IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 10 Ver. VII (Oct. 2017), PP 76-81
www.iosrjournals.org

Molecular Characterization of TEM, SHV and CTX-M Extended Spectrum Beta Lactamase Among Escherichia *coli* And Klebsiella *pneumoniae* In Urinary Isolates.

R.Sujatha[1*], Anil Kumar[2], Anil Kumar[3]
([1.] Professor and Head, Department of Microbiology, Rama Medical college, Kanpur, India.)*
([2.] Ph.D Research scholar, Rama Medical College Kanpur, India.)
([3] Assistant professor, Central Research Laboratory, Rama Medical College Kanpur, India.)
**Corresponding author*: Dr. R. Sujatha

Abstract:
Objective: *To determine the prevalence and molecular characterization of ESBLs produced by E.coli and K.pneumoniae from urinary isolates.*
Material And Method: *Urinary isolates of patients in Rama Medical College, Kanpur (U.P), India from November 2015 to December 2016. ESBLs were determine by disk diffusion test , double disk diffusion test, E-testand Vitek 2 ESBLs .All ESBLs producing isolates were investigated for the presence of bla(TEM), bla(SHV) and bla(CTX-M) genes by polymerase chain reaction (PCR).*
Results: *Total of 341 isolates of Escherichia coli and Klebsiella pneumoniae isolated from urinary isolates were processed. 207 were E. coli and 134 were K. pneumoniae. ESBL screening test was performed. Of the 90 screening test positive K. pneumoniae isolates, ESBL production was detected in 48.8% (44/90) by E-test and the beta-lactamase genes were 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.*
Conclusion: *The double disk diffusion test should be added routinely in the antibiotic susceptibility test for the Enterobacteriaceae. It is simple to perform, easy to interpret, and economical. The presence of bla(CTX-M), bla(SHV) and bla(TEM) in ESBL-producing E. coli and K. pneumoniae indicastes the high prevalence of these genes in North India.*
Keywords: *Extended spectrum beta lactamase (ESBL), E-Test, bla(TEM) bla(CTX-M-like) and bla(SHV)*

Date of Submission: 29 -07-2017 Date of acceptance: 30-09-2017

I. Introduction

In the battle against β-lactamase mediated bacterial resistance, development of third generation cephalosporins in early 1980s was a major breakthrough. But soon, a new plasmid encoded β -lactamase capable of hydrolyzing the extended spectrum cephalosporins was reported[1-3]. The ESBL genes are mostly plasmid encoded. Most ESBL genotypes are TEM, SHV, CTX-M. 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[4-5].The ESBL enzyme that are most produced by *Escherichia coli*. and *Klebsiella* spp . ESBLs were first reported in Klebsiella pneumoniae in 1983, from Germany.[4]

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 [4]. Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended- spectrum β-lactamases (ESBLs) [5]. 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, blaTEM and blaCTX-M genes in these multi-drug resistant isolates.

II. Material And Methods

The present study was conducted in the Department of Microbiology, Rama Medical College, Kanpur over a period from November 2015 to December 2016. A total of 341 isolates of Escherichia coli and Klebsiella pneumoniae isolated from urinary isolates from various clinical departments including OPD and IPD of all age groups including both the genders. Out of them 207 were E. coli and 134 were K. pneumoniae were obtained All samples were routinely cultured on Blood agar, Macconkey agar and CLED agar plates at 37°C aerobically for 18 hours and gram negative isolates were further characterized by standard biochemical test. Isolates were screened for ESBL production by using disc diffusion test on Muller Hinton agar according to CLSI guidelines, 2016.[11] Isolates showing inhibition zone size of ≥22mm with ceftazidime (30µg), ≥25mm with ceftriaxone (30µg), ≥ 27mm with cefotaxime (30µg), ≥27 mm with aztreonam (30 µg) were suspected for ESBL production. All screening test positive isolates were further confirmed by Phenotypic confirmatory test for ESBL producers by double disc diffusion test (DDDT) by two methods which were ceftazidime and ceftazidime plus clavulinic acid (CAZ/CAZC) and cefotaxime and cefotaxime plus clavulinic Acid (CTX/CTXC). And Phenotypic confirmatory test for ESBL was done by E- Test and Vitek- 2 method. E. coli ATCC 25922 was used as negative control and *Klebsiella pneumoniaee* ATCC 700603 used as positive control. The phenotypically confirmed ESBL were tested genotypically.

