Original Article

Detection and Antibiogram of Extended Spectrum Beta Lactamase (ESBL) producing Escherichia coli isolated from urine samples at Goverment Medical College, Kota, Rajasthan

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***Abstract*: The spread of ExtendedSpectrum Beta-Lactamases(ESBLs) producing bacteria has become strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required.The therapeutic options for the infections which are caused by these organisms have also become increasingly limited.ESBLs producing strains have emerged as a significant challengeto counter with present antibiotics.The aims of this study were to detect prevalence of ESBL production among E. coli isolates in urine samples and to detect their antibiotic susceptibility pattern at Govt. Medical College, Kota.This study demonstrate the importance of regular review of empirical antibiotic therapy for UTI in view of the evolving resistance of ESBL producing E. coli to commonly used antimicrobial agents.**

A total of 500 consecutive, nonrepetitiveE. coli isolates were studied.These isolates were confirmed for ESBL production by the double disc synergy test (DDST) and the phenotypic confirmatory disc diffusion test (PCDDT) and Twenty-five randomly selected isolates were further confirmed by the E-test ESBL strip randomly.Out of 500 E. coli isolate, 310(62%) were ESBL producers and 190(38%) were Non ESBL producers.Out of the 310 isolates which were tested,228(73.55%)were found to be ESBL producers by PCDDT,193(62.26%)were found to be ESBL producers by DDSTand19(76%), were found to be ESBL producers by the E-test ESBL stripwhich showed a highly significant correlation with PCDDT.As results showed that there was a high prevalence of ESBL production in our setup so, it is essential to report the ESBL production along with the routine sensitivity reports, which will help the clinician in selection of proper antibiotics.

***Keywords*: ESBL, UTI, Escherichia Coli, DDST, PCDDT, E-Test**

INTRODUCTION:

UTI remains one of the most common bacterial infections and second most common infectious disease in the communitypractice. Approximately, about 150 million people were diagnosed with UTI each year. In present scenario, the essenceof antimicrobial drug resistance of major uropathogens hasposed a global threat[2]. Of the various uropathogens, the most common organisms are E. Coli, Enterococcus fecalis,Staphylococcus aureus,Enterococci spp and Klebsiellaspp.[14,18]But most of these organisms have developed resistance to antimicrobial agents. Inappropriate and empirical usage of wide spectrum antibiotics, insufficient hygiene, immune suppression and prolonged hospitalization are some of the major aetiological factors that elevate the chances of infection.[14,18] Extended spectrum beta lactamases (ESBLs) producing bacteria are typically resistant topenicillins, first and second generation cephalosporins as well as the third generation oxyiminocephalosporins (e.g., Ceftazidime, Ceftriaxone) and Monobactam (Aztreonam) except cephamycinsandcarbapenems[19].First isolated in 1983 in Germany, ESBLs spread rapidly to Europe,United States and Asia and are now found all over the world[26]. Since ESBL positive isolates show false susceptibility to extended spectrum cephalosporins in standard disk diffusion tests[15]. It is difficult to reliably detect ESBL production by the routine disk diffusion techniques. Specific detection methods such as double disk methods recommended by CLSI (2016) have to be adopted[6].ESBLs are inhibited by βlactamase inhibitors like clavulanic acid, sulbactam and tazobactam and this property of specific inhibition can be utilized for the detection and confirmation of ESBLs.Laboratory detection of ESBL production can be problematic[8]but its detection is important because its spread within the hospital may lead to endemic occurrence and repeated outbreaks from time to time. Another important implication of ESBL production is failure to treat ESBL producing organisms because of limited therapeutic choices[29].

AIMS & OBJECTIVE:

Theaims of this study were to detect prevalence of ESBL production among E. coli isolates in urine samples and to detect their antibiotic susceptibility pattern at Govt. Medical College, Kota.This study demonstrate the importance of regular review of empirical antibiotic therapy for UTI in view of the evolving resistance of ESBL producing E. coli to commonly used antimicrobial agents.This study is conducted to aid in early detection and treatment of ESBL producing E. coli and its prevention in community.

