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Antibiotic Resistance Pattern Of *E. Coli* And *Klebsiella* Species Producing Extended Spectrum Beta Lactamase (ESBL) In Urinary Isolates: A Cross Sectional Study.

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ABSTRACT

Urinary tract infections (UTIs) are one of the most common infectious diseases encountered in the clinical practice. Even if wide spread availability of antibiotics, UTI remains to be one of the most common infectious diseases diagnosed. Further world wide data shows that there is an increasing resistance among UTI pathogens to routine antibiotics. Resistance have emerged even to newer and more potent antimicrobial agents. Therefore, the aim of the present study is to determine the incidence of extended spectrum beta – lactamase in urinary isolates of *Escherichia coli* (*E. coli*) and Klebsiella species. Urine samples from patients suspected to have UTI (Total of 542) were processed. Escherichia coli and Klebsiella species isolated in significant numbers were included. Standard microbiological procedures and AST was done according to CLSI guidelines were followed for their identification. The isolates were tested for susceptibility to third generation cephalosporins and then for Double disc synergy test (DDST) & Phenotypic confirmatory disc diffusion test (PCDDT). Number of urinary isolates were 175 over a study period of six months. *E.coli* was the predominant isolate 101 (57.7%) followed by Klebsiella species 49 (28.33%) A total of 78 *E.coli and* Klebsiella species resistant to third generation cephalosporins were tested for ESBL production by two methods. ESBL production was seen in 18(10.28%) isolates. Routine ESBL testing of all uropathogens along with conventional antimicrobial susceptibility is recommended for all cases as this will help in the proper treatment of the patients and also prevent further development of bacterial drug resistance.

Keywords: Urinary tract infections, antimicrobial resistance, ESBLs

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INTRODUCTION

In spite of the wide spread availability of antibiotics, UTI remains to be one of the most common infectious diseases diagnosed [1]. World wide data shows that there is an increasing resistance among UTI pathogens to conventional drugs. Resistance have emerged even to newer more potent antimicrobial agents [1, 2].

Escherichia coli and *Klebsiella* species account for most of the cases of community as well as hospital acquired UTI and they have ability to produce ESBL in large quantities [2, 3]. In India, there have been several reports on the prevalence of ESBLs in recent years. ESBL production has been observed in large percentage of urinary isolates and majority of ESBL producing strains worldwide are of Klebsiella spp. & *E. coli* [2, 3].

Extensive use of third generation cephalosporins has contributed to the evolution of extended spectrum beta lactamases (ESBLs) [2]. ESBL's are defined as β lactamases capable of hydrolyzing oxyiminocephalosporins and are inhibited by β lactamase inhibitors [1].

These plasmid mediated groups of enzymes are the product of point mutations at the active site of TEM, SHV, and OXA enzymes [4, 5]. Early identification of ESBL production is becoming increasingly important in terms of appropriate treatment and effective infection control in hospitals. With reports on high prevalence of ESBL production in the members of *Enterobacteriaceae* family and paucity of information especially on uropathogens from our country [2], the present study was undertaken to find out prevalence of ESBL producers in urinary isolates of *E.coli* and *Klebsiella* species and to study their antimicrobial susceptibility pattern.

MATERIAL AND METHODS

It was a cross sectional, observational study conducted from 1st August 2021 to 31st March 2022.Total 542 urine samples from symptomatic UTI cases (both IPD and OPD) received in the Department of Microbiology, Dr. Vitthalarao VikhePatil Foundation's Medical college Ahmednagar, were processed.

Most of the samples were Clean catch midstream urine sample (CCMSU) and others included aseptically collected catheterized urine sample and suprapubic aspirates. Urine samples were inoculated on 5% sheep blood agar and Mac-Conkey's agar and incubated at 37° C for 24 hours. Semi quantitative urine culture by using a calibrated loop was done on blood agar and Mac- Conkey's agar plates. We followed Kass criteria which depicts, significant monomicrobic bacteriuria was defined as culture of a single bacterial species from the urine samples at a concentration of > 10^5 CFU/ml [4, 6, 7]. Microorganisms were identified by standard biochemical procedures [4, 8, 9]. All *Escherichia coli* and Klebsiella species isolated in significant numbers were included in the study for detection of ESBL production.

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique using commercially available disc procured from Hi-media, according to CLSI guidelines. The antibiotics tested were Co-trimoxazole (25µg), Ampicillin (10µg), Amoxycillin-clavulanic acid (20µg), Gentamycin (30µg), Ciprofloxacin (5µg), lomefloxacin(10µg), Norfloxacin(10µg), Nitrofurontoin(300µg), Piperacilline-tazobactem (100/10µg), Cefazolin(30µg), Amikacin (30µg), Tetracyline (30µg) and Imipenem(10µg).

