Molecular Characterization of blaCTX-M, blaTEM and blaSHV Beta Lactamases Produced by Uropathogens

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Authors' contributions

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ABSTRACT

Background: This prospective study was carried out to look for the frequency of isolation of Extended spectrum β lactamase (ESBL) producing bacteria from urine samples and study their susceptibility pattern. The detection of ESBL genes responsible for their resistance was done by Polymerase chain reaction (PCR).
Methods: The study was carried out over a period of one year from January 2016 to December 2016. Urine specimens from patients were processed as per standard protocol. Antimicrobial susceptibility testing was performed by disk diffusion method as per CLSI guidelines 2016.
Urine isolates obtained were screened for ESBL, by cefotaxime, ceftazidime disk and confirmation

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was made by Double disk diffusion test method. The detection of ESBL genes responsible for their resistance was done by Polymerase chain reaction (PCR) for *bla*CTX-M, *bla*TEM and *bla*SHV genes.

Results: Prevalence of ESBL producing uropathogens were found to be 20.47% with most common organism to be isolated was *Escherichia coli* (*E. coli*) followed by *Klebsiella pneumoniae.* Nitrofurantoin and Imipenem were the most effective antibiotic agents against urinary isolates. Most common gene responsible for ESBL production was *bla*CTX-M (71.42%).

Conclusion: A large number of ESBL producing strains are creating significant therapeutic problems. Therefore, monitoring of ESBL production, judicious use of antibiotics and infection control measures are necessary to avoid treatment failures in patients with Urinary Tract Infections (UTI).

1. INTRODUCTION

With over 150 million annual diagnosis of Urinary Tract Infections (UTIs) worldwide, it remains one of the most common community- acquired as well as nosocomial infections [1]. Bacteria are the major causative organisms responsible for more than 95% of UTI cases. *Escherichia coli* is the most prevalent causative organisms of UTI solely responsible for more than 80% of the infections. Multiple drug resistance has significantly increased in recent years. There is a growing concern for multidrug- resistant Gram-negative bacteria which produce extended-spectrum β-lactamases (ESBLs). ESBLs are Class A β-lactamases that hydrolyse penicillin, oxyimino-cephalosporins, and monobactams but not cephamycins or carbapenems. They are inhibited *in vitro* by clavulanate. ESBLs are often plasmid-mediated enzymes and have various genotypes. The most common are the *bla*SHV, *bla*TEM, and *bla*CTX-M types. ESBLs are primarily produced by the Enterobacteriaceae family of Gram-negative organisms, in particular *Klebsiella pneumoniae, K. oxytoca* and *E. coli*. They are also produced by other Gram-negative organisms, such as *Acinetobacter baumannii, Proteus* spp, *Pseudomonas aeruginosa* and *Salmonella* spp. [2].

β Lactam antibiotics are widely used for the treatment of UTIs, therefore emergence of β Lactamase producers, has become a matter of concern. The first plasmid mediated β lactamase in Gram negative bacilli was *bla*TEM-1 discovered in 1960s followed by *bla*SHV-1 in 1983 [3]. Nowadays *bla*CTX-M enzymes are being discovered and widely becoming the most prevalent β-lactamases [4], causing multidrug resistance leading to restricted treatment options. Therefore, this study attempts to investigate the prevalence of Extended spectrum β lactamase (ESBL) production among Gram negative uropathogens by phenotypic methods and their molecular characterization so as to tackle the menace of drug resistance effectively.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

A total of 5513 non-repeat urine samples obtained from patients suspected of suffering from UTI were assessed. These urine samples were received in the microbiology laboratory for culture and antimicrobial sensitivity testing were put on Cysteine Lactose Electrolyte Deficient (CLED) medium and incubated at 37°C for 24 hours. A sample was considered positive for UTI if the colony count was more than 10^5 CFU/ml and direct microscopy revealed pus cells more than 8/cu mm[3].

Samples growing more than two organisms were not included in the study and were discarded as mixed growth.

2.2 Identification of Bacterial Pathogens

Preliminary identification of bacteria was done by standard identification techniques. All isolates exhibiting ambiguous taxonomic classification were confirmed by the VITEK 2 – compact system (BioMerieux, France) following the manufacturers instruction.

