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A Study Of Prevalence Of Urinary Isolates And Their Antimicrobial Susceptibility With Special Reference To Extended Spectrum Beta Lactamases Detection.

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ABSTRACT

Urinary tract infections (UTIs) are one of the most common infectious diseases encountered in the clinical practice. Extended spectrum beta lactamases (ESBLs) production in gram negative bacteria, have emerged as a major problem in hospitalized as well as community based patients. Bacterial resistance to β-lactam antibiotics has risen dramatically, with ESBL contributing to this increase. Early identification of ESBL production is becoming increasingly important. The objective of this study was to determine the resistance patterns of the micro-organisms isolated from cases of UTI and to detect ESBLs production in gram negative bacteria. Uropathogens from symptomatic UTI cases were identified by conventional methods, over the period of eight months. Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method. Gram negative isolates resistant to third generation cephalosporins were tested for ESBL production by two methods (Modified Double disc synergy test (DDST) and 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%). A total of 170 Gram negative isolates resistant to third generation cephalosporins were tested for ESBL production. It was observed that ESBL production was present in13 isolates by DDST method and in 18 isolates by PCDDT 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

Urinary Tract Infection (UTI) forms the largest single group of hospital acquired infections in many hospitals and accounts for approximately 35% of all hospital acquired infections [1, 2]. Resistant bacteria are emerging worldwide as a threat to the favourable outcome of common infections in community and hospital settings. β lactamases production by several gram negative and gram-positive organisms is perhaps one of the most important single mechanism of resistance to penicillins and cephalosporins [3, 4]. Extensive use of third generation cephalosporins has contributed to the evolution of extended spectrum beta lactamases (ESBLs).[3] 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 [3, 5].

Over the years, many new β-lactam antibiotics have been developed, however, with each new class of antibiotic, a new β-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and overuse of new antibiotics in the treatment of patients has resulted in the emergence of new variants of β-lactamase [1, 6]. Therefore, antimicrobial resistance surveillance is necessary to determine the size of problem and to guide empirical selection of antimicrobial agents for treating the infected patients. Hence, the present study was conducted in a tertiary care teaching hospital. The purpose of the study is to find out the prevalence of ESBL producers in urinary isolates of gram negative bacteria and also their susceptibility to commonly used antibiotics.

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 Vikhe Patil Foundation's Medical college Ahmednagar, were included in study.

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 [3, 7, 8]. Only a single positive culture per patient was included in the study. Microorganisms were identified by standard biochemical procedures [9, 10].

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:

Gram negative isolates 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) [3, 11]

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 Amoxicillin-clavulanic acid (20ug amoxicillin / 10ug clavulanic acid). The cefotaxime and ceftazidime disc were placed 16mm center to center from amoxiclav disc. Plate was incubated at 37° 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 No.1)

Phenotypic confirmatory disc diffusion test (PCDDT) [3, 12]

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. (Fig No.2)

RESULTS

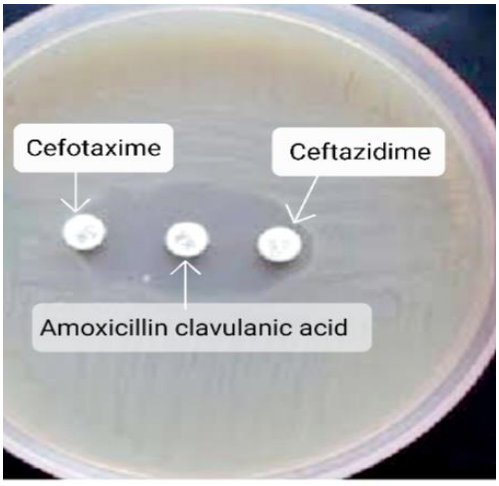
A total of 175 uropathogens were isolated from symptomatic UTI patients. *E.coli* was the predominant isolate 101 (57.7%) followed by *K.pneumoniae* 49 (28.3%). Other isolates are shown in Table No.1.