Ethical clearance was taken from ethical committee. Rama Medical College, Kanpur.

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 isolates. 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 pneumoniae strains was extracted by using bacterial gDNA isolation kit (CHROMOUS BIOTECH) [5].

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 TEM-R 5- TACGATACGGGAGGGCTTAC	717
CTX-M	CTX-M.- F 5' ACCGCCGATAATTCGCAGAT CTX-M. R- 5' GATATCGTTGGTGGTGCCATAA	588

III. Results And Discussion

In the present study total of 341 isolates of Escherichia coli and Klebsiella pneumoniae isolated from urinary isolates were processed. 207 were E. coli and 134 were K. pneumoniae. ESBL screening test was performed. Of the 90 screening test positive K. pneumoniae isolates, ESBL production was detected in 48.8% (44/90) by E-test, Vitek-2 and the beta-lactamase genes were 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).

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 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 disagreed with the resultsof studies by Eftekhar et al., 2012, in which SHV (43.1%)exceeded TEM (35.2%),[4] by Shahid et al., 2011, in which CTX‑M (28.8%) exceeded SHV (13.7%),[3] 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%Klebsiella).[11] Several other studies performed throughout the 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

Total urine sample	E.coli	Klebsiella pneumoniae	Others
1169	207	134	398

Table-3 ESBL screening method

Organisms	Positive	Negative
Escherichia coli	161	46
Klebsiella pneumoniae	90	44

Table-4 ESBL confirmatory method

Organisms	Double Disk Diffusion test	E-Test	Vitek- 2
Escherichia coli	**59**	**62**	**65**
Klebsiella pneumoniae	**42**	**44**	**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

Table-5: Individuals and combination of bla genes among ESBL- Escherichia coli and ESBL – Klebsiella pneumoniae

	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

Table- 6: (a and b) contingency table

(a)

PCR	DDST		E-TEST		VITEK 2	
	POS	NEG	POS	NEG	POS	NEG
POS - 64	TP 56	FN 9	TP 62	FN 3	TP 63	FN 2
NEG- 143	FP 3	TN 139	FP 0	TN 142	FP 2	TN 140
Total - 207	**59**	**148**	**62**	**145**	**65**	**142**

(b)

TP: true positive, TN: True negative, FP: False positive, and FN: False negative.

PCR	DDST		E-TEST		VITEK 2	
	POS	NEG	POS	NEG	POS	NEG
POS -44	TP 39	FN 10	TP 44	FN 5	TP 48	FN 0
NEG- 90	FP 3	TN 82	FP 0	TN 85	FP 1	TN 85
Total - 134	**42**	**92**	**44**	**90**	**49**	**85**

Table: 7 ESBL producing Escherichia *coli*

	DDST	E-TEST	VITEK 2
Pravelance	31.4%	31.4%	31.4%
Sensitivity	0.86(0.74- 0.93)	0.95(0.86- 0.98)	0.96(0.88-0.99)
Specificity	0.97 (0.93 -0.99)	1(0.96-1)	0.98(0.94- 0.99)
Probability positive	0.28	0.29	0.31
Probability Negative	0.71	0.70	0.68
Positive predictive value	0.94	1	0.96
Negative predictive value	0.93	0.97	0.98
Likely hood ratio			
Conventional (C)	40.77	0	68.81
Weighted by prevalence(W)	18.66	0	31.5

Table: 7 ESBL producing Klebsiella *pneumoniae*

	DDST	E-TEST	VITEK 2
Pravelance	36.5%	36.5%	36.5%
Sensitivity	0.79(0.65 -0.89)	0.89 (0.76- 0.96)	0.97 (0.87- 0.99)
Specificity	0.96 (0.89- 0.99)	1(0.94 - 1)	1(0.94 - 1)
Probability positive	0.31	0.32	0.35
Probability Negative	0.68	0.67	0.64
Positive predictive value	0.92	1	1
Negative predictive value	0.89	0.94	0.98
Likely hood ratio			
Conventional (C)	22.55	0	0
Weighted by prevalence(W)	13	0	0

