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PREVALENCE AND MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM Β-LACTAMASES PRODUCING ESCHERICHIA COLI RECOVERED FROM URINE SAMPLES OF PATIENTS ATTENDING TERTIARY CARE HOSPITAL, BATHINDA.

Microbiology

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ABSTRACT

Background: Prevalence of Extended-spectrum-β-lactamases (ESBLs)-producing uropathogenic Escherichia coli (E. coli) isolates has been found to be increasing rapidly across the world. β-lactam antibiotics are mainly used for treatment of infections caused by E. coli , however, the emergence of species with plasmid-borne beta-lactamases has decreased the efficacy of these antibiotics. So, continuing studies on the resistance pattern of different regions is necessary for assessment of proper antimicrobial therapy protocols. **Material and methods :**Urinary isolates of E. coli were collected from Adesh Institute of Medical Sciences and Research, Bathinda over one and a half year period. Specimens were processed as per standard protocol and verified using VITEK 2. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method as per CLSI guidelines 2019. Urinary isolates obtained were screened for ESBLs by CLSI recommendations for ESBLs screening and confirmation was made by Combination disk diffusion method. Existence of genes encoding for ESBLs production were identified using polymerase chain reaction (PCR) for blaSHV, blaTEM and blaCTX-M genes (blaCTX-M- I group and blaCTX-M- IV group genes). **Results:** Prevalence of ESBLs producing E. coli was found to be 41.6%.Carbapenems were the most effective antibiotic group against E. coli isolates. Most common gene responsible for ESBLs production in E. coli was blaCTX-M (53.3%). **Conclusion:** The study shows that the prevalence of ESBLs producing E. coli spread in this region is alarming causing significant therapeutic problems, so it mandates implementation of regulatory programs to reduce the spread of ESBLs producing E. coli in the community. Therefore monitoring of ESBLs production, judicious use of antibiotics and infection control measures are necessary to avoid treatment failures in patients with UTIs.

KEYWORDS

blaCTX-M, blaTEM, blaSHV, E. coli, Extended-spectrum β-lactamases.

INTRODUCTION

With over 150 million annual diagnosis of UTIs worldwide, it remains one of the most common community acquired as well as nosocomial infection [1]. There are approximately one million patients that get hospitalized due to UTIs annually worldwide [2]. E. coli is the main causative pathogen in UTIs [3] causing 80-90% of community-acquired urinary medical issues and 30-50% of nosocomial UTIs [4].

The incidence of ESBLs producing E.coli has been increasing worldwide. It varies according to geographic location and is directly linked to use and misuse of antibiotics [5]. The production of ESBLs by urinary E. coli strains is a major public health concern in both hospital and community settings [6]. ESBLs are plasmid mediated β-lactamases which have mutated from pre-existing broad-spectrum β-lactamases (TEM-1, TEM-2, and SHV-1) and have ability to hydrolyze penicillins, oxyimino-cephalosporins and monobactams but not cephamycins or carbapenems. Additionally, these enzymes are repressed by β-lactamase inhibitors as clavulanic acid, sulbactum and tazobactam[7]. And also most ESBLs producing uropathogens are also resistant to aminogylcosides, fluororquinolones, sulfonamides [8, 9]. Dissemination of ESBL-producing organisms is caused not only by horizontal transfer of plasmids, but also by clonal spread of ESBL-producing strains [10].

More than 350 different natural ESBLs variants are known, which have been classified into nine distinct structural and evolutionary families based on amino acid sequence[11]. Almost all the known generations of β-lactamases have been found to be circulating in India, and particularly the tertiary care hospitals encounter massive antimicrobial resistance (AMR) with prevalence ranging from 6 to 86 per cent among E. coli and K. pneumoniae isolates [12]. There is a probability that if antibiotic resistance keeps on rising then by 2050, 10 million individuals would disease each year because of shortage of existing management & there would be two to three percent low GDP [13]. The challenge is more in a country like India where bacterial disease burden is highest in the world [14]. There is, thus, a need for geographical surveillance of ESBLs production in E. coli to guide appropriate antimicrobial therapy.

