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Research Article

PREVALENCE OF BLA$_{TEM}$ GENE IN UROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM TERTIARY CARE HOSPITALS IN COIMBATORE, SOUTH INDIA

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ABSTRACT

Background: Extended spectrum β-lactamases (ESBLs) have been observed in virtually all the species of family *Enterobacteriaceae.* The enzymes are predominantly plasmid mediated and are derived from broad-spectrum β-lactamase by a limited number of mutations. Data on TEM type extended-spectrum β-lactamase (ESBL) produced by uropathogenic *E. coli* by molecular methods are limited from South India. This study was conducted to investigate the prevalence of TEM type ESBL producing uropathogenic *E .coli* from patients attending tertiary care hospitals in and around Coimbatore, South India.

Methods: A total of 512 *E. coli* isolates were obtained from tertiary care hospitals in Coimbatore and assessed for ESBL production phenotypically. Phenotypically ESBL positive isolates were subjected to PCR for bla$_{TEM}$ gene using uniplex primers for the detection of TEM gene.

Results: Out of 512 *E. coli* isolates, 258 (58 %) were positive for ESBL phenotypically. All of them were examined by PCR using uniplex primers for the presence of bla$_{TEM}$ genes. Among the 258 phenotypic positive isolates, 9 (90 %) were positive for bla$_{TEM}$ genes.

Conclusion: The present finding shows the evidence of the high prevalence of multidrug resistant TEM gene among UTI patients in this region. This is alarming and calls for the judicious use of carbapenems, especially in developing countries like India.

Keywords: β-lactamase, *E. coli*, ESBL, bla$_{tem}$, Uropathogenic *E. coli*, Antibiotic resistance

INTRODUCTION

Most ESBLs are mutants of classical plasmid and transposon mediated TEM and SHV β-lactamases[1]has facilitated its spread to other species of bacteria[2]. In the 80's and early 90's, various studies on ESBL producers focussed on the outbreak of nosocomial infections caused by *Klebsiella* and *Enterobacter sp*. which produced various TEM or SHV ESBL enzymes[3]. TEM was reported first in *E. coli* isolated from a patient named Tenomeira in Greece in 1960s[4]. TEM enzymes which are mostly found in *E. coli* and *K. pneumonia* can also occur in other members of the family *Enterobacteriaceae* and in non enteric organisms such as *Acinetobacter sp*[5].

TEM enzymes have an extended profile of conferring resistance to ampicillin, amoxycillin, carbenicillin and ticarcillin[6]. Knowledge and understanding the responsible sources for continuous failure in treating patients by appropriate antibiotics and also in preventing the spread of pathogens possessing these β-lactamases is a herculean task, moreover, the above activity requires expensive control effects[7,8,9]. ESBL infected UTI patients are at greater risk of fatal outcome, if they are not treated properly. When ESBLs are detected from clinical samples, their complications and mortality rates indicates the need for appropriate antibacterial agents. With the above stated information, the present study aimed to detect the presence of TEM gene in uropathogenic *E. coli* strains isolated from UTI patients attending tertiary care hospitals in and around Coimbatore, South India.

MATERIALS AND METHODS

ESBL detection and Antibiotic susceptibility test

All the 512 *E. coli* strains were screened for ESBL production as recommended by the Clinical and Laboratory Standards Institute[10]guidelines. Isolates showing increased zone of inhibition to third generation cephalosporins i.e., ceftazidime (30 mg), cefotaxime (30 mg) and to fourth generation cephalosporins, cefepime (30 mg), were screened for ESBL production. ESBL production was confirmed by using ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic disks with and without clavulanic acid (10 µg) and by Double Disk Synergy Test (DDST) (Jarlier et al. 1988).

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Hi-Media, India) by Kirby Bauer disk diffusion technique as per the CLSI guidelines[10]. The following antibiotic disks (drug concentration in µg); amikacin (30), piperacillin (10), gentamicin (15), tobramycin (10), ampicillin (10), cefixime (5), rifampicin (5), norfloxacin (10), cephalothin (30), carbenicillin (100), cefepime (30), imipenem (10), meropenem (10), piperacillin/tazobactam (100/10), cefotaxime (30), ceftazidime (30) and cefoxitin (30) were used. The antibiotic disks used were obtained from Hi-Media Pvt. Ltd, Mumbai, India.

Preparation of genomic DNA

Genomic DNA was isolated from bacterial cells using DNA purification kit (PureFast® Bacterial Genomic DNA purification kit, HELINI Biomolecules, Chennai). The purified DNA was stored at -20°C. The samples were run on agarose gel and stained with ethidium bromide. The stained gel was examined for presence of bands under UV-light using molecular weight marker (HELINI Biomolecules, Chennai).