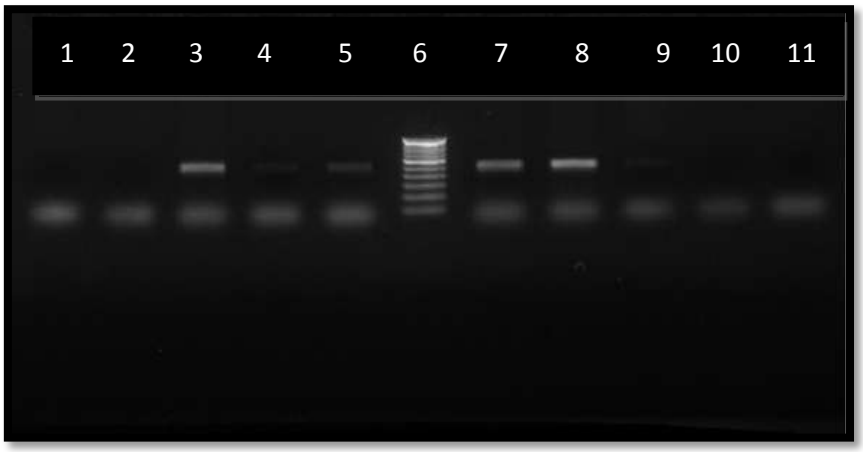

Figure 1: Detection of bla CTX-M gene. 6 th lane is DNA ladder and 3, 4, 5. 7, 8 and 9th lane is sample positive for CTX-M gene.

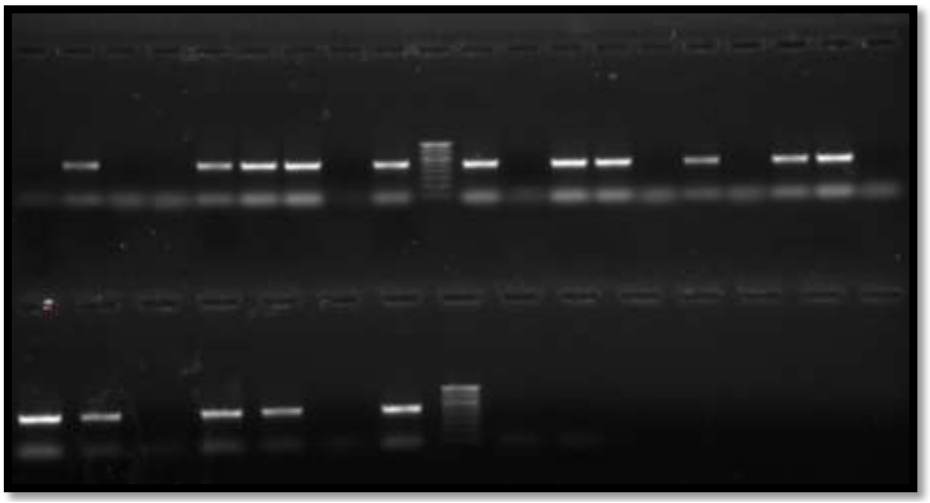

Figure 2: Detection of bla SHV gene. 10 th lane is DNA ladder

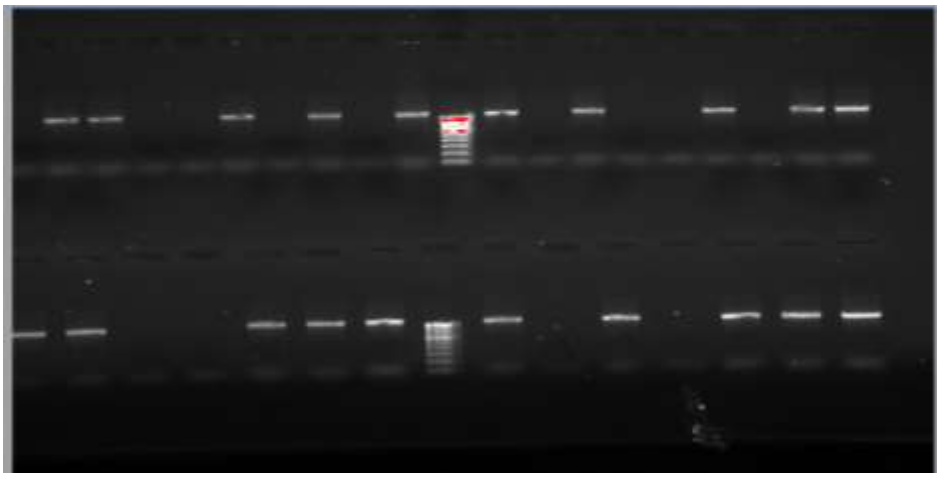

Figure 3: Detection of bla TEM gene. 6 th lane is DNA ladder and 1, 2, 3. 4, 5, 7, 8, 9, 10 and 11th lane is sample positive for TEM gene.