2.3 ESBL Detection

i. **ESBL screening:** All the isolates of *Escherichia coli* (*E. coli), Klebsiellla*

pneumoniae (K. pneumoniae) and Proteus mirabilis (P. mirabilis) were screened for ESBL production by Kirby- Bauer disc diffusion method on Mueller -Hinton Agar with Ceftazidime (30 µg), Cefotaxime (30 µg). Zones size ≤ 22 mm for Ceftazidime and ≤ 27 mm for Cefotaxime indicate potential ESBL producer. If any strain was suspected to be an ESBL producer, then confirmatory test was done.

ii. **Phenotypic confirmatory test for ESBL detection:** The screened ESBL producers were subjected to confirmatory phenotypic testing for ESBL production by the combined disc diffusion test.

2.4 Combined Disc Diffusion Test

Pairs of discs containing ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic discs with and without clavulanic acid were placed on the plate inoculated with the test organism.

An ≥5 mm Increase in the zone diameter of either antimicrobial agent tested in combination with clavulanic acid verses its zone when tested alone was taken as indicative of ESBL production.

Quality of media and antibiotic discs were controlled by the reference strains:

Escherichia coli ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 for ESBL. Antimicrobial susceptibility pattern of isolated *E. coli, K. pneumoniae and P. mirabilis* was performed by Kirby Bauer Disc Diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Polymerase Chain Reaction (PCR) for *bla*CTX-M, *bla*SHV and *bla*TEM gene: Samples which were confirmed ESBL positive were subjected to PCR for the determination of genes responsible for drug resistance namely *bla*CTX-M, *bla*SHV and *bla*TEM.

i. **Bacterial DNA Extraction:** The bacterial DNA for the test and control strains was extracted using the method in the manufacture's kit. Bacterial DNA extraction was performed using the QIAMP DNA MINI kit (QIAGEN, Dueseeeldorf, Germany).

ii. **PCR Amplification:** The primers were procured from Imperial Life Sciences Ltd. (Gurgaon, India) As shown in Table 2, PCR reagents were prepared in 200 (µl) PCR tube by adding each component as follows:

The cycling conditions for amplifying the genes were as follows (Table 3):

Table 1. Template DNA

SL No	Reagents	Amount in PCR tube (µl)
1	DEPC* treated water	7.5
2	PCR master mix (2x -HiMedia)	12.5
3	Forward primer (10pM)	1
4	Reverse primer (10pM)	1
5	Template	3
Total Volume		25

DEPC-Diethypyrocarbonate

Table 2. Primers used for PCR to detect genes responsible for ESBL production

Primer	Sequence (5' →3')	Gene	Size of PCR product
TEM-F[†]	ATGAGTATTCAACATTTCCG	TEM	867
TEM-R[‡]	CTGACAGTTACCAATGCTTA		
SHV-F	GGTTATGCGTTATATTCGCC	SHV	867
SHV-R	TTAGCGTTGCCAGTGCTC		
CTX-M-F	ATGTGCAGYACCAGTAARGT[C]	CTX-M	593
CTX-M-R	TGGGTRAARTARGTSACCAGA		

[†]*F-Forward primer*
[‡]*Reverse primer*

Table 3. PCR cycles

No of cycles- 30

Stage 1		
95°C		5 minutes
Stage 2		
95°C		30 Sec
55°C	30 cycle	30 Sec
72°C		30 Sec
Stage 3		
72°C		5 minutes
4°C		∞

iii. Analysis of PCR Results: The analysis of the PCR results was done by running the amplified products on Gel Electrophoresis. The amplified PCR products were analyzed in a 1.5% agarose gel and visualized by BIO-RAD GEL DOC set with UV /VISIBLE TRANSILLUMINATOR.

3. RESULTS

The total number of urine samples that came to the laboratory for culture and sensitivity during the study period of roughly one year were 5513, which constitute majority of the samples received in our lab. Out of the overall 5513, 4586 (83.20%) showed no growth, 449 (8.14%) showed significant growth of pathogens including bacteria and yeast, 283 (5.13%) were contaminated or showed polymicrobial growth and 195 (3.53%) showed insignificant growth.

Out of 449 pathogenic organisms grown from collected urine samples 417 (92.87%) were bacterial isolates and 32 (7.10%) were yeast (Candida sp.). Amongst the bacteria isolated 342 (76.20%) were Gram negative bacilli and 75 (16.70%) were Gram positive cocci. The foremost common organism among Gram negative bacilli was *E. coli* followed by *Klebsiella sp. Enterococcus* was the foremost common organism among the Gram positive cocci and *Non Candida albicans* dominated among the Yeast organism.