MATERIAL AND METHODS:

The present study was conducted in the clinical microbiology laboratory of the Govt. Medical College, Kota from period of 1st January, 2018 to 31th December 2018 to evaluateprevalence of ESBL production among E. coli isolates in urine samples and to detect their antibiotic susceptibility pattern.A total of 500consecutive, non-repetitive E. coli isolates were studied during this period.All samples were cultured on MacConkey Agar and Blood Agar and incubated at 37 °C for 24–48 hr.The isolateswere identified and confirmed using standard microbiological methods including Gram staining, colonial morphology on media, growth on selective media, lactose and mannitol fermentation, H2S production, catalase, oxidase, coagulase, indole and citrate utilization, and urease test. Antibiotic sensitivity testing of all E. coli isolates was performed on Muller Hinton agar (MHA) plates by Kirby-Bauer disk diffusion technique with guidelines established by the Clinical Laboratory Standards Institute (CLSI). All E.coli isolates were included in the study.Antibiotic Susceptibilitytestinto various antimicrobial agents was determined by Disc diffusion method of Kirby Bauer on MHA (Hi-media) as described by the Clinical Laboratory and Standard Institute (CLSI) guidelines[6].The following antibiotic discs (drug concentration in µg) were used: Amikacin (30), Ceftazidime (30), Cefotaxime (30), Ceftriaxone (30), Cotrimoxazole (25), Imipenem (10), Ciprofloxacin (5), Cefoperozone–sulbactam(30), Piperacillin–tazobactam (30), Nitrofurantoin (30),Piperacillin (100), Amoxyclav (20/10), Azithromycin (15).The diameter of zone of inhibition was measured and interpreted according to CLSI guidelines (2016)[6].

Test forESBL Production:-

A. Screening Test-ESBL detection was done for all isolates according to latest CLSI criteria**.** All E.coli isolates were subjected to screening tests by using Cefotaxime (30 µg) and Ceftriaxone (30 µg) discs. Those isolates with Cefotaxime zone <=27 mm and Ceftriaxone zone <=25 mm were considered as ESBL producer and then those isolates were subjected to confirmatory tests.

B.Confirmatory Test:-

1.Double Disc Synergy Test (DDST): Accordingto the British Society for Antimicrobial Chemotherapy (BSAC) guidelines[17]isolates which were presumed to be ESBL producers on the basis of the screening test results, were picked up and emulsified in saline to a 0.5 McFarland's turbidity standard. Discs of Ceftazidime (30 µg), Cefotaxime (30 µg) and Amoxyclav (20 µg Amoxycillin and 10 µg Clavulanic acid) were placed at a distance of 20 mm from center to centerin a straight line, with the Amoxyclav disc in the middle on a plate of MHA being inoculated with the test strain. The plates were incubated at 37 °C aerobically overnight. Isolates which showed an enhancement of the zone of inhibition as greater than 5 mm on the Amoxyclavside of the disc as compared to that which was seen on the side without Amoxyclav, were confirmed as ESBL producers[23].(Figure 1).

Figure 1:Double disc Synergy Test (DDST)-Organism showing enhanced zone of inhibition between ceftazidime and cefotaxime and amoxicillin/clavulanic acid containing disc indicating ESBL production.

2.CLSI Confirmatory Test (PCDDT-Phenotypic Confirmatory Double Disc Test):

For this test disc of Ceftazidime (30 µg) and Ceftazidimeplus Clavulanic acid (30/10 µg) were placed on MHA and incubated. An increase of > 5 mm in the zone of inhibition of the combination discs in comparison to the Ceftazidime disc alone was considered to be a marker for ESBL production. E. coli ATCC25922 and K. pneumonia ATCC 700603 wereused as negative and positive controls,respectively[23]. (Figure 2)

Figure 2:PCDDT(Phelnotiypic Confirmatory Double Disk Diffusion Test)-A > 5 mm increase in zone of inhibition for ceftazidime/clavulanic acid (CAC) versus its zone diameter when tested alone by ceftazidime confirmed an ESBL producing organism.