Detection of bla$_{TEM}$ gene by PCR

Among 298 ESBL producing uropathogenic *E. coli*, only 10 isolates were taken for molecular detection of bla$_{TEM}$ which was performed by using uniplex Polymerase Chain Reaction (PCR). The primers used for detection of bla$_{TEM}$ gene are shown in Table 1 which is obtained from Helini Biomolecules, Chennai. For PCR amplifications, about 1 ml of DNA was added to 25µl mixture containing 1µl of 10mM dNTPs mixer, 1µl of each primer and 2U of Taq DNA polymerase (Helini Biomolecules, Chennai) in 10X PCR buffer. Amplification was performed in a thermocycler (Corbett Research, Australia) with cycling parameters comprising initial denaturation at 94ºC for 3 min followed by 35 cycles each of denaturation at 94ºC for 30 sec, annealing at 56°C for 30 sec, amplification at 72°C for 30 sec and final extension at 72°C for 5 min, for the amplification of bla$_{TEM}$. The amplified products were separated using 2% agarose. The gel was visualized by staining with ethidium bromide (0.5 mg/ml) in a dark room for 30 min. A 100 bp ladder molecular weight marker (Helini Biomolecules, Chennai) was used to measure the molecular weight of amplified products. The images of ethidium bromide stained DNA bands were digitized using a gel documentation system (Helini Biomolecules, Chennai). In this study we did not attempt to detect group specific TEM genes.

RESULTS AND DISCUSSIONS

The antibiotic resistance patterns of the ESBL producing uropathogenic *E. coli* according to their genotypes are presented

in Table 3. All of the TEM producing UPEC strains retained favourable susceptibility to imipenem (100%). The report of the present findings is in accordance to other authors who also reported a rate of 100% sensitivity to imipenem[11,12,13].[14,15]. The ESBL positive *E. coli* carrying TEM genes showed maximum (100%) resistance to meropenem, cefotaxime, cephalothin, norfloxacin, cefixime, gentamicin followed by 88.9 % to rifampicin, cefepime, piperacillin/tazobactam and 77.8% to ampicillin and carbenicillin. Five TEM isolates (50%) showed resistant to 16 of the 17 antibiotics tested, followed by one strain each showing resistant to 13, 12, 10 and 7 antibiotics respectively (Table 4).

Table 1: Primers used and the expected size for detection of bla$_{TEM}$

PCR target	Primer sequence/ name	Primer sequence (5'-3')	Amplicon size
bla TEM	TEM-F	TTCTGCTATGTGGTGCGGTA	444bp
	TEM-R	GCAGAAGTGGTCCTGCAACT	

Table 2: Genotypes of ESBL producing uropathogenic *E. coli*

Organism	Total No. Of Isolates	TEM (+)	TEM (-)
E. coli	10	9 (90 %)	1 (10%)

Table 3: Antimicrobial susceptibility pattern of ESBL producing Uropathogenic *E. coli* carrying bla$_{TEM}$ gene

Antibiotics	**TEM** (n=9) (%)		
	Resistance	**Intermediate**	**Sensitive**
Amikacin	5 (55.55)	-	4 (44.45)
Piperacillin	5 (55.55)	1 (11.1)	3 (33.35)
Gentamicin	9 (100)	-	-
Tobramycin	5 (55.55)	-	4 (44.45)
Ampicilin	7 (77.8)	-	2 (22.2)
Cefixime	9 (100)	-	-
Rifampcin	8 (88.9)	-	1 (11.1)
Norfloxacin	9 (100)	-	-
Cephalothin	9 (100)	-	-
Carbenicillin	7 (77.8)	-	2 (22.2)
Cefepime	8 (88.9)	1 (11.1)	-
Imipenem	-	-	9 (100)
Meropenem	9 (100)	-	-
Piperacillin/ Tazobactam	8 (88.9)	1 (11.1)	-
Cefotaxime	9 (100)	-	-
Ceftazidime	9 (100)	-	-
Cefoxitin	6 (66.65)	3 (33.35)	-

Table 4: Resistance patterns of UPEC carrying ESBL genotypes – TEM

Organism	Resistant to	Resistance patterns	No. of isolates
E. coli	16	AK, PC, G, TB, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ, CX	5
	13	G, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ, CX	1
	12	G, A, CFX, R, NX, CH, CB, CPM, MRP, PIT/TAZ, CTX, CAZ	1
	10	G, CFX, R, NX, CH, CPM, MRP, PIT/TAZ, CTX, CAZ	1
	7	G, CFX, NX, CH, MRP, CTX, CAZ	1