3.1 Distribution of ESBL producing Uropathogens

Out of the 342 isolates of Gram negative Uropathogenic organisms, 175 were screened as positive ESBL producers by Kirby-Bauer disc diffusion method using Cefotaxime and Ceftazidime. These 175 positive screened isolates were confirmed for ESBL production by disc combination method using ceftazidime and clavulanic combination disc's. As per CLSI guidelines recommendation, detection of ESBLs, only *E. coli, K. pneumoniae., P. mirabilis* were included. Of these isolates of *E. coli, K. pneumoniae* and *P. mirabilis* were screened and confirmed for ESBL production by tests described in materials and methods. *E. coli* was the commonest isolate. 70 (20.47%) isolates were confirmed to be ESBL producers.

3.2 Bacteriological Profile of ESBL Producing Uropathogens

As per CLSI guidelines recommendation, detection of ESBLs, only *E. coli, Klebsiella sp., Proteus mirabilis* were included. Amongst these *E. coli (63/70)* came intent on to be the foremost predominant organism (90%) followed by *Klebsiella pneumoniae (5/70) (7.15%) and Proteus mirabilis (2/70) (2.85%).*

3.3 Antibiotic Susceptibility Pattern of ESBL Producing Uropathogens

There were 4 classes of antibiotics tested for antimicrobial susceptibility in Enterobacteriaceae. These included β-lactam (Penicillins, Monobactams, Cephalosporins and Carbapenems), Aminoglycosides (Amikacin, Tobramycin, Gentamicin), Fluoroquinolones (Levofloxacin, Norfloxacin) and Tetracyclines.

***Escherichia coli*:** Amongst all the classes, Carbapenems emerged as the most effective class of drugs against *E. coli*, followed by Aminoglycosides. In Carbapenems, Imipenem was found to be most effective drug (100%), followed by Meropenem (84.13%). In Aminoglycosides, Amikacin (85.71%) was the foremost effective agent. Nitrofurantoin was found to be 100% sensitive against the urinary *E. coli* isolates. Penicillin and Cephalosporins exhibited an appreciable resistance.

Table 4. Bacteriological profile of ESBL producing uropathogens

S. no	Organism	Number	Percentage %
1	*Escherichia coli*	63	90
2	*Klebsiella pneumoniae*	5	7.15
3	*Proteus mirabilis*	2	2.85
Total		70	100

Klebsiella: Amongst all the antibiotics, Imipenem (100%) was found to be most effective against *Klebsiella*. It had been followed by Meropenem (80%) and so by Tobramycin (60%), Amikacin (60%) and Gentamicin (60%). All the *Klebsiella* isolates were resistant to Amoxicillin/ Clavulanic acid and Ampicillin / Sulbactum. Also the Cephalosporins showed high resistance (95%). Aztreonam and Ticarcillin also showed 80% resistance.

Proteus mirabilis: *P. mirabilis* were sensitive to a number of antibiotics which including Ampicillin/ Sulbactum, Ticarcillin/ Clavulanic acid, Tobramycin, Tetracycline, Meropenem and Imipenem. All of these drugs showed 100% sensitivity. Amoxicillin/ Clavulanic acid, Levofloxacin, Gentamycin, Aztreonam, Norfloxacin showed 50% sensitivity. Whereas, Cephalosporins were 100% resistant.

Distribution of *bla*CTX-M, *bla*TEM & *bla*SHV genes among phenotypically confirmed ESBL producing isolates of *E. coli*, *K. pneumoniae* and *Proteus mirabilis* isolates.

ESBL confirmed isolates were subjected to PCR for *bla*CTX-M, *bla*TEM and *bla*SHV. Fig. 1 shows the analysis of PCR product by gel electrophoresis showing bands at particular nucleotide denoting *bla*CTX-M, *bla*TEM and *bla*SHV genes. Table 5 shows the distribution of those three genes accountable for ESBL production in 70 isolates. The foremost common gene found in these isolates was *bla*CTX-M which was found in 45 (71.4%) isolates of *E. coli*, 3 (60%) isolates of *K. pneumoniae* and 2(100%) isolates of *P. mirabilis.* The following most typical gene was *bla*TEM which was found in 32(50.7%) isolates of *E. coli* and 1(50%) isolate of *P. mirabilis*.