3.E-Test: The E-test ESBL strips (AB Biodisk, Sweden) carry two gradients, ceftazidime (0.5-32 µg/ml) on the one end and ceftazidime plus clavulanic acid (0.064-4 µg/ml) in a different concentration gradient on the other end, along with a fixed concentration of clavulanic acid (4 µg/ml). A lawn culture of the test organism was plated on Mueller Hinton Agar (MHA) on which the E-test ESBL strip was placed on the centre of the plate. The plates were incubated aerobically at 37oC for 16-18 hours. The MIC was interpreted at the point of intersection of the inhibition eclipse with the E-test strip edge. The presence of ESBL was confirmed by the appearance of a phantom zone or by the deformation of the TZ eclipse or when the ceftazidime MIC was reduced by >3 log2 dilutions (ratio TZ/TZL, >8) in the presence of clavulanic acid as per the manufacturer's guidelines[1]. (Figure 3)

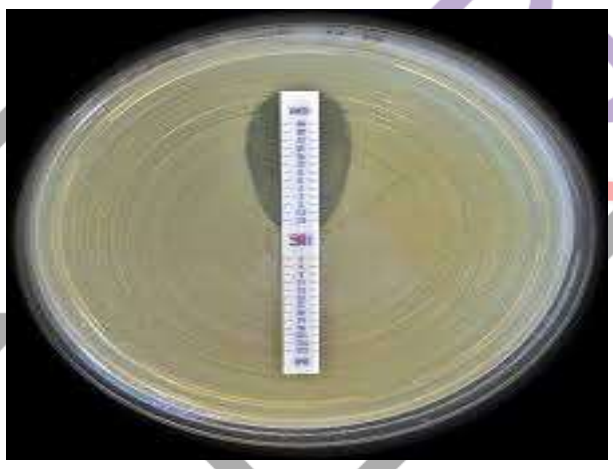


Figure 3:Epsilon test(E-test):-E-test ESBL strip showing ESBL positive organism showing ceftazidime (TZ) MIC is reduced by >3 log2 dilutions (ratio TZ/ TZL, >8) in the presence of clavulanic acid.

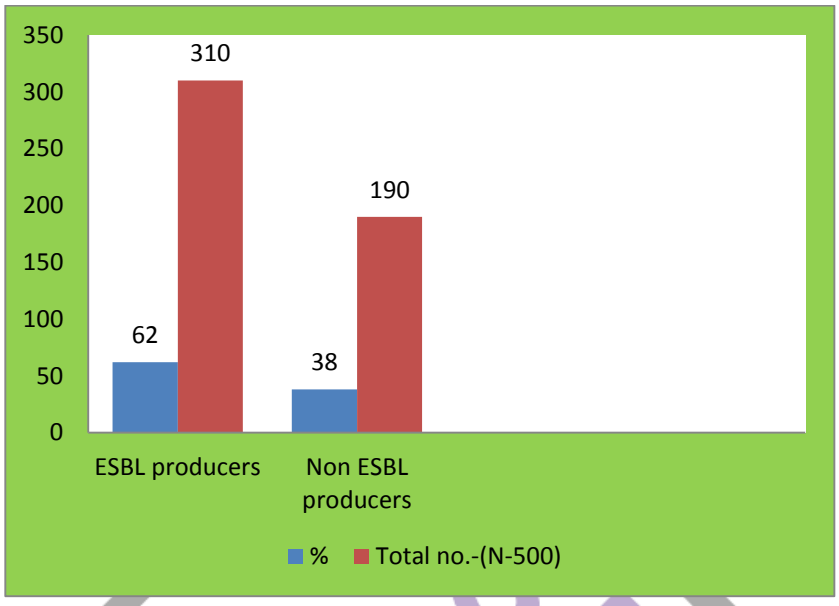
Quality control:β-lactamase negative Escherichia coli ATCC 25922 was used as the negative control and ESBL-producing Klebsiellapneumoniae ATCC 700603 was used as the positivecontrol throughout the study[6]. Statistical analysis: Statistical analysis was performed by the Chi square test and a p value of less than 0.05 was considered as statistically significant.

RESULT:

A total of 500 consecutive, nonrepetitive E. coli isolates were studied. Antimicrobial susceptibility test was performed on Muller Hinton agar plates using Kirby Bauer disk diffusion method. ESBL detection was done for all isolates according to latest CLSI criteria. These isolates were confirmed for ESBL production by the double disc synergy test (DDST) and the phenotypic confirmatory disc diffusion test (PCDDT) and they were further confirmed by the E-test ESBL strip randomly. Twenty-five randomly selected isolates were confirmed by the E-test ESBL strip.Out of 500 E. coli isolate, 310(62%) were ESBL producers and 190(38%) were Non ESBL producers.