MATERIAL AND METHODS

Urinary isolates of E. coli were collected from Adesh Institute of Medical Sciences and Research, Bathinda over one and a half year period. Specimens were processed as per standard protocol and verified using VITEK 2. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method as per CLSI guidelines 2019. Urinary isolates obtained were screened for ESBLs by CLSI recommendations for ESBLs screening and confirmation was made by Combination disk diffusion method [15]. DNA was extracted from control strains and isolates which were confirmed for ESBLS production as per the procedure given by Gill et al. 2001[16]. Existence of genes encoding ESBLs production were identified using polymerase chain reactions which were performed in BIO-RAD T100 thermo cycler using isolated DNA samples. The conserved primers (Forward primer and reverse primer) adopted from the previous studies were used for the amplification of bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ genes as shown in Table 1:

TABLE 1

Primer orientation	Sequence (5′ –3′)	Amplicon size	Reference
blaSHV (F)1	AGCCGCTTGAGCAA ATTAAAC	786 bp	Dallenne et al. [17] (2010)
blaSHV (R)2	GTTGCCAGTGCTCG ATCAGC	786 bp	Strauss et al. [18] (2015)
blaTEM (F)	CATTTCCGTGTCGC CCTTATTC	846 bp	Dallenne et al. (2010)
blaTEM (R)	CCAATGCTTAATCA GTGAGGC	846 bp	Strauss et al. (2015)
aCTX-M group I CTXM1-F3	GACGATGTCACTGG CTGA GC	499 bp	Pitout et al. [19] (2004)
CTXM1-R2	AGCCGCCGA CGC TAA TAC A	499 bp	Pitout et al.(2004)
bCTX-M group IV CTXM914F	GCT GGA GAA AAGCAG CGG AG	474bp	Pitout et al.(2004)
CTXM914R	GTA AGCTGA CGC AAC GTC TG	474 bp	Pitout et al.(2004)

1. Forward primer 2. Reverse primer
a. Group I includes CTX-M-1, -3, -10 to -12, -15 (UOE-1), -22, -23, -

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28, -29, and -30.

b. Group IV includes CTX-M-9, -13, -14, -16 to -19 and -21, and -27 and Toho-2.

PCR assay of bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ genes - PCR reagents were prepared in 200 (µl) PCR tube by adding each component as shown in Table 2

TABLE 2

Constituent	Volume
PCR master mix	10 µl
DNA sample(Template)	3 µl
Forward primer (10pM)	0.8 µl
Reverse primer (10pM)	0.8 µl
MilliQ water	5.4 µl
Total	20 µl

The ready-to-use PCR master mix (Bio-Rad) was used. Conditions for the amplification of genes is shown in Table 3

TABLE 3

Steps	Temperature (°c)				Time				Cycles
	bla shv	blat em	ctx-m group I	ctx-m group IV	blas hv	blat em	ctx-m group I	ctx-m group IV	
Initial Denaturation	95	95	95	95	5 min	5 min	5 min	5 min	-
Second Denaturation	95	95	95	95	40 sec	40 sec	40 sec	40 sec	30X
Primer annealing	58	55	55	62	40 sec	40 sec	40 sec	40 sec	-
Final extension	72	72	72	72	40 sec	40 sec	40 sec	40 sec	-
Chain elongation	72	72	72	72	5 min	5 min	5 min	5 min	-

RESULTS

Phenotypic distribution of ESBLs producing E. coli

A total of 2030 urine samples were processed for culture and sensitivity during the study period. Out of the overall 2030, 1584(78.03%) samples showed no or insignificant growth, 446 (21.97%) samples showed significant growth of pathogens ($\geq 10^5$ CFU/ml).

Out of 446 samples, 32 (7.17%) samples showed polymicrobial growth (54 bacterial isolates and 10 candida spp.) and 414 samples (92.83%) were pure bacterial isolates. Amongst the 468 bacteria isolated (414 pure and 54 from mixed) 257 (54.91%) E. coli were isolated.

Amongst these 41.6 % E. coli (107/257) were confirmed for ESBLs production by tests described in materials and methods.