4. DISCUSSION

Urinary tract infections are frequent cause of morbidity in outpatients and are also responsible for healthcare – associated infection [5]. The most common uropathogens belong to the family Enterobacteriaceae such as *Escherichia coli, Klebsiella pneumoniae* and Proteus sp. [6] UTI is a serious ailment in humans due to its recurrence and difficulty in eradication. There are many enzymes responsible for drug resistance in bacteria, one of which is the production of ESBLs.

Extended spectrum β-lactamase producing organisms are those that hydrolyze the oxyimino beta – lactams and monobactams, but have no effect on the cephamycins and carbapenems [7,8] β-lactamase inihibitors such as clavulanic acid, sulbactam and tazobactam inhibit ESBL producing strains and are acquiring a transmissible form of antibiotic resistance [9].

Occurrence and distribution of ESBLs differs in different geographical areas and also from hospital to hospital [10]. Initially ESBL producing organisms were usually isolated from healthcare – associated infections but these organisms are now also being isolated from community [11]. The continuous pressure exerted by the use of newer expanded-spectrum β-lactams promoted the development of new *bla*TEM and *bla*SHV genes. There are so many types of ESBL's like *bla*TEM, *bla*SHV, *bla*CTX-M, OXA AmpC but majority of the ESBLs are derivatives of TEM or SHV enzymes and these enzymes are most commonly found in *E. coli* and *K. pneumoniae* [12]. Monitoring the antibiotic resistance of Gram negative organisms isolated from specimens is a useful tool to get information about the prevalence of ESBLs and are required for controlling the spread of resistance in bacteria.

The current Clinical and Laboratory Standards Institute guideline recommends detection of ESBLs in *E. coli, Klebsiella sp.* and *Proteus mirabilis*, which includes an initial screening test with any two of the following β-lactam antibiotics: cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone. Isolates exhibiting resistance to any one of these antibiotics should be confirmed phenotypically by double disc diffusion test (DDDT) using ceftazidime alone and ceftazidime-clavulanic acid combination.
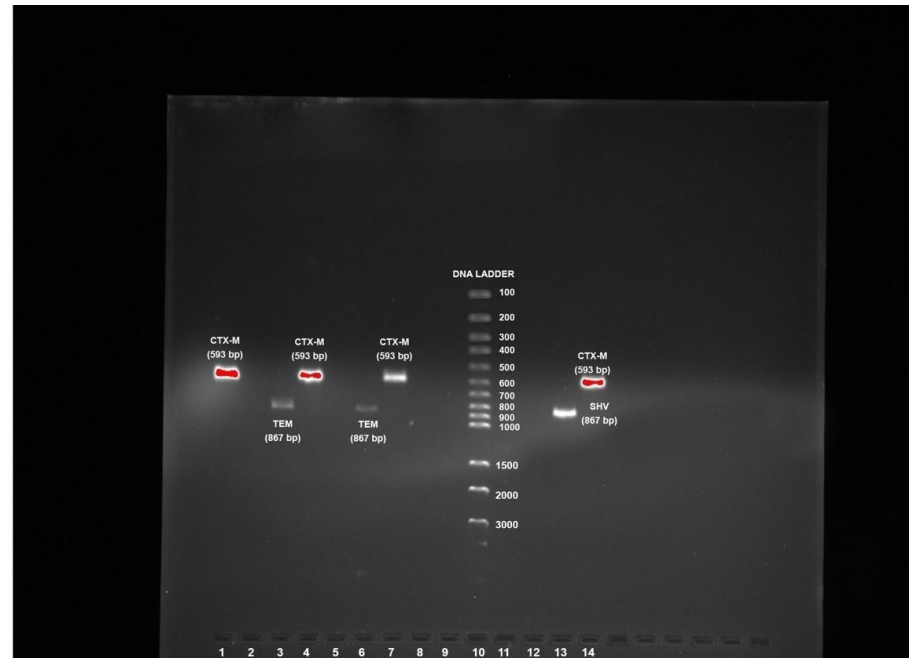
Fig. 1. Representative agarose gel of PCR amplification of TEM, SHV and CTX-M beta-lactamase genes

Table 5. Distribution of *bla*CTX-M, *bla*TEM & *bla*SHV genes among phenotypically confirmed ESBL producing isolates of *E. coli, K. pneumoniae* and *P. mirabilis*