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), *K.pneumoniae* (n=29) and other gram negative bacilli resistant to third generation cephalosporins (ceftazidime) were tested for ESBL production by two methods.

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%). (Table3)

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)

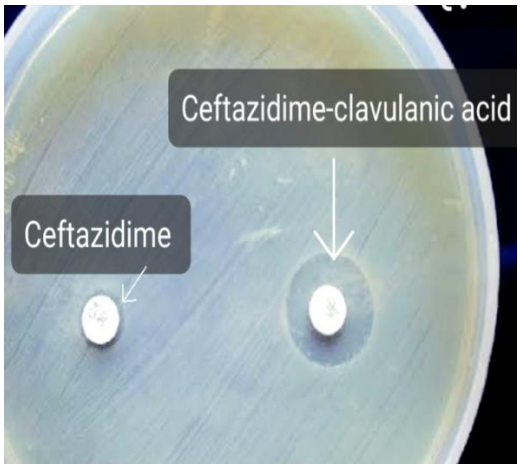

Table 1: Bacterial isolates in symptomatic UTI cases

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
Total gram negative bacteria		170	97.14
6	Staphylococcus aureus	01	0.57
7	Enterococcus feacalis	04	2.28
Total gram positive bacteria		05	0.02%
	Total	175	

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

Antibiotics	E. coli	Klebsiella pneumonia	Pseudomonas aeruginosa	Proteus mirabilis	Acinetobacter spp
	(N=101)	(N=49)	(N= 15)	(N=4)	(N= 1)
Ampicilline	73	36	07	02	01
Amoxycillin/ clavulanic acid	68	27	04	01	01
Piperacillin+ Tazobactum	48	24	03	-	-
Co-trimoxazole	72	34	05	02	-
Ciprofloxacin	69	39	04	02	-
Norfloxacin	58	32	05	03	-
Lomefloxacin	52	25	02	-	-
Nitrofurantoin	37	23	03	-	-
Gentamycin	69	39	04	01	01
Amikacin	65	28	05	02	01
Imipenem	63	29	05	01	01

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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 cephalosporin	Modified DDST	PCDDT
1	E. coli	49 (28%)	7 (4%)	10 (5.7%)
2	K. pneumoniae	29 (16.57%)	6 (3.4%)	8 (4.5%)
3	Pseudomonas aeruginosa	6 (3.4%)	1 (0.5%)	1(0.5%)
4	Proteus mirabilis	01 (0.5%)	-	-
5	Acinetobacter	01 (0.5%)	-	-
	Total	86 (49.14%)	14 (8%)	19 (10.85%)

DISCUSSION

Extensive use of expanded spectrum cephalosporins since 1980s has contributed significantly in the emergence of plasmid encoded ESBLs [11, 13]. Failure to detect these enzymes has contributed to their uncontrolled spread and sometimes to therapeutic failures [3, 14]. Incidence of these organisms is being continuously increasing throughout the world with limited therapeutic alternatives [13, 15]. 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 [16] Gupta V et al [17]. Abu Hena et al [18] and Kulkarni et al [13]. Our findings, however contrast with the study of Bajaj et al [19]. Where *Klebsiella species* predominated *E.coli*. Both host and bacterial factors have been associated with the pathogenesis of UTI.

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 (i.e.ceftazidime) were tested for ESBL production by two methods. ESBL production was detected in 14 (8%) isolates by modified DDST whereas; additional [20], ESBL producers were detected by CLSI PCDDT (10.85%). 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 [21]. 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 [13] as well as study conducted by Kaur et al [22]. High prevalence rate of ESBL producing strains have been reported in *Klebsiella spp* by Gupta V et al [17] and Akata F et al [23]. One of isolate of *Ps. aeruginosa was found to be ESBL producer in our study* Acinitobater species & proteus mirabilis were negative for ESBL by both the methods. Overall incidence of ESBL production in uropathogens is less (10.85%) in our study which is comparable with the study of Lee D et al [24]. Tankhiwale et al have reported higher incidence of ESBL production among urinary isolates [25].

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 at least 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.

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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