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DISTRIBUTION OF TEM, SHV AND CTX‑M β-LACTAMASE PRODUCING GENES AMONG *Escherichia coli* AND *Klebsiella pneumoniae*, IN URINE ISOLATES AT A TERTIARY CARE CENTRE IN KANPUR

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ABSTRACT

To determine the prevalence of ESBLs(blaTEM, blaCTX‑M, and blaSHV) produced by *E.coli* and *K.pneumoniae* from urinary isolates.In our study, ESBL production was detected in 48.8% (44/90)of *K.pneumoniae* that carried (bla(SHV) (34/90, 37.7%), bla (TEM) (25/90, 27.7%), and bla (CTX-M-like) (35/90, 38.8%) and for 161 screening test positive E. coli, ESBL production was detected in 36.6% (59/161) of *E. coli* that carried bla(TEM) bla(CTX-M-like) and bla(SHV) genes in 5.5% (9/161), 28.57% (46/161) and 22.36% (36/161), respectively. Definitive identification of ESBL genes is only possible by molecular detection methods. Phenotypic tests need to be evaluated periodically as their performance may change with the introduction of new enzymes.

KEYWORDS: Extended spectrum beta lactamase (ESBL),bla(TEM) bla(CTX-M-like) and bla(SHV)

Beta –lactamases are enzymes that are major cause of bacterial resistance to the beta-lactam family of antibiotics such as penicillins, cephalosporins,Cephamycins and Carbapenems.[Naseer U etal., 2011, shahid M etal., 2011 and Lal P etal. 2007]. The ESBL genes are mostly plasmid encoded. Most ESBL genotypes are TEM, SHV, CTX-M.On the basis of primary structure,beta –lactamase are grouped into four classes A,B,C and D enzymes.Enzyme of classes A,C and D have serine at the active site Whereas the class B enzymes are Zine-metaloenzyme. Classical ESBLs have been evolved from the widespread plasmid encoded enzyme families Temoniera (TEM), Sulfhydryl variable (SHV) and Oxacillin (OXA), have an extended substrate profile which allows hydrolysis of all cephalosporins, penicillins, and aztreonam[El-Fileyetal., 2007].The ESBL enzyme are mostly produced by *Escherichia coli* and *Klebsiella.* ESBLs were first reported in Klebsiellapneumoniae in 1983, from Germany.[El filey etal.,2007]

Production of beta lactamase is the most common mechanism of antibiotic resistance tobeta lactam antibiotics. These are produced by aerobic Gram positive,Gram negative bacteria andalso in anaerobes . Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended- spectrum β-lactamases (ESBLs) [Cheesbrough 1984]. During the last three decades, ESBLs among urinary E. coli have been reported worldwide, and their occurrence has increased in both outpatients and inpatients diagnosed with UTIs. The present study aims to determine the prevalence of ESBL-producing E. coli of both community and nosocomial origin isolated from urine samples taken from patients diagnosed with UTIs, to detect their drug resistance pattern to commonly used antibiotics in medical practice and to detect blaSHV, bla TEM and blaCTX-M genes in these multi-drug resistant isolates.

MATERIALS AND METHODS

The present study was carried out in theDepartment of Microbiology, Rama Medical College, Kanpur over a period from November 2015 to December 2016. It wasapproved by the Institutional Ethical Committee. A total of 341 isolates of *Escherichia coli* and*Klebsiella pneumoniae* isolated from urinary isolates from various clinical departmentsincluding OPD and IPD of all age groups including both the genders. Out of them90 were screening test positive *K. pneumonia* isolates and161 were screening test positive E. coli.Identification of bacterial isolates was carried out using conventional biochemical methods and automated system (Vitek‑2 compact, BioMerieux, France). Following identification, the isolates were stored at 4°C on nutrient agar. All the E. coli and Klebsiellapneumoniae isolates were phenotypically tested for ESBL production by double disk diffusion test, According to CLSI guidelines, 2016. Ceftazidime plus clavulanic acid

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(30/10 mcg) and cefotaxime plus clavulanic acid (30/10 mcg) discs were also included along with ceftazidime (30 mcg) and cefotaxime (30 mcg) discs on MullerHinton agar. An organism was considered as ESBL producer if there was a ≥5 mm increase in the zone diameter of ceftazidime/clavulanic acid disc and that of ceftazidime disc alone and/or ≥5 mm increase in the zone diameter of cefotaxime/clavulanic acid disc and that of cefotaxime disc alone. E. coli ATCC25922(Hi-Media) were used as negative and *K.pneumoniae* ATCC 700603(Hi-Media) used as positive control.

Genotypic characterization of ESBL genes by PCR

The Deoxyribonucleic acid (DNA) was extracted from all phenotypic ESBL confirmatory test positive *Escherichia coli and Klebsiella pneumoniae*i solates. Polymerase chain reaction (PCR) amplification was done with specific gene primers for TEM, SHV and CTX-M types.The genomic DNA from *E.coli and Klesiella pneumonia* strains was extracted by using bacterial gDNA isolation kit (CHROMOUS BIOTECH) .

PCR cycling temperature for SHV

- Denaturation at 94 for 2 min
- Denaturation at 94°C for 1 min
- Annealing at 52°C for 30 sec 30 cycles
- Extension at 72°C for 45 sec
- Final Extension at 72°C for 5 min
- Holding at – 40C for 5 min

PCR cycling temperature for TEM

- Denaturation at 94°C for 2 min
- Denaturation at 94°C for 1 min
- Annealing at 58°C for 1 min 30 cycles
- Extension at 72°C for 1 min
- Final Extension at 72°C for 7 min
- Holding at – 4°C for 5 min

PCR cycling temperature for CTX-M

5 min at 94°C and 32 cycles of amplification consisting of 30 s at 95°C, 1 min at 54°C, and 2 min 72°C, with 5 min at 72°C for the final extension.

