ORIGINAL ARTICLE

Molecular Characterization of Extended Spectrum B-Lactamase Producing *Escherichia Coli* Isolated From Urine Samples

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Abstract

Aim: The upsurge in multiple antibiotic resistance strains of pathogenic bacteria has become one of the biggest concern for the past 25 years. Research states that the developments of resistance to multiple drugs are primarily due to the antibiotic resistant genes acquisition through grouped in multifaceted clusters, transposons, and plasmids. **Materials and Methods:** The collected urine samples were polymerase chain reactions (PCR)-screened for the confirmation of *Escherichia coli* and the presence of multiple antibiotic resistant markers *bla*CTX-M coding the most common resistant phenotypes established in enteric bacteria group. **Results and Discussion:** During the present study, pathogenic isolates of extended-spectrum β-lactamase (ESBL) producing *E. coli* causing urinary tract infections (UTI) infections from different hospital environments were detected for their virulence gene using PCR; further, they were examined for antibiotic sensitivity patterns against varied therapeutic drugs available in the marketplaces. It was found that the antibiotic sensitivity was high for Carbapenems followed by Ofloxacin and Doxycycline hydrochloride. Moreover, least sensitivity was recorded for Cephalosporin. The present study found that UTI initiated by Pathogenic ESBL producing *E. coli* among nosocomial infections which are to be high among the persons with immunologically suppressed. **Conclusion:** Drug resistant E. coli may be freely come across in hospital locations during every day clinical practices and the urologists must act untimely. The organization of this infection is exceedingly significant for forthcoming research with specific allusion to prevent of fresh antibiotic resistant pattern.

Key words: *Bla*CTX-M, Extended-spectrum β-lactamase, ***Escherichia coli***, Polymerase chain reactions, Urinary tract infections

INTRODUCTION

During the current scenario, elevated bacterial drug resistance is of the greatest horror among human diseases like respiratory tract infections, diarrhoea, sexually transmitted infections and meningitis. The difficulty of the spread of this drug resistance among the human pathogens may not be resolved without seeing the flow of resistant determining factor in the environments.[1,2] Resistant factors are transmitted from one bacterium to another bacterium over gene transfer mechanism.[3] Bacteria are able to also transfer from one of the host to another host over direct through the food chain or by contact indirectly through water supply for further spreading the resistance.[4] Direct transfer of resistant bacteria from hospitals to patients has become one of the big threat in medical field.[5] Information of high amounts of resistant among *Escherichia coli* from the people with high level of urinary tract infections (UTI) infection has augmented with the overall communities.[6] *E. coli* belonged to Enterobacteriaceae family and is a communal occupant of

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Received: 27-04-2022
Revised: 09-07-2022
Accepted: 30-07-2022

the healthier gastrointestinal tracts of all the mammal. *E. coli* is one of the facultative anaerobe and also Gram-negative rods. 700 *E. coli* (antigenic types) serotypes are identified based upon (cell wall) O, (flagella) H, and (capsule) K antigens.[7] Although most of the bacteria are non-pathogenic commensal, but *E. coli* is pathogenic type that can effect intestinal and extraintestinal infections, both in human and animal.[8] *E. coli* of pathogenic type are accountable for three type of human infection: neonatal meningitis, gastroenteritis, and UTI. About 90% of known UTIs are relevant to particularly of the kidneys and bladder that are caused by *E. coli* infection.[9] Those infections are much shared, with 1 in 3 women and 1 in 20 men contaminated during their lifetimes. The majority of extended-spectrum β-lactamase (ESBL) producing *E. coli* have been reported from hospitalized patients in ICU's and the UTI infection by these ESBL generating *E. coli* have also increased in the recent years.[10] ESBLs may be difficult to distinguish, because they have diverse steps of activity against varied cephalosporin.[11] Therefore, the choice of that antimicrobials agent to be used becomes critical. This study deals with the attempt to isolate and confirm the ESBL producing *E. coli* from UTI infected patients and to analyze them at molecular levels for the presence of particular genes which are responsible for the resistance against multiple antibiotics.