Criteria for selection of ESBL producing strains

All *Escherichia coli* and Klebsiella species having zone size of <22mm for ceftazidime (third generation cephalosporins) by using standard disc diffusion method were selected as suspicious for ESBL production as recommended by CLSI guidelines.

These potential ESBL producing strains were further tested by two methods.

Modified Double disc synergy test (DDST) [4, 10]

Lawn culture of test strain on Mueller Hinton agar (Himedia) after adjusting turbidity to McFarland 0.5 standard was exposed to discs of cefotaxime (30 mcg), ceftazidime (30 mcg), and the disc of amoxiclav (20ug amoxicillin / 10ug clavulanic acid). The cefotaxime and ceftazidime disc were placed 16mm center to center from amoxiclav disc. Plate was incubated at 37^0 C overnight. The test isolate was considered to produce ESBL, if the zone size around the cefotaxime and ceftazidime disc increased towards the Amoxicillin-clavulanic acid disc. (Fig-1)

Phenotypic confirmatory disc diffusion test (PCDDT) [4, 11]

Lawn culture of test isolates was done on Muller Hinton agar. Antibiotic used were Ceftazidime (30mcg) and combination of ceftazidime–clavulanic acid (30mcg). Discs were placed opposite to each other in Muller Hinton agar plate and incubated overnight at 37^0 C. Next day zone of inhibition around ceftazidime and ceftazidime clavulanic acid was measured. If zone of inhibition around ceftazidime-clavulanic acid is increased by more than 5mm than that of ceftazidime disc alone. It is confirmed that isolate was ESBL producer.

E.coli ATCC 25922 was used as ESBL negative control and *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control.

RESULTS

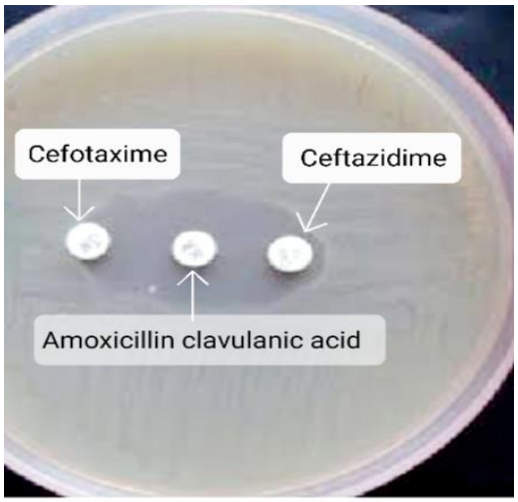
A total of 175 uropathogens were isolated from symptomatic UTI patients. *E.coli* was the predominant isolate 101 (57.7%) followed by *K.pneumoniae* 49 (28.3%). Other isolates are shown in Table No.1

Antibiotic resistance pattern showed *E.coli* to be maximum resistant to ampicilline [78 (77.22%)] followed by Co-trimoxazole [72(71.28%)]. Most effective antibiotic against *E.coli* was nitrofurantoin [37(36.63%)]. For *K.pneumoniae*, Nitrofurantoin, Piperacillin+ Tazobactum and Lomefloxacin were found to be effective (Table No.2). E.coli (n=49) and *K.pneumoniae* (n=29), resistant to third generation cephalosporins (ceftazidime) were tested for ESBL production by two methods.(Table No. 2).

ESBL production by 'Phenotypic confirmatory disc diffusion test' (PCDDT) was seen in 19 (10.85%) isolates out of a total of 86 tested isolates. Maximum ESBL production was seen in *E.coli* (5.7%) followed by *K.pneumoniae* (4.5%) (Table No.3)

DDST failed to detect ESBLs in three isolates of *E.coli* and two isolates of Klebsiella *pneumoniae*. The ESBL positive strains were isolated from almost all the wards of the hospital, mainly from the surgical wards.

Modified Double disc synergy test (DDST)



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Phenotypic confirmatory disc diffusion test (PCDDT)

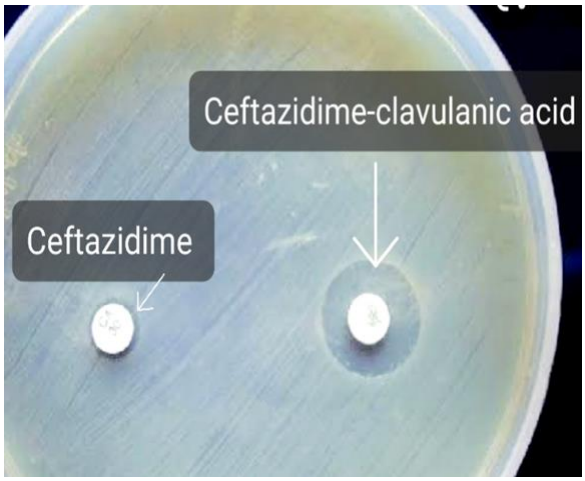

Table 1: Bacterial isolates in symptomatic UTI cases

S.No	Bacterial isolates	No. (N=175)	Percentage
1	E. coli	101	57.7
2	Klebsiella pneumoniae	49	28.33
3	Pseudomonas aeruginosa	15	8.5
4	Proteus mirabilis	04	2.28
5	Acenatobacter spp	01	0.57
6	Staphylococcus aureus	01	0.57
7	Enterococcus feacalis	04	2.28
	Total	175	