Analysis of PCR products (amplicons)

After amplification, the amplicons were visualized on 1.5% agarose gel for the presence of band. The agarose gel were scanned under UV illumination, visualized and digitized with the gel documentation system.

Table 1: Primers used in a master cycler

Primers	Primer sequence (5'- 3')	Product size (bp)
SHV	SHV-F 5- TCAGCGAAAAACACCTTG SHV-R 5- TCCCGCAGATAAATCACC	471
TEM	TEM-F 5-CTTCCTGTTTTTGCTCACCCA	717
	TEM-R 5- TACGATACGGGAGGGCTTAC	
CTX-M	CTX-M.- F 5' ACCGCCGATAATTCGCAGAT	588
	CTX-M. R- 5' GATATCGTTGGTGGTGCCATAA	

RESULTS AND DISCUSSION

Total of 341 isolates of Escherichiacoli and *Klebsiella pneumoniae* isolated from urinary isolates were studied.In our study, ESBL genes were detectedin 90 screening test positive K. pneumoniae isolates, ESBL production was detected in 48.8% (44/90)of *K.pneumoniae* that carried(bla(SHV) (34/90, 37.7%), bla(TEM) (25/90, 27.7%), and bla(CTX-M-like) (35/90, 38.8%) and for 161 screening test positive E. coli, ESBL production was detected in 36.6% (59/161) of *E. coli* that carried bla(TEM) bla(CTX-M-like) and bla(SHV) genes in 5.5% (9/161), 28.57% (46/161) and 22.36% (36/161), respectively.

In our study blaTEM + bla CTX-M both genes were found in 2 isolates of *Escherichia coli* and 4 isolates of *Klebsiella pneumoniae*. bla TEM+ bla SHV both genes were found in 1 isolates of *Escherichia coli* and 2 isolates of Klebsiellapneumoniae. bla SHV+ bla CTX-M both genes were found in 8 isolates of Escherichia coli and 8 isolates of *Klebsiella pneumoniae*. bla TEM+ bla SHV+ bla CTX-M genes were found in 4 isolates of Escherichia coli and 18 isolates of

Klebsiella pneumoniae were detected. The results are in accordance with a study by Yazdi et al., 2012 (87.1% TEM,followed by 70.6% SHV)[12] but lesser when compared with the results of studies by Eftekhar et al., 2012, in which SHV (43.1%)exceeded TEM (35.2%) by El Filewyetal., 2007, in whichCTX-M (28.8%) exceeded SHV (13.7%),[Lal P etal., 2007] and by Ahmedet al., 2013, in which CTX-M (71.4%in E. coli and 68.4%in *Klebsiella*) exceeded TEM (55.1% E. coli and 58%K*lebsiella*).[Yazdi M etal., 2012] Several other studies performed throughoutthe world showed variable results. In a Chinese study, the TEM gene predominated followed by SHV. A report from Canada showed SHV as the main group of ESBLs. However,reports from South America, Israel, Spain, New York, the United Kingdom, and several parts of Indian subcontinent revealed CTX-M as the predominant gene.

Until the year 2000, TEM was the most prevalent ESBL gene in the Indian bacterial population but was replaced by CTX-M in the following decade. In urine isolates in our setting,CTX-M is again predominant. The differences between our study results and those of other authors indicated that the prevalence and type of ESBL genes may vary from one geographical region to another. The present study clearly demonstrates the dramatic change in the gene pool in Indian Enterobacteriaceae.

Table-2 ESBL screening method

Organisms	Positive	Negative
Escherichia coli	161	46
Klebsiella pneumoniae	90	44

Table-3ESBL(Phenotypic) method

Organisms	Double Disk Diffusion test	Vitek- 2
Escherichia coli	**59**	**65**
Klebsiella pneumoniae	**42**	**44**

Table-4 : Distribution of various genes in the ESBL producers

Distribution of various genes in the ESBL producers			
Organisms	***Bla*CTX-M**	***Bla*- TEM**	***Bla*- SHV**
Escherichia coli	46	9	36
Klebsiella pneumoniae	35	25	34

Table5:Individuals and combination of bla genes among ESBL- *E.coli* and ESBL – *Klebsiella pneumonia*

	Genes	Escherichia *coli*	Klebsiella*pneumoniae*
1.	***bla*- CTX-M**	32	5
2.	**bla- SHV**	23	6
3.	**bla- TEM**	2	1
4.	**bla- TEM + CTX-M**	2	4
5.	**bla- TEM + SHV**	1	2
6.	**bla- SHV + CTX-M**	8	8
7.	**bla- TEM + SHV + CTX-M**	4	18
	Total samples	64	44

CONCLUSION

Inappropriate identification of antibiotic resistance may lead towrong antibiotic prescription, which may in turnchoose for new resistance genes. Phenotypic tests for ESBLdetection only confirm whether an ESBL is produced butcannot detect the ESBL subtype and cannot detect those genes whose expression is hidden or masked. Therefore, the genotypic method is suggested as the method of choice for detection of ESBL-producing strains of Enterobacteriaceae. Molecular methods are sensitive, but they are expensive and require specialized equipment andexpertise.Furthermore,genotypic methods can only detect those genes with known sequences. Phenotypic tests need to be evaluated periodically: Their performance may change with the introduction of a new enzyme, and they may detect new enzymes not included within the laboratory's test algorithm.For best results, phenotypic methods of ESBL detection should be improved.

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