Isolates (n= 70)	*bla*TEM	*bla*SHV	*bla*CTX-M	*bla*CTX-M+*bla*TEM	*bla*TEM+*bla*SHV	*bla*CTX-M+*bla*SHV	*bla*TEM+*bla*SHV+*bla*CTX-M	No gene
E. coli	10	3	24	18	3	2	1	2
K. pneumoniae	-	2	3	-	-	-	-	-
Proteus mirabilis	-	-	1	1	-	-	-	-
Total	10	5	28	19	3	2	1	2

In the present study the most common uropathogen was *E. coli* that is 229 out of 342(66.95%) followed by *K. pneumoniae* that is 43 out of 342(12.5%). As CLSI recommends detection of ESBL only in *E. coli*, *K. pneumoniae* and *P. mirabilis*, we included only these isolates in our study. All these isolates of *E. coli*, *K. pneumoniae* and *P. mirabilis* were screened and confirmed for ESBL production by tests described in materials and methods. *E. coli isolates were most prevalent than K. pneumoniae and P. mirabilis isolates.* This finding is in agreement with previous studies conducted by Moyo et al. [13] Tansarli et al. [14], Ophori et al. [15], Yismaw et al. [16] in which similar organisms were isolated from UTI with *E. coli* strains were isolated at high rates.

The present study found the prevalence of ESBL producing uropathogens to be 20.47% (70/342). Increasing resistance to broad spectrum cephalosporins predominantly due to production of ESBL and AmpC beta lactamases reported in different studies ranging from 21% to 64% across the globe. This wide variation could be due to difference in the population, study design, geographical distribution, differential clonal expansion, drug pressure in community and due to the wide use of these drugs empirically as they are relatively cheap and easy to administer orally [16]. The prevalence of ESBL producers was found to variable in different geographical areas and in different institutes. Previous studies from India have reported ESBL production varying from 21% to 84% [17].

A study from Coimbatore, Tamil Nadu showed the presence of ESBLs to be 40% while a study from Nagpur showed it as 50% from the urinary isolates [18].

In the present study, the prevalence of ESBL among uropathogens was 20.47% which is similar to other studies. A large survey of 1610 *E. coli* and 785 *K. pneumoniae* isolated from 31 centers in 10 European countries found that the prevalence of ESBL ranged from as low as 1.5% in Germany to as high as 39-47% in Russia, Poland and Turkey [19].

Other countries, in comparison to India, show a reduced incidence of ESBL producers. A study has shown that the prevalence of ESBL producing isolates of *E. coli* is 13.3% in Lebanon, 9.2% in Korea and 10.3% in Saudi Arabia. However, there is a constant increase in the population of ESBL producing strains in communities of other countries [20].

In the present study the most common ESBL producing uropathogen is *E. coli* (63/70) 90% followed by *Klebsiella pneumoniae* (5/70) 7.5% (Table 4). Previous studies have shown that ESBL-producing *E. coli* are the highest in India (60%), Hong Kong (48%) and Singapore (33%). In another study done by Aruna et al 2012, 96 of the 195 isolates (49.23%) were identified as *E. coli*, of which 36 isolates (49.32%) were ESBL producers. Overall, studies have shown *E. coli* to be one of the most frequently encountered drug-resistant uropathogen. The study however showed only 8 out of 29 (27.5%) *Klebsiella pneumoniae* isolates to be ESBL producers, which is otherwise the second most commonest uropathogen and ESBL producer (20). Another study in Chennai reported ESBL production among 47% *E. coli* and 37% *Klebsiella pneumoniae* in a tertiary care center [21].

In this study, 63 *E. coli* were isolated from urine samples. Amid these, all isolates were found to be sensitive to Imipenem (100%) followed by Amikacin (85.71%), Meropenem (84.13%), Gentamicin (55.56%), Levofloxacin (55.56%) and Tobramycin (55.56%). Nitrofurantoin, Imipenem were the most effective (100%) drug against urinary isolates.

Five *Klebsiella pneumoniae* were isolated in our study. All of these isolates were 100% susceptible to Imipenem, whereas 80% were susceptible to Meropenem. Resistance against Amikacin and Tobramycin was 40% whereas 100% resistance to Amoxicillin/clavulanic acid and Ampicillin/sulbactam was observed. Ninety five percent of the isolates were resistant against cephalosporins and 80% were resistant to Aztreonam, Norfloxacin, Ticarcillin, Ticarcillin/ Clavulanic acid and Tetracycline.