MATERIALS AND METHODS

The present study was carried out to isolate and confirm the ESBL producing *E. coli* collected from 246 urine samples of patients in Micro Diagnostics, Hosur, TN, India during January 2020–December 2020. The isolated *E. coli* was subjected to identification and confirms by molecular polymerase chain reactions (PCR) method according to 16S rRNA.[12]

Isolation and identification of *E. coli*

Different urine samples were collected from UTI infected patients from various hospitals of Hosur. The urine samples were inoculated on MacConkey agar mediated plate and hatched 37°C for 24 h. The colonies of sequestered organism were subcultured on nutrient agar plate and pure culture was achieved in various discerning media such as EMB agar media as well as other chemicals including antibiotics, buffers, and media which were procured from Hi Media. Green metallic sheen colored isolates were reflected to be *E. coli* and the probable colonies were biochemically verified for growth on for oxidative/fermentative degradation of glucose triple sugar iron agar (TSI), urease production, citrate utilization, indol fermentation, lysine iron agar tryptophan degradation, glucose degradation (methyl red), and motility test. The *E. coli* isolates were deposited in tryptic soy broths with 15% glycerol at −20°C. Colony confirmation was performed employing molecular method (PCR). Molecular validation of bacterial clones was dogged according to the 16S rRNA gene regions from *E. coli.*

Antimicrobial susceptibility pattern determination by disk diffusion methods

The indifference of the verified isolate against 12 antibiotic, signifying different modules of beta-lactams, was dogged employing the disk diffusion methods rendering to the Clinical and Laboratory Standard Institutes (CLSI) guideline.

Confirmatory test employing disc diffusion for ESBL

The double disks synergy tests employed in our study and were amended from Jarlier's double-disks synergy methods. Disks of the cefepime, cefatazidime, and cefotaxime were placed surrounding an amoxicillin and clavulanic acid disks within a distance of 20 mm Positive strain ESBL production and was distinct by the scrutiny of keyholes.

Combined disk test

CLSI recommended confirmatory test required for the use of cefotaxime and ceftazidime, without and with clavulanic acids. Disks employed were ceftazidime clavulanic acids (30/10 μg), ceftazidime (30 μg), cefotaxime clavulanic acids (30/10 μg), and cefotaxime (30 μg). In any situation, uncertainty the zone diameter was increased by ≥ 5 mm after any antibiotic, it was united with clavulanic acids and was considered as indication for the existence of ESBL producer bacterium.

Method of molecular documentation of ESBL by genotype study

PCR method was employed to notice the presence of antibiotic resistant genes among *E. coli* viewing the phenotypic resistance toward beta-lactamase.[13-15] Magnification condition for all the reactions during PCR method were as follows: initial denaturation at 94° c for 5minutes followed by 30 cycles of denaturation at 94° c for one minute, forging or annealing at 62° c for forty five seconds, elongation at 72° c for one minute and final extension at 72° c for 10 minutes. These settings were smeared to all multiplex or the product PCR reaction not including for the following primer pair:, CTX-M-2, CTX-M-15, and CTX-M-1 that were forged at 56°C, 43°C, 46°C, 50°C, and 50°C, correspondingly.

Nucleotides sequence of amplified blaCTX M gene

To ratify, the character of blaCTX M gene noticed by sequencing reactions was accomplished using a ABI PRISM® BigDyeTM Terminators Cycle Sequencing Kits with AmpliTaq® DNA polymerases (FS enzyme) (Applied Biosystem). The fluorescent labeled fragment was purified from the un-incorporated terminator with an ethanol precipitation protocols. The samples were again suspended in distilled water and subjected to the electrophoresis in an ABI 3730xll sequencers. All the obtained sequence were visually considered and modified for minor electropherogram error, then were matched to sequence database by BlastX and BlastN with evasion parameters and settings.[16-18]

RESULTS AND DISCUSSION

The present study carried out by selection of 246 clinical isolate convalesced from the urine for antibiotic-resistant *E. coli* in and around Hosur, Tamil Nadu, India.

The different age group-wise patients were infected by UTI infection caused by *E. coli* that is given in the Figure 1. The maximum 39 positive samples were obtained from the age group of 45–60, and the least numbers (12) of positive samples got from 01 to 15-year-old children. Twenty-four positive samples were collected from reproductively active adults in the age of 16–30 years. Eighteen positive samples were obtained from middle age people (31–45). From the data, reproductively active (16–30 years old) and aged people (45–60) reports shown the maximum positive samples of UTI. Below 15 years and middle age people had less numbers of positive samples when compared to others [Figure 1]. Hassuna *et al*.[19] worked on the molecular characterization of β-lactamase generating *E. coli* in Upper Egypt and studied with 583 urine samples collected from UTI infected patients, out of which, 400 isolates were found to be positive ethics and 134 isolates were confirmed as *E. coli* at 33.5% significance. Out of the 134 isolates, 60% were belonged to ESBL makers by CHROMagar ESBL screening method and 75 were found at 56% significance employing Amended Double Disk Synergistic Test (MDDST). No statistical significance was found in between the MDDST recognition of ESBL creation and CHOROM agar methods ($P > 0.5$; $\chi^2 = 0.235$).