Genotypic distribution of ESBLs producing E. coli

Among 107 phenotypically ESBLs producing E. coli isolates blaCTX-M, blaSHV and blaTEM were detected in 73% (78/107) isolates. blaCTX-M type was the most prevalent found in 53.3% (57/107) isolates and blaTEM in 27.1% (29/107) isolates and none of them carried genes for blaSHV.

Out of them 23.4% (25/107) were positive for blaCTX-M genes from the CTX-M- I group alone , 16.8% (18/107) had blaCTX-M genes for both the CTX-M-I group and CTX-M-IV group , followed by 5.6% (6/107) isolates for blaCTX-M genes from the CTX-M-IV group only. Among them 19.6% (21/107) carried gene for blaTEM alone. There was co-occurrence of CTX-M and TEM β-lactamases in 7.5 % (8/107) isolates. CTX-M- I group and blaTEM in 6 isolates and CTX-M-IV group and blaTEM in 2 isolates.

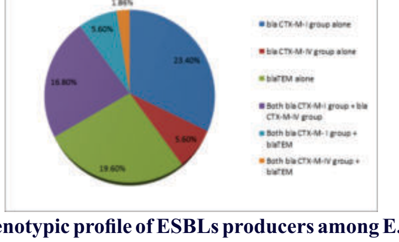

Figure1. Genotypic profile of ESBLs producers among E.coli

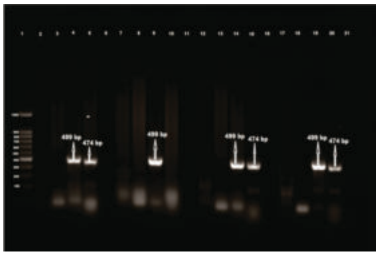

Figure2. A gel photograph showing amplified segment of gene bla CTX-M group I and bla CTX-M group IV .Lane 1 DNA ladder 100+ bp; Lane 2-5 (sample1); Lane 7-10 (sample 2); Lane 12-16 (sample3); Lane 17-20 (sample4)

Antibiotic Susceptibility Pattern of ESBLs Producing E. coli isolates

The sensitivity to antibiotics in E.coli isolates was: Cefotaxime (24%), Ceftazidime (24%), Cefepime(34%), Amoxicillin-clavulanate (51%), Piperacillin tazobactum (76%), Cefaperazone /sulbactum (79%), Gentamicin (58%), Amikacin (71%), Norfloxacin (51%), Nitrofurantoin(56%), Tigecycline (87%), Cotrimoxazole (59%), Imipenum (100%), Ertapenem (100%).

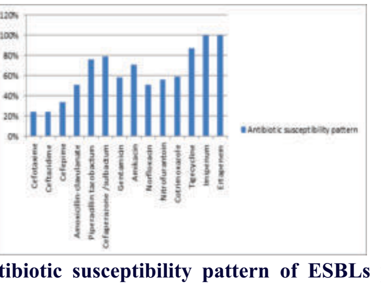

Figure3. Antibiotic susceptibility pattern of ESBLs producing strains of E.coli.

DISCUSSION

UTIs are one of the frequent causes of morbidity in outpatients and are also responsible for healthcare associated infections [20].Beta-lactam resistance in Gram-negative bacteria, especially E.coli, is a main clinical problem. It is often caused by the production of β-lactamases, particularly ESBLs [21]. ESBLs producing strains are acquiring a transmissible form of antibiotic resistance [22]. Distribution and occurrence and of ESBLs differs in different geographical areas and also from hospital to hospital [23]. Nowerdays ESBLs producing organisms are not only isolated from healthcare associated infections but are also now being isolated from community [24].

In the present study the prevalence of E. coli is 54.91%. All isolates were screened and confirmed for ESBLs production by tests described in materials and methods. This finding is in agreement with previous studies conducted by Ravishankar et al. [25], Awari et al [26], Mallikarjun et al. [27], Amatullah et al. [28], Bhargava et al. [29], Somashekara et al. [30], Khan et al. [31], Nepal et al. [32] in which similar prevalence was isolated from UTIs with E.coli strains.