Table:1ESBL producers among E. Coliisolates from Urine samples

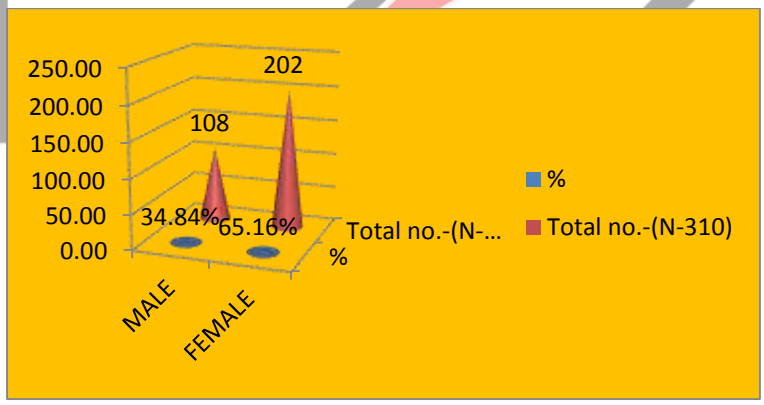
ESBL producers among E. coli isolates	ESBL producers		Non ESBL producers	
	Number	%	Number	%
500	310	62	190	38



Graph:2 ESBL producers among E. Coli isolates from Urine samples

Table:2Sex wise distribution of ESBL positive E. Coliisolates from urine samples

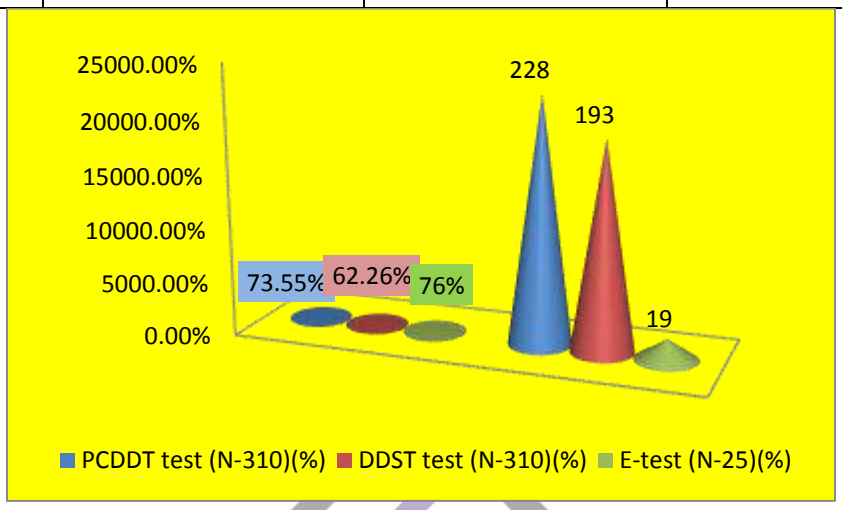
Total number of ESBL isolates	ESBL producers in males		ESBL producers in females	
	Number	%	Number	%
310	108	34.84%	202	65.16%



Graph:2 Sex wise distribution of ESBL positive E. Coli isolates from urine samples

Table:3ESBL Producing E. coli from Urine samples by Various Confirmatory Test.