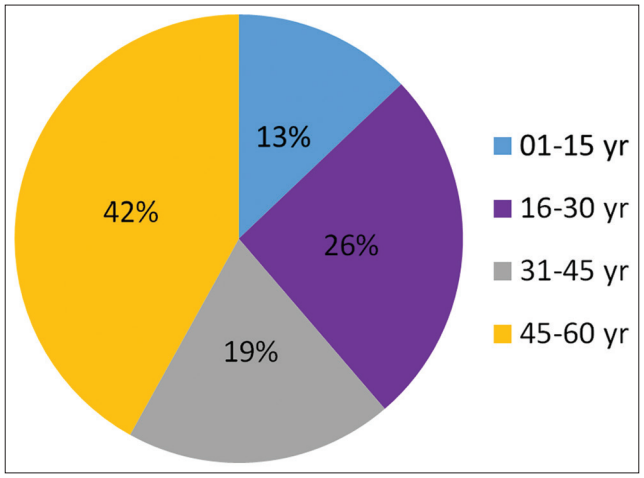

Figure 1: Isolation of *E. coli* from different aged group of UTI infected people

Most of the ESBL generating *E. coli* was surveyed by the PCR for its occurrence of *bla*CTX-M gene. The genomic analysis of a multidrug-resistant strain of enterohemorrhagic *E. coli* O157:H7 causing a family outbreak in Japan was discussed by Ahmed *et al*. 2005.[9] Out of the 23 strains of *Escherichiacoli* sequestered during the study periods, 9 (39%) were found positive for *bla*CTX-M gene from the CTX-M-I groups, signifying that CTXM- 1-like lactamase and the remainder (14 [61%]) were found to be negative for *bla*CTX-M gene. The drug resistant pathogens were becoming more resistant toward number of antibiotics and there is jeopardize in new antibiotics which were described by Akinbowale *et al*. (2006)[10] and other workers (Muthukrishnan *et al*., 2019 a, b; Bhat *et al*., 2015; Majeed *et al*. 2021).[20-23]

The molecular description of extended spectrum β-lactamase producing *E. coli* sequestered from urine sample/s was detected based the used primers, CTXM1-F3 and CTXM1-R2 [Table 1]. Figure 2 describes about different genes of same molecular weight along with ladder DNA.

1. The Group I includes CTX-M-
2. The sequences of primers as synthesized 3_to 5_
3. The nucleotides position in bases pairs for the GenBank agreement number sequences
4. The agreement number of the sequences used for primer designs.

Table 1: Nucleotide sequences of PCR primers used for amplification of DNA isolated from *E. coli* of UTI infected people

Gene	Primer	Sequence	Product size (bp)	Annealing temp (°C)	Nucleotide positions (bp)c	GenBank
CTX-M group I	CTXM1-F3	GAC GAT GTC ACT GGC TGA GC	914–896	55	1, –3, –10––12, –15	X92506
CTX-M group I	CTXM1-R2	AGC CG C CGA CGC TAA TAC A	416–435	55	–10––12, –15	X92505

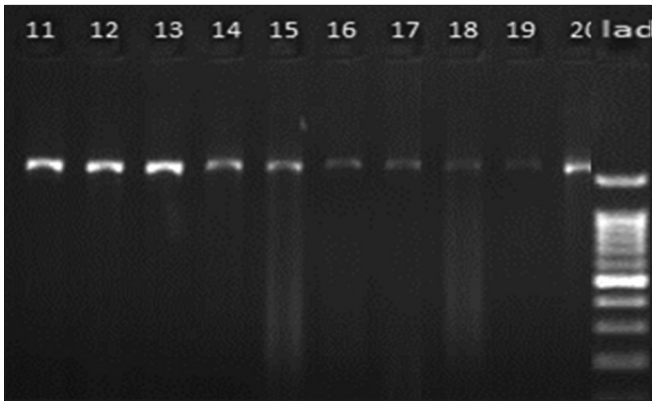

Figure 2: Different genes of same molecular weight along with ladder DNA

CONCLUSION

Percentage of UTI infection caused in Community by *E. coli* is high. The percentage of UTI infection among people was found high in old aged patients. Our current study supported the findings that the UTI caused by Pathogenic ESBL producing *E. coli* in nosocomial infections was found to be very high. Molecular analysis confirmed the ESBL producing *E. coli* in the UTI infected people. It also explained that the need of strict observing and tailing for effective processes of sanitary practice and hygiene within the hospitals particularly in ICU and LTCF's.

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Source of Support: Nil. **Conflicts of Interest:** None declared.