In this study, *Proteus* was isolated in 2 urinary samples. It was found to be 100% sensitive to Ampicillin/sulbactam, Imipenem, Meropenem, Tobramycin, Tetracycline and Ticarcillin/ clavulanic acid. In contrast to this, it was found to be 100% resistant against Cephalosporins while Aztreonam, Norfloxacin, Levofloxacin, Gentamycin, Amikacin, Ticarcillin and Amoxicillin/ clavulanic acid showed 50% resistance each.

In the present study, 63 *E. coli, 5 K. pneumoniae* and *2 P. mirabilis* isolates were further run on PCR for detection of *bla*CTX-M, *bla*TEM AND *bla*SHV genes using specific primers.

We had performed uniplex PCR as multiplexing could not be carried out because the amplicon size of both *bla*TEM and *bla*SHV was 867 bp and it would not have been possible to differentiate between the two amplicons due to their same size. We selected only genes in the families of *bla*TEM, *bla*SHV and *bla*CTX-M being highly prevalent in India [22].

*bla*CTX-M was the commonest gene which was found in 50 isolates (71.42%), out of which 45 were *E. coli*, 3 were *K. pneumoniae* and 2 were *P. mirabilis*. In India, till date, *bla*CTX-M has been reported to be the most prevalent ESBL [22] which is also the case in our study. The next most common gene was *bla*TEM, which was found in 33 isolates (47.14%), out of which 32 were *E. coli*, 0 were *K. pneumoniae* and 1 were *P. mirabilis. bla*SHV gene was the least commonest, found in only 5 isolates(7.14%), out of which 3 were *E. coli*, 2 were *K. pneumoniae*. Multiple genes were also present in many isolates, with the most common combination being, *bla*CTX-M+*bla*TEM in 19 isolates, succeeded by *bla*TEM+*bla*SHV in 3 isolates and *bla*CTX-M+*bla*SHV in 2 isolates. Co-exsistence of all three genes was only seen in 1 isolate (Table 5). Occurence or association of more than one β-lactamase within the same isolate has also been reported in many other studies [23].

In this present study we noticed the occurrence of these β-lactamase gene singly and also in various combinations, which shows their wider dissemination mostly due to mobilization of genetic elements. However, there were 2 ESBL producing *E. coli* isolates in which no gene was detected. The probable explanation may be because of the presence of other ESBL like VEB, SFO, TOHO, SME, IMI, PER etc. Similar findings were observed by studies done by Borah et al. in 2016 [24], Poovendran and Ramanathan in 2015 [25] and Bora et al. in 2014 [26] showing comparative percentage prevalence of individual ESBL genes and their coprevalence.

5. CONCLUSION

This study illustrates the moderate prevalence of ESBL among uropathogens of the family Enterbacteriaceae. This situation demands the implementation of an efficient infection control program and regular surveillance of ESBL producing uropathogens and their antimicrobial resistance pattern in order to formulate a rational antibiotic policy for the better management of these infections.

CONSENT

As per international standard informed and written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

Ethical clearance was obtained from the Institutional Ethical committee of Sharda University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prakash D, Saxena RS. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. ISRN Microbiology. 2013;2013: 1-13.
2. Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL production among *E. coli* and *Klebsiella spp*. causing urinary tract infection: A hospital based study. The Open Microbiology Journal. 2017;11:23-30.
3. Sekar U, Shanthi M. The expanding family of beta-lactamases. J Acad Clin Microbiol. 2009;11:72-86.
4. Liu WE, Chen L, Li H, Duan H, Zhang Y, Liang X, Li X, Zou M, Xu L, Hawkey PM. Novel CTX-M β-lactamase genotype distribution and spread into multiple species of Enterobacteriaceae in Changsha, Southern China. Journal of Antimicrobial Chemotherapy. 2009;63:895-900.
5. Shanthi J, Kayathri S. Incidence, distribution and antibiogram of uropathogens isolated from patients with urinary tract infections. Adv Applied Sci Res. 2012;3:3410-4.
6. Banerjee S, Padmashri VP. The study of urinary tract infections and antibiogram of uropathogens in and around Ahmadnagar, Maharashtra. Int J Infect Dis. 2011;9:1528-8366.
7. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of extended spectrum beta-lactamase producing uropathogens and their antibiotic

resistance profile in patients visiting a tertiary care hospital in central India: Implications on empiric therapy. Indian Journal of Pathology and Microbiology. 2014;57:407-12.

