDR *E. coli* uropathogens were tested and 46 (59.74%) isolates were found to be ESBL producers.

Plasmid profiling

Plasmid DNA was extracted from 46 ESBL-producing E. coli uropathogens by alkaline-lysis method. All the isolates showed that the presence of plasmids and multiple plasmids (bands) was found in 38 isolates. Of the 46 isolates, 8 isolates showed the presence of single plasmid. One plasmid having molecular weight 23.13 Kb was present in 31 of the isolates tested.

Prevalence of ESBL gene

All the ESBL-producing isolates confirmed by phenotypic methods were also analyzed by molecular methods (PCR) to understand the frequency of ESBL genes. All the 46 ESBL-producing E. coli isolates were found to have at least one bla gene, of which 8.69% harboured all the three bla genes. The blaTEM was the predominant (93.47%) followed by blaCTX-M (82.6%) and blaSHV (4.34%). Co-existence of two of the genes was observed at the highest range with blaTEM and blaCTX-M in 23 (50%) isolates followed by blaTEM and blaSHV in eight (17.39%) isolates and blaCTX-M and blaSHV in six (13.04%) isolates.

DISCUSSION

The frequency of use of antibiotics and even the dosages and period of administration vary greatly from country to country, region to region, and to some degree even in a locality. This has led to large differentials in the emergence of resistant patterns. Therefore, it is essential to study and report trends in antimicrobial resistance regularly.[12] Multidrug resistant E. coli isolates from UTI are increasingly found worldwide, which is a serious problem in many countries. In our study, it was observed that 68.75% E. coli isolates were MDR supported by the study of Adwan et al.[13] from Palestine reported that 76% E. coli isolates were MDR.

The present study on the susceptibility pattern of MDR isolates shows 3.89% resistance towards colistin. However, a much higher rate of colistin resistant (82%) in ESBLproducing uropathogen E. coli was reported by Rezai et al.[14] Imipenem is highly b-lactamase stable and has an uncommon property of causing a post antibiotic effect on Gram-negative bacteria. It was observed from the present study that imipenem was resistant up to 14.28% to the MDR uropathogens, which was quite higher when compared with the analysis by Hassan et al. (15) which is only 9%. In our study, we observed that the rate of resistance to ceftazidime was 100% which is much higher when compared with other finding from Malaysia, where resistance percentage of ceftazidime was 11% by Thong et al.[16], and from China, where it was found to be 28% for ceftazidime.[17]

Moreover, ESBL producers show resistance not only to b-lactam agents but also to other antimicrobial agents such as tetracycline, fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole.[18] Similar resistance pattern was also observed in our study. There are so many factors responsible for such elevated rate of antibiotic resistance which includes enormous use and misuse of antimicrobial agents by health practitioners in hospitals in addition to the self-prescription by the community.[18] Colistin, imipenem, nitrofurantoin, netilimicin, ceftazidime/clavulanic acid, and amikacin constitute the reasonable treatment option for UTI, as these antibiotics showed a lower rate of resistance to the uropathogenic E. coli isolates.

UTI remains the commonest type of community acquired Gram-negative sepsis, especially in developing countries.[20] In our study, most of the ESBL producers were from outpatients 31 (67.39%) than hospital acquired 15 (32.6%). One study by Tada et al. [21] reported that 16.7 and 30% isolates were ESBL positive from community and hospital acquired UTI, respectively.

Recommendation of proper antimicrobial regimens for the treatment of infectious diseases caused by ESBL-producing E. coli drastically get hampered due to the presence of other antibiotic resistance genes and the existence of a conjugative plasmid allied with the ESBL phenotype. In this situation, carbapenems represent a good choice for the treatment option in case of serious infection condition.[22]

The incidence of ESBL-producing E. coli varies from country to country and from centre to centre. Our study showed that the prevalence of ESBL was 59.74%, which was higher when compared with the study from Sudan with 30.2% ESBL producers in E. coli.[23] Statistics have shown that prevalence of ESBL-producing E. coli are highest in India (60%), followed by Hong Kong (48%) and Singapore (33%) as reported by Hsueh et al.[24] Molecular characterization of b-lactamase gene would be crucial for a reliable epidemiological analysis of antimicrobial resistance.[19] From many different studies, it was observed that the predominant of ESBL gene was diverse. Earlier reports mentioned that the most prevalent type of ESBL genes is SHV, TEM, and CTX-M. During the past decade, TEM and SHV types were reported to be the most common types of b-lactamase genes, but recently, CTX-M type has been widespread worldwide compared to TEM and SHV genotypes.[25] CTX-M was predominant in many regions; several reports were made from Iran (74%).[26], Morocco, North Africa (70%).[19], and India (93.7%).[27] However, the present study showed that TEM type b-lactamase gene was the predominant ESBL gene in uropathogenic E. coli which corroborated with the reports of several other previous studies (28; 29). TEM-type b-lactamase gene was the predominant in Italy (45.4%) [30], Portugal (40.9%).[31], and Turkey (72.7%).[32] Our study also corroborates with another study from India, where TEM was predominant followed by SHV in E. coli and Klebsiella pneumonia from UTI and also they reported CTX-M gene was found lowest.[33] Several investigators reported the co-existence of various b-lactamase genes within the same isolates.[34] Co-existence of all the three bla genes was observed in two (4.34%) isolates in our study, which was lower than the report from Tamilnadu, India, where they observed only 60% isolates, harboured all the three genes. The major co-existence of both the genes was CTX-M and TEM (50%) in our isolates. This result is also in agreement with others and Sharma et al. [34] in India who reported both TEM + CTX-M as the most common type.

In conclusion, ESBL-producing uropathogenic *E. coli* is a rising problem and the dissemination occurs over the whole country. The predominant gene type was TEM, but CTX-M gene was also increasing. Colistin, imipenem, nitrofurantoin, netilimicin, ceftazidime/clavulanic acid, and amikacin constitute the reasonable treatment option for UTI as based on state of findings. Since dissemination of MDR and ESBL-producing E. coli isolates decreases the treatment options and increases the hospital cost, it is necessary to be updated with the prevailing resistant pattern of any locality which will help in appropriate antimicrobial therapy.

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