Table 2: Antibiotic resistant pattern of gram-negative urinary isolates from symptomatic UTI cases

Antibiotics	E. coli	Klebsiella pneumonia
	(N=101)	(N=49)
Ampicilline	73	36
Amoxycillin/ clavulanic acid	68	27
Piperacillin+ Tazobactum	48	24
Co-trimoxazole	72	34
Ciprofloxacin	69	39
Norfloxacin	58	32
Lomefloxacin	52	25
Nitrofurantoin	37	23
Gentamycin	69	39
Amikacin	65	28
Imipenem	63	29

Table 3: Comparison of Modified DDST and PCT for ESBL detection in gram negative isolates
(N=175)

Sr.No.	Bacterial isolates	No of isolates resistant to 3rd Generation Cephalosporins	Modified DDST	PCT/PCDDT
1	E. coli	49 (28%)	7 (4%)	10 (5.7%)
2	K. pneumoniae	29 (16.57%)	6 (3.4%)	8 (4.5%)
	Total	78 (44.57%)	13(7%)	18(10.28%)

DISCUSSION

Microorganisms responsible for urinary tract (UTI) such as E.coli and Klebsiella spp. have the ability to produce ESBL's in large quantities. These enzymes are plasmid borne and confer multiple drug resistance making urinary tract infection difficult to treat [1]. In India, there have been several reports on the prevalence of ESBLs in recent years. ESBL production has been observed in large percentage of urinary isolates and majority of ESBL producing strains worldwide are of Klebsiella spp. & *E. coli* [2, 1]. In our study *E.coli* was the predominant organism isolated from urine sample followed by *K.pneumoniae*. This is in concordance with the studies of other workers like Varma N et al [13], Gupta V et al [14], Abu Hena et al [15], and Kulkarni et al [2]. Our findings, however contrast with the study of Bajaj et al [22] where *Klebsiella species* predominated *E.coli*.

Uropathogenic strains of *E.coli* are believed to display a variety of virulence properties that help them to colonize the host mucosal surfaces and circumvent host defences to allow invasion of normally sterile urinary tract [4, 16, 17]. By routine disc diffusion susceptibility tests, 86 (49.14%) out of 175 gram negative isolates showed resistance to ceftazidime. All gram negative bacteria resistant to third generation cephalosporins (ceftazidime) were tested for ESBL production by two methods. ESBL production was detected in 14 (8%) isolates by modified DDST whereas; additional ESBL producers were detected by CLSI PCDDT (10.85%) [18]. Various factors like precise placement of the discs, correct storage of the clavulanate containing disc and performance of appropriate control tests are critical to the sensitivity of DDST [19]. In comparison to this, PCT is simple, cost effective and easy test to perform; therefore it can be used as a routine test for ESBL detection.

Maximum incidence of ESBL production was seen in *E.coli* (13.26%) isolates followed by *K. pneumoniae* (22.41%). It is in concordance to the study conducted by Kulkarni et al.[2] as well as study conducted by Kaur et al [20]. High prevalence rate of ESBL producing strains have been reported in *Klebsiella spp* by Gupta V et al [21] and Akata F et al [22].

CONCLUSION

Our study clearly highlight that all the isolated ESBL producers were resistant to third generation cephalosporins like ceftazidime, but are still sensitive to carbapenems like Imipenem and also highly responds to combination of drugs like Piperacillin / Tazobactum**.** The results of the present study showed an increase in the prevalence of resistance to a number of commonly used antibiotics to an alarming level. Many isolates were found to be resistant to atleast 3 - 5 antibiotics. In view of this emerging drug resistance the practice of routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases which will help in the proper treatment of the patient and also prevent further development of bacterial drug resistance.

Drug resistance surveillance in hospital is necessary to know the impact of higher drug resistance of the urinary isolates prevailing in their population which will lead to the formation of a strict antibiotic policy thereby reducing the resistance level.

Certain general precautions are to be followed to control the outbreaks of infections due to ESBL organisms. A proper use of antimicrobial agents will mostly control the emergence of resistant strains. Control measures and education programs are necessary to avoid the problem of ESBL's. In addition to that clinicians must depend on more laboratory guidance, while laboratory must provide resistant patient data for better management more rapidly.

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