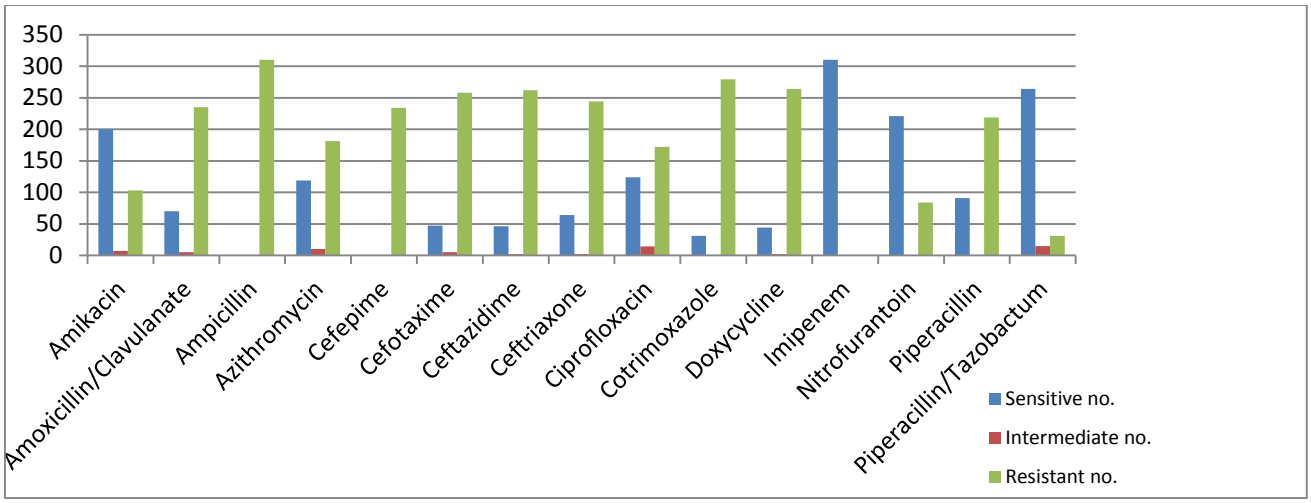
Organism (N-310)	PCDDT test (N-310)(%)	DDST test (N-310)(%)	E-test (N-25)(%)
Escherichia coli (310)	228 (73.55%)	193 (62.26%)	19 (76%)



Graph:3 ESBL Producing E. Coli in Urine samples by Various Confirmatory Test.

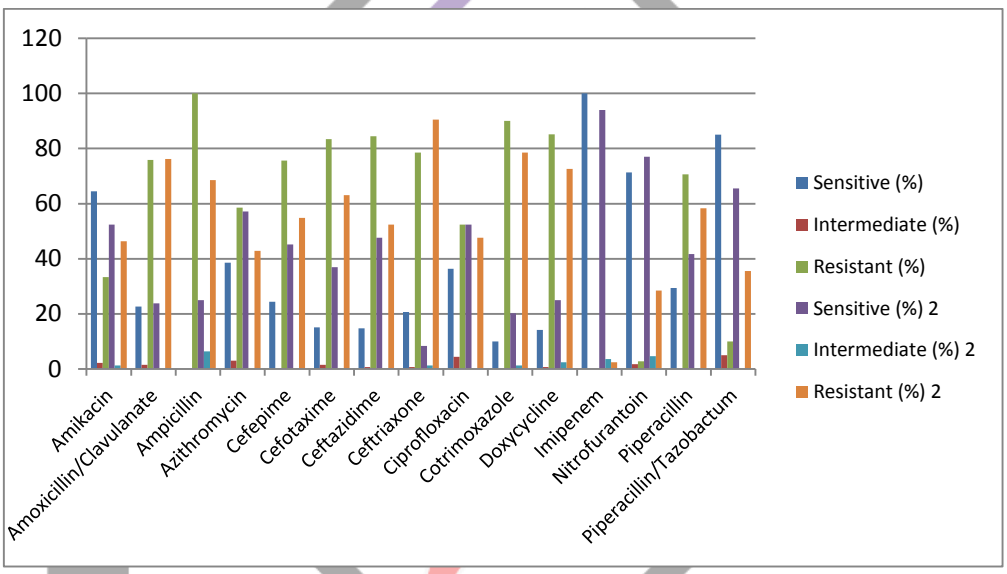
Table:4Antibiotic susceptibility pattern of ESBL producing E. Coliisolates from Urine samples

