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DETECTION OF SHV, TEM AND CTX-M β-LACTAMASE PRODUCING GENES AMONG *Escherichia coli* AND *Klebsiella pneumoniae* IN URINE SAMPLES.

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ABSTRACT
Prevalence of ESBLs (blaSHV, blaTEM, blaCTX-M,) *E.coli* and *K.pneumoniae* from urinary samples. In this study, production of ESBL was measured 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) (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) 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 technique. 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).

INTRODUCTION
Bacterial resistance is major role of due to that beta –lactamases are enzymes and beta- lactam family of antibiotics such as penicillins, cephalosporins,Cephamycins and Carbapenems. [1,2]. The ESBL genes are mostly plasmid encoded. Most ESBL genotypes are SHV, TEM, CTX-M .On the of structure,beta –lactamase are classified 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 Zinc-metalo enzyme. Classical ESBLs have been evolved from the widespread plasmid encoded enzyme families Temoniera (TEM) [3]., Sulfhydryl variable (SHV) and Oxacillin (OXA), have an extended substrate profile which allows hydrolysis of all cephalosporins, penicillins, and aztreonam [3].The mostly beta-lactamase produced by *Escherichia coli* and *Klebsiella in the hospital acquired infections* ESBLs were first reported in Klebsiella pneumoniae in 1983, from Germany.[4]. Production of beta lactamase is the most common mechanism of antibiotic resistance to beta lactam antibiotics. These are produced by aerobic Gram negative and Gram positive bacteria and also in anaerobes . Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended- spectrum β-lactamases [5]. Last three decades, ESBLs among urine samples E.

coli have been reported worldwide, and the 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 nosocomial and community origin isolated from urine samples was collected in the microbiology department Rama medical college kanpur to detect their drug resistance pattern to commonly used antibiotics in medical practice and to detect blaSHV, bla TEM and blaCTX-M genes in urine samples.

MATERIALS AND METHODS

The present study was done in Department of Microbiology, Rama Medical College, Kanpur November 2015 to march 2016 and Ethical clearance was taken from Institutional Ethical Committee. A total of 341 isolates of *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine samples from various clinical department including inpatient and outpatient of all age groups including both the male and females genders. Out of them 90 were screening test positive *K. pneumonia* isolates and 161 were screening test positive E. coli. Identification of bacterial isolates was carried out using different biochemical test 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 Klebsiella pneumoniae isolates were phenotypically tested for ESBL production by double disk diffusion test, According to CLSI guidelines, 2014 [6]. . Ceftazidime plus clavulanic acid 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. and 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[7]. .

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) [8,9].

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 [8,9]

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 [10,11]. .

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

A Total of 341 urinary isolates of Escherichiacoli and *Klebsiella pneumoniae* studied.In present study, ESBL genes were detected in 90 screening test positive *Klebsiella 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 the present 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 Klebsiella pneumoniae. 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 [14]. , 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*) [15]. 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 [15]. .

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-3 ESBL (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*pneumonia e***
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

Figure-1

***1000bp**
DNA ladder
Detection of *bla* CTX-M gene. 6 th lane is DNA ladder and 3, 4, 5, 7, 8 and 9th lane is sample positive for CTX-M gene.products (band size 588bp) of ESBL gene.

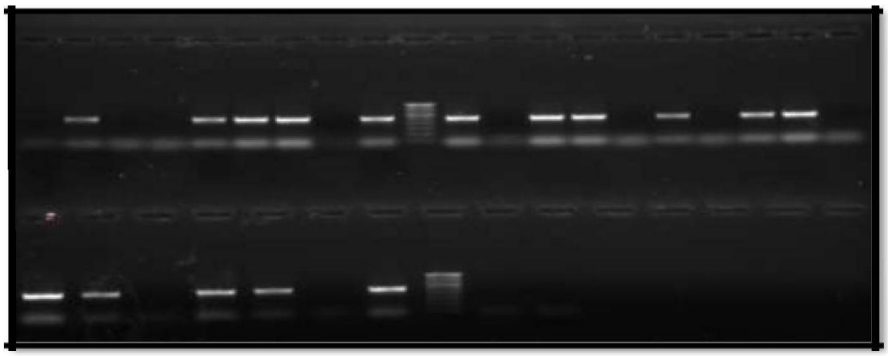
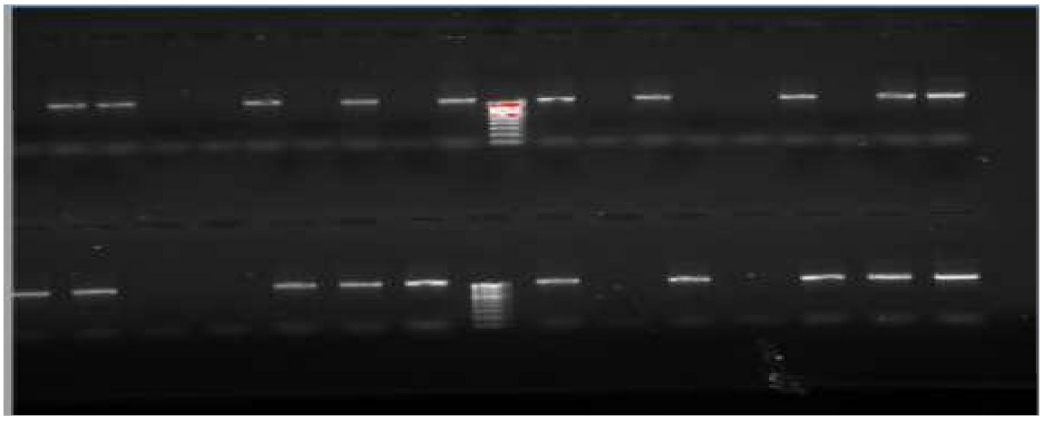


Figure-2

***1000bp**
DNA ladder

10 th lane is DNA ladder Detection of *bla* SHV gene. products (band size 471bp) of ESBL gene.



***1000bp** **Figure-3**
DNA ladder

Detection of *bla* TEM gene. 6 th lane is DNA ladder and 1, 2, 3. 4, 5, 7, 8, 9, 10 and 11th lane is sample positive for TEM gene. products (band size 717bp) of ESBL gene.

CONCLUSION

Inappropriate identification of antibiotic resistance may lead to wrong antibiotic prescription, which may in turn choose for new resistance genes. Phenotypic tests for ESBL detection only confirm whether an ESBL is produced but cannot 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 and expertise. 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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