The national prevalence of ESBLs phenotypes of E. coli in United States in 2017 was 15.7% and was geographically distributed. [33]. Previous studies from India have reported ESBLs production varying from 21% to 84% [34]. Our study reported 41.6 % (107/257) ESBLs producing E. coli isolates resembling those reported by Sahu et al.[35], Bajpai et al.[36], Taneja et al. [37], Ranjini et al.[38], Kumar et al.[39], Ramakrishna et al.[40] and Koshesh et al [41] unlike those reported by Maya et al [42], Rajowar [43], Kammili et al[44], Mukherjee et al. [45], Singhal et al [46], Ramesh et al. [47], Kesavaram et al. [48], Natrajan et al. [49] , Dash et al. 50], Gupta et al. 51], Bakshi et al. 52], Tankhiwale et al. [53], Aruna and Mobashshera [54] ,Gururajan et al.[55], Jain et al.[56], and Mustafa et al. [57]. The reports presented by different authors clearly indicate that the prevalence of ESBLs producing organisms among clinical isolates vary greatly geographically and rapidly changing over time. [58, 59].

A large survey of 1610 E.coli and 785 K. pneumoniae isolated from 31 centers in 10 European countries found that the prevalence of ESBLs ranged from as low as 1.5% in Germany to as high as 39-47% in Turkey, Russia, and Poland [60]. In comparison to India, other countries show a reduced incidence of ESBLs producers. A study has shown that the prevalence of ESBLs producing isolates of E. coli is 9.2% in Korea, 10.3% in Saudi Arabia and 13.3% in Lebanon. However, there is a constant increase in the population of ESBLs producing strains in communities of other countries [61]. Previous studies have shown that ESBLs-producing E. coli to be 76.5 % in Ethopia [62], 62.31% in Nepal[63] , 60% in Uganda[64], 50.5 % in Turkey[65], 38.07% in China [66] , 37.6% in Iran [67] , 36% in Egpyt [68].

In this study, amongst all the classes, Carbapenems emerged as the most effective class of drugs against E. coli, followed by tigecycline and Aminoglycosides. In Carbapenems both Imipenem and Ertapenum was found to be equally effective drugs (100%). In Aminoglycosides, Amikacin (71%) was the foremost effective agent. Nitrofurantoin was found to be 57% sensitive against the urinary E.coli isolates. Cephalosporins exhibited an appreciable resistance. Overall, studies have shown E. coli to be one of the most frequently encountered drug-resistant uropathogen and antibiotic resistance as a serious problem in the Asia-Pacific region. [69].

In the present study, 107 E. coli isolates were further run on PCR for detection of blaCTX-M, blaTEM and blaSHV genes using specific primers. blaCTX-M type was the most prevalent in 53.3% (57/107) isolates which is similar to studies by Koirala et al and Barguigua et al [70,71]. blaCTX-M has been reported to be the most prevalent ESBLs in studies done by Bouamri et al. [72], Seyedjavadi et al [73], Adwan et al [74], Nandagopal et al [75]a380, Alqasim et al[76], Mohamed et al [77], Sadeghi et al [78] which is also the case in our study unlike studies done by Jena et al[79], Dirar et al [80] Valadbeig et al. [81] in which blaTEM was highest. In our study blaTEM was next most common gene, which was found in 27.1% (29/107) isolates.

Multiple genes were also present in many isolates, with the most common combination being, CTX-M-I group+ CTX-M-IV group with 16.8% (18/107) isolates succeeded by blaCTX-M-I group +blaTEM in 5.6% (6/107) isolates . Occurrence or association of more than one β-lactamase within the same isolate has also been reported in other studies [82, 83, 84]. However, there were few ESBLs producing E. coli isolates in which no gene was detected. The probable explanation may be because of the presence of other ESBLS's like VEB,SFO, TOHO, SME, IMI, PER etc. Similar findings were observed by studies done by Eftekhar et al. [85], Rezai et al [86].

CONCLUSION

As this study shows the rising rate of ESBLS production in E.coli is observed. Thus every hospital should have antibiotic policy. Antimicrobial susceptibility testing should be compulsory to avoid treatment failure in patients with UTI. Monitoring of beta lactamses production should be regularly done.

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