8. Mukherjee M, Basu S, Mukherjee SK, Majumder M. Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata, India. Journal of Clinical and Diagnostic Research: JCDR. 2013;7:449-53.

9. Subashini J, Kannabiran K. Screening and identification of extended spectrum β-lactamase (ESBL) pathogens in urine sample of UTI patient's. Trop Med Surg. 2013;1:2-8.

10. Ali AM, Abbasi S, Ahmed M. Frequency of extended spectrum beta lactamase (ESBL) producing nosocomial isolates in a tertiary care hospital in Rawalpindi. A Journal of Army Medical Corps. 2009;3:9630-48.

11. Marchaim D, Chopra T, Bhargava A, Bogan C, Dhar S, Hayakawa K, Pogue JM, Bheemreddy S, Blunden C, Shango M, Swan J. Recent exposure to antimicrobials and carbapenem-resistant Entero-bacteriaceae: The role of antimicrobial stewardship. Infection Control & Hospital Epidemiology. 2012;33:817-30.

12. Sharma J, Sharma M, Ray P. Detection of TEM & SHV genes in *Escherichia coli & Klebsiella pneumoniae* isolates in a tertiary care hospital from India. Indian Journal of Medical Research. 2010;32:332-7.

13. Moyo SJ, Aboud S, Kasubi M, Lyamuya EF, Maselle SY. Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary Hospital in Tanzania. BMC Research Notes. 2010; 3:348.

14. Tansarli GS, Poulikakos P, Kapaskelis A, Falagas ME. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among Enterobacteriaceae in Africa: Evaluation of the evidence—systematic review. Journal of Antimicrobial Chemotherapy. 2014;69:1177-84.

15. Ophori E, Imade P, Johnny E. Asymptomatic bacteriuria in patients with type-2 diabetes mellitus. African Journal of Bacteriology Research. 2010;2:14-7.

16. Yismaw G, Asrat D, Woldeamanuel Y, Unakal CG. Urinary Tract Infection: Bacterial etiologies, drug resistance profile and associated risk factors in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia. European Journal of Experimental Biology. 2012;2:889-98.

17. Naz R, Hussain A, Khan S, Farooqui MK, Malik A. Antibiotic susceptibility pattern of ESBL producing uropathogens. Int J Curr Microbiol App Sci. 2016;5:825-31.

18. Arora S, Munshi N. Comparative assessment of antibiotic susceptibility pattern of gram negative pathogens isolated from intensive care unit patients in Pune. Microbiology Research Journal International. 2015;18:1-9.

19. Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. Indian Journal of Community Medicine: Official Publication of Indian Association of Preventive & Social Medicine. 2012;37:39.

20. Agrawal P, Ghosh A, Kumar S, Basu B, Kapila K. Prevalence of extended-spectrum β-lactamases among *Escherichia coli and Klebsiella pneumoniae* isolates in a tertiary care hospital. Indian Journal of Pathology and Microbiology. 2008;51:139-42.

21. Aruna K, Mobashshera T. Prevalence of extended spectrum beta-lactamase production among uropathogens in south Mumbai and its antibiogram pattern. EXCLI Journal. 2012;11:363.

22. Gururajan G, Kaliyaperumal KA, Ramasamy B. Prevalence of extended spectrum beta lactamases in uropathogenic *Escherichia coli and Klebsiella species* in a Chennai suburban tertiary care hospital and its antibiogram pattern. Research Journal of Microbiology. 2011;6:796.

23. Ensor V, Shahid M, Evans J, Hawkey P. Occurrence, prevalence and genetic environment of CTX-M β-lactamases in Enterobacteriaceae from Indian hospitals. Journal of Antimicrobial Chemotherapy. 2006;58:1260-3.

24. Borah VV, Saikia KK, Chandra P, Hazarika NK, Chakravarty R. New Delhi metallo-β-lactamase and extended spectrum β-lactamases co-producing isolates are high in community-acquired urinary infections in Assam as detected by a novel multiplex polymerase chain

reaction assay. Indian J Med Microbiol. 2016;34:173-82.

25. Poovendran P, Ramanathan N. Molecular Characterization of *bla*CTX-M, *bla*TEM, *bla*SHV beta Lactamase produced by uropathogenic *Escherichia coli* isolates. International Journal of Microbiological Research. 2015;6:67-73.

26. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of blaTEM, blaSHV and blaCTX-M genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian Journal of Pathology and Microbiology. 2014;57:249.

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