III. Conclusion

The double disk diffusion test should be added routinely in the antibiotic susceptibility test for the Enterobacteriaceae. It is simple to perform, easy to interpret, and economical. The presence of bla(CTX-M), bla(SHV) and bla(TEM) in ESBL-producing E. coli and K. pneumoniae indicates the high prevalence of these genes in North India. 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.

References

[1]. Sharma J, Sharma M, Ray P. Detection of TEM and SHV genes in Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital from India. Indian J Med Res 2010;132:332‑6.

[2]. Naseer U, Sundsfjord A. The CTX‑M conundrum: Dissemination of plasmids and Escherichia coli clones. Microb Drug Resist 2011;17:83‑97.

[3]. Shahid M, Singh A, Sobia F, Rashid M, Malik A, Shukla I, et al. Bla (CTX‑M), bla (TEM), and bla (SHV) in Enterobacteriaceae from North‑Indian tertiary hospital: High occurrence of combination genes. Asian Pac J Trop Med 2011;4:101‑5.

[4]. Lal, P., Kapil, A., Das, B.K., Sood, S. 2007. Occurrence of TEM and SHV gene in extended spectrum beta-lactamases (ESBLs) producing Klebsiella sp. isolated from a tertiary care hospital. Indian J. Med. Res., 125:173-8.

[5]. El-Fiky, S., Elgandour, A., Harfoush, R., 2007. Phenotypic and Molecular Detection of TEM and SHV beta-lactamases produced by organisms of the family Enterobactericeae; Study on blood culture clinical isolates from intensive care unit. Egyptian J. Med. Microbiol., 16(2): 211-221.

[6]. Eftekhar F, Rastegar M, Golalipoor M, Mansour Samaei N. Detection of extended spectrum beta‑lactamases in urinary isolates of Klebsiella pneumonia in relation to BlaSHV, BlaTEM, BlaCTX‑M gene carriage. Iran J Public Health 2012;41:127‑32.

[7]. Collee JG, Fraser AG, Marmian BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology.14th ed. New York: ChurchillLivingstone; 1996. [Reprinted 1999].

[8]. Cheesbrough M. Microbiology. Medical Laboratory Manual for Tropical Countries. Vol. 2. Cambridgeshire, England: Tropical Health Technology,Norfolk; 1984. p. 985.

[9]. Thomson KS. Extended‑spectrum‑β‑lactamase, AmpC, and carbapenemase issues. J Clin Microbiol 2010;48:1019‑25.

[10]. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493‑6.

[11]. Clinical Laboratory Standards Institute. Performance Standard for Antimicrobial Susceptibility Testing. 24th Information Supplement.NCCLS Document M100‑S23‑24. Wayne, Pennsylvania, USA: Clinical Laboratory Standards Institute; 2013‑2014.

[12]. Moosavian M, Deiham B. Distribution of TEM, SHV and CTX‑M genes among ESBL‑poducing Enterobacteriaceae isolates in Iran. Afr J Microbiol Res 2012;6:5433

[13]. Ahmed AB, Omar AO, Asghar AH, Elhassan MM. Prevalence of TEM, SHV and CTX‑M genes in Escherichia coli and Klebsiella spp. urinary isolates from Sudan with confirmed ESBL phenotype. Life Sci J 2013;10:191‑5.

[14]. Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M, Sharifi SH. Genotypic versus phenotypic methods to detect extended‑spectrum beta lactamases (ESBL's) in uropathogenic Escherichia coli. Ann Biol Res 2012;3:2454‑8.

R.Sujatha1*. "Molecular Characterization of Tem, Shv And Ctx-M Extended Spectrum Beta Lactamase Among Escherichia Coli And Klebsiella Pneumoniae In Urinary Isolates." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) , vol. 16, no. 10, 2017, pp. 76–81.