Antibiotics(N-500)	ESBL producer(N-310)			Non ESBL producer(N-190)		
	Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	200 (64.5%)	7(2.3%)	103(33.2%)	100(52.6%)	2(1.1%)	88(46.3%)
Amoxicillin/Clavulanate	70(22.6%)	5(1.6%)	235(75.8%)	45(23.7%)	0(0%)	145(76.3%)
Ampicillin	0(0%)	0(0%)	310(100%)	48(25.3%)	12(6.3%)	130(68.4%)
Azithromycin	119(38.4%)	10(3.2%)	181(58.4%)	108(56.8%)	0(0%)	82(43.2%)
Cefepime	76(24.5%)	0(0%)	234(75.5%)	86(45.3%)	0(0%)	104(54.7%)
Cefotaxime	47(15.2%)	5(1.6%)	258(83.2%)	70(36.8%)	0(0%)	120(63.2%)
Ceftazidime	46(14.9%)	2(0.6%)	262(84.5%)	90(47.4%)	0(0%)	100(52.6%)
Ceftriaxone	64(20.7%)	2(0.6%)	244(78.7%)	16(8.4%)	2(1.1%)	172(90.5%)
Ciprofloxacin	124(40%)	14(4.5%)	172(55.5%)	100(52.6%)	0(0%)	90(47.4%)
Cotrimoxazole	31(10%)	0(0%)	279(90%)	38(20.%)	2(1.1%)	150(78.9%)
Doxycycline	44(14.2%)	2(0.6%)	264(85.2%)	48(25.3%)	4(2.1%)	138(72.6%)
Imipenem	310(100%)	0(0%)	0(0%)	178(93.7%)	7(3.7%)	5(2.6%)
Nitrofurantoin	221(71.3%)	5(1.6%)	84(27.1%)	146(76.9%)	9(4.7%)	35(18.4%)
Piperacillin	91(29.4 %)	0(0%)	219(70.6 %)	79(41.6%)	0(0%)	111(58.4%)
Piperacillin/Tazobactum	264(85.2%)	15(4.8%)	31(10%)	124(65.3%)	0(0%)	66(34.7%)



Graph:4 Antibiotic susceptibility pattern in ESBL producers found in number of cases.

(Total no.-(N-500), ESBL producer (N-310))



Graph:5Comparative study ofAntibiogram in both ESBL producers and non ESBL producers
(Total no. -(N-500) 1.ESBL producer(N-310) 2. Non ESBL producer(N-190))

Table1 and Graph1 showthe number and percentage of ESBL and Non ESBL producing *E. coli* isolates.Out of 500 *E. coli* isolate, 310(62%) are ESBL producers and 190(38%) are Non ESBL producers.Table2 and Graph2 shows the number and percentage of ESBL producing *E. coli* isolates in males and females. Out of 310 ESBL producing *E. coli* isolates, 108(34.84%) were found in males and 202 (65.16%) in females.Table3 and Graph3 shows theESBL Producing E. coli from Urine samples by Various Confirmatory Test. Out of 310 ESBL producing *E. coli* isolates, 228(73.55%)were found to be ESBL producers by PCDDT and 193(62.26%) were found to be ESBL producers by DDST and out of 25 samples which were further confirmed by the E-test ESBL strip,19(76%)were found to be ESBL producers. Table4, Graph4 and Graph 5 shows the antibiotic susceptibility pattern of ESBL producing *E. coli* isolates from Urine samples. The isolates were highly susceptible to Imipenem (100%), Piperacillin/Tazobactum(85.2%),Nitrofurantoin(71.3%) and amikacin (64.5%), in the decreasing order, were the most active and reliable agents for the treatment of the infections which were caused by the ESBL producing organisms and were resistant to Ampicillin (100%),Cotrimoxazole(90%)and Doxycycline(85.2%) drugs. There is a scarcity of information on ESBL prevalence particularly in developing countries like India, hence present study was conducted for early detection & prevention of ESBL producer organisms in UTI patients.

DISCUSSION:

The present study was conducted in the Department of Microbiology & Immunology,Govt. Medical College,Kota. The patients included in the study were of OPD and IPD from all associated hospitals ofGovt. Medical College, Kota reporting for diagnosis of UTI in Department of Microbiology from 1st January, 2018to 31th December 2018 to evaluate prevalence of ESBL production among E. coli isolates in urine samples and to detect their antibiotic susceptibility pattern. The samples were collected and processed as per routine recommended methods of technical guidelines.In all, 500 patients were screened. The observations were made with reference to age, sex, constitutional symptoms, various risk groups and investigations. ESBL prevalence varies with geographical distribution and social characteristic of population groups.Prevalence of ESBL varies across continents, countries and hospitals as demonstrated by various studies. Urinary tract infections are the most common bacterial infection[11]. Escherichia coliis the most common organism causing urinary tract infection (UTI). Extended spectrum beta - lactamases (ESBLs) are on the rise in hospital settings across the globe[27].The antimicrobial resistance patterns of organisms-causing UTI are changing over the years, including resistance due to ESBL producing pathogens. Correct identification of ESBL producing organisms in due time is necessary not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organism[21].Our study was a step ahead in this direction with the purpose of providing authentic scientific data based on the affected population attending our hospitals.ESBL-positiveE. coli which indicates great variation in ESBL positivity throughout India and world. (Table 5)