AK- Amikacin; PC- Piperacillin; G-Gentamicin; TB-Tobramycin; A- Ampicillin; CFX- Cefixime; R- Rifampicin; NX- Norfloxacin, CH- Cephalothin; CB-Carbenicillin; CPM- Cefepime; MRP-Meropenem; PIT/TAZ- - Piperacillin/ Tazobactam; CTX- Cefotaxime; CAZ-Ceftazidime; CX- Cefoxitin

Previous studies have shown that, when ceftazidime is used to treat infections due to TEM/SHV type ESBL producing *Enterobacteriaceae*, the clinical outcomes are generally poor[16], even in the current study 100 % of resistance against ceftazidime was observed. The resistant pattern of the TEM producing strains to cefepime was found to be 88.9%.

Most of the TEM producing isolates detected in the study were also resistant to fluoroquinolones, signifying the presence of specific genes indicating resistant to quinolones. The occurrences of TEM along with impermeability results in resistant to carbapenem group of drugs meropenem[17,18]where 100% resistant was observed in this study. Most ESBL producing strains particularly those with the TEM genotypes are resistant to aminoglycosides[19].

The study result suggests that only imipenem can be used to treat UTI caused by TEM producing *E. coli*. Amikacin, piperacillin and tobramycin may be used in situations where the other antibiotics used in the current settings are unsuitable to treat UTI because of the resistance observed against them.

The ESBL producing uropathogenic *E. coli* were genotyped for β-lactamase gene TEM. Of the ten ESBL positive *E. coli* isolates subjected for the detection of bla$_{TEM}$ by uniplex PCR, nine isolates (90%) were found to carry TEM gene (Table 2 and Figure 1). The main ESBL genotypes in *K. pneumoniae* is TEM in Europe[19,20]. In India, 52 and 60.9% of ESBL producing *E. coli* isolates were found to be positive for bla$_{TEM}$ gene by PCR[21,22]. Xiong et al (2002) detected TEM genes in *E. coli* isolates in China indicates that ESBL strain producing multiple β-lactamases in East Asia[23]. Similar prevalence of TEM gene have also been reported from Turkey, Iran, Thailand are 20.6, 63, 72.7 and 85.6 %[24,25,26].[27]respectively. These reports suggesting a significant increase in the number of *E. coli* producing TEM gene in the hospitals studied.

In this study one ESBLs producing uropathogenic *E. coli* showed negative results in PCR method for TEM genes, therefore other β-lactamases gene may be involved in ESBLs resistance. These isolates are usually multi-drug resistant because they became resistant to available antibiotics and they can also transfer the genes to other strains. According to Shahid et al. (2008) bla_{TEM} is the post prevalent ESBL gene in the clinical isolates[21].

According to published reports, SHV and TEM type ESBL's were predominantly associated with nosocomial outbreaks, which dramatically changed the epidemiology pattern during the last twenty years. The spread and differentiation of *E. coli* in the community and hospitals illustrates the importance of movements of patients between the community and the health care system[28].

Phenotypic tests for ESBL detection confirm only whether an ESBL is produced but cannot detect the subtypes of ESBL[22]. Though the detection of ESBL subtypes by molecular methods are sensitive, but they are also expensive, time consuming, requiring specialized equipment and expertise[29]. Therefore, the definitive identification of ESBL is possible only by molecular methods. Detection of ESBLs by phenotypic tests is needed to be evaluated from time to time, as their performance may change with the introduction of new enzyme[30]. Grover et al. (2006) concluded that PCR based methods are reliable for the detection of ESBL[31]. Identification and confirmation of β-lactamases by molecular techniques would be very important for reliable investigation of antimicrobial resistance. Early and rapid detection of these strains assumes significant importance in clinical microbiology laboratories. The present report gives a few insights on the clinical patterns of health care associated infection caused by ESBL carrying TEM gene.

CONCLUSION

In the present study, we have targeted the detection of TEM gene of uropathogenic *E. coli*. This finding is quite interesting because the therapeutic options for ESBL producing bacteria might be limited. The study also emphasizes the clinical implications of ESBL types, importance of antibiotic therapy and preventing the spread of TEM harboring strains in both hospital and community settings.

Authors' Contributions

Vidhya Natarajan has conceived and designed the work and performed strain isolation and identification, antibiotic susceptibility testing, PCR tests and prepared the manuscript. **Sudha SS** has participated in the designing of the work and correction of manuscript. Both the authors read and approved the final manuscript.

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