Table:5Various studies showing the prevalence of ESBL producing E. Coli isolated from UTI

S. No	Studies	Place	Year	Preva-lence (%)	S. No	Studies	Place	Year	Preva - lence (%)
1	PurvaMathur et al.[18]	New Delhi	2002	68%	9	Manoharan et al.[17]	Vellore	2011	78%
2	C Rodrigues et al.[20]	Mumbai	2004	53%	10	Dissanayake et al.[9]	Srilanka	2012	29%
3	S Singhal et al[23]	Gurgaon	2005	64%	11	Chaudhary NK et al.[4]	Mysore	2013	54.5%
4	Kumar et al.[15]	Hyderabad	2006	24.8%	12	Dugal S et al.[10]	Mumbai	2013	24.4%
5	Hawser SP et al.[13]	Asia Pacific Region	2007	79%	13	Datta P et al.[7]	Nepal	2014	21.4%
6	Sridhar Rao et al.[24]	Karnataka	2008	62.9%	14	Sapna et al.[5]	Dehradun	2015	53%
7	Goyal et al.[12]	Lucknow	2009	63.6%	15	Singh N et al.[22]	Bhubaneswar	2016	82.6%
8	MaheshE et al.[16]	Bengaluru	2010	56.2%	16	Present Study	Kota	2018	62%

Our findings were in accordance with various authors as above.Comparison of studies conducted by other researchers showed slight variations in prevalence of ESBL prevalence.This study agrees with previous studies in other countries as well as India.Previous studies from India have reported the prevalence of the ESBL producers to be 6.6 to 68%. The our results were correlated with the Previous studies in India byPurvaMathur et al.[18], S Singhal et al[23], Sridhar Rao et al.[24], Goyal*et al*.[12], Mahesh E et al.[16], Chaudhary NK et al.[4] and Sapna et al.[5] in which ESBL prevalence were 68%,64%,62.9%,63.6%,56.2%,54.5% and 53% respectively whereas in our study it was 62%. The our results were not correlated with the Previous studies in other countries studies by Hawser SP et al.[13] at Asia Pacific Region,Dissanayake et al[9] at Srilankaand andDatta P et al.[7] at Nepal in which ESBL prevalence were 79%, 29% and 21.4% respectively. The antibiotic susceptibility pattern of ESBL producing E. coli in our study was slightly different from other researcher's studies in sensitive and resistant pattern of antibiotics.

SUMMARY AND CONCLUSION:

The emergence and rapid spread of ESBL producing bacteria has become a worldwide problem indicating that continuous monitoring systems and effective infection control measures are absolutely required In conclusion, the present study found 62 % ESBL producing *E. coli* isolate in UTI. Most of the ESBL producing *E. coli* isolates were multidrug resistant making available therapeutic choices limited. Our study also demonstrates the importance of regular review of empirical antibiotic therapy for UTI in view of the evolving resistance of ESBL producing *E. coli* to commonly used agents.Clinicians must depend on more laboratory guidance, while laboratories must provide resistance pattern data for optimal patient management more accurately. Additionally, improper antimicrobial use and strengthened infection control measures are required to prevent the spread and reduce the emergence of antibiotic resistance.It is essential to report ESBL production along with the routine susceptibility testing, which will help the clinician in prescribing proper antibiotics. To reduce the prevalence of antimicrobial resistant pathogens, including ESBL-producing E.coli, effective infection control measures such as hand washing and proper hospital hygiene are required.There is a need to formulate strategies to detect and prevent the emergence of ESBL producing strains for the effective treatment of infections which are caused by them.A committee must be formed at all hospitals, which should provide guidelines for the judicious use of antibiotics and should formulate policies which will help in minimizing the emergence of resistant bacteria among the patients.

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