Original article:

Bacteriological profile and antibiogram of uropathogens with special reference to extended spectrum beta lactamases (ESBLs) detection in gram negative bacilli

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Abstract

Introduction-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 bacilli, have emerged as a major problem in hospitalized as well as community based patients. ESBLs producing bacteria may not be detected by routine disc diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure.

Aims-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 bacilli.

Material and Methods-Urinary isolates from symptomatic UTI cases (both in patients and out patients) attending the, Rural Medical College and Pravara Rural hospital, Loni were identified by conventional methods. Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion method. Clinical and laboratory standard institute (CLSI) recommendations to identify potential ESBL producing isolates using standard disc diffusion techniques were followed. All the potential ESBL producers were further subjected to detection of ESBL by two methods-Modified double disc synergy test (DDST) and CLSI phenotypic confirmatory test (PCT).

Results-Number of urinary isolates from patients with symptomatic UTI was 350 over a study period of one year. *E.coli* was the predominant isolate (57.7%) both in IPD as well as OPD patients. A total of 187(54.84%) gram negative bacilli were found to be potential ESBL producers according to CLSI criteria. ESBL production was confirmed in 41 (21.93%) isolates. Maximum ESBL production was seen in *K. pneumoniae* (22.22%) isolates followed by *E.coli* (13.76%).

Conclusion-This study showed *E.coli* to be the predominant urinary pathogen isolated from UTI cases. Overall incidence of ESBL producing microorganisms was 21.93%.

Key Words- Urinary tract infections, uropathogens, antimicrobial resistance, ESBLs

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases encountered in the clinical practice, mainly associated with different members of the family Enterobacteriaceae (Ruiz *et al,* 2002). Bacteria responsible for UTI, often originate from the faecal and perineal flora (Kaper *et al*, 2004; Wullt *et al*, 2002). Antibiotics are usually given empirically before the laboratory results of urine

culture are available (Tankhiwale *et al*, 2004). Resistant bacteria are emerging world wide as a threat to the favorable outcome of common infections in community and hospital settings. β lactamases production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins(Chaudhary and Aggarwal, 2004). Extensive use of third generation cephalosporins has contributed to the evolution of extended spectrum beta lactamases (ESBLs). These plasmid mediated groups of enzymes are the product of point mutations at the active site of TEM, SHV, and OXA enzymes (Menon *et al*, 2006).

To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility pattern is mandatory (Grubenberg *et al*, 1984). Since patterns of antibiotic resistance in a wide range of pathogenic organisms may vary over short periods, depend on the site of isolation and on different environments, periodic evaluation of antibacterial activity is needed to update this information (Jones and Thornsberry, 1982; Fu and Neu, 1978; Nokashino and Nakamuro, 1988). This study was conducted in a rural tertiary care hospital with an aim to determine the resistance patterns of the micro-organisms isolated from cases of UTI and to detect ESBLs production in gram negative bacilli.

Materials and Methods

A prospective study of 1084 urine samples from symptomatic UTI cases (both IPD and OPD) received in the Department of Microbiology of our institute was carried out over a period of one year . Majority of the samples were midstream urine (MSU) specimens (945) and others included catheterized urine samples (127) and suprapubic aspirates (12). Urine samples were microscopically studied by wet mount preparation and gram staining, inoculated on 5% sheep blood agar and Mac-Conkey's agar and incubated at 37^{o} C for 24 hours.

Semi quantitative urine culture using a calibrated loop was done on blood agar and Mac-Conkey's agar plates. Following Kass criteria, significant monomicrobic bacteriuria was defined as culture of a single bacterial species from the urine samples at a concentration of $> 10^5$ CFU/ml (Beckford, 2006; Girou *et al*, 2006). Only a single positive culture per patient was included in the study. Micro-organisms were identified by standard biochemical procedures (Crichton, 1996; Forbes *et al*, 2002). Antibiogram of the isolates was done by Kirby Bauer's disc diffusion method using antibiotic discs from Himedia laboratories pvt ltd, Mumbai, India. Antibiotics used for gram negative bacilli were amino penicillin (100 µg), cephalexin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), amikacin (30 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), norfloxacin (10 µg), co-trimoxazole (25 µg), and gentamicin (10 µg). For *Pseudomonas aeruginosa*, piperacillin (100 µg) was also used. For gram positive cocci, vancomycin (30 µg) was additionally used. *E. coli* ATCC 25922, *P.aeruginosa* ATCC 27853 and *S.aureus* ATCC 25923 were used as control strains.

Gram negative bacilli with reduced susceptibilities to cefotaxime (zone diameter of ≤ 27 mm) and /or ceftazidime (zone diameter of ≤ 22mm) were provisionally regarded as ESBL producing organisms according to guidelines for laboratory detection of ESBLs from Clinical and

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Laboratory Standards Institute (CLSI) (Wayne, 2006).Potential ESBL producers were subjected to ESBL detection by two methods .

1) *Modified Double disc synergy test (DDST)* (Jarlier, 1988): Lawn culture of test strain on Mueller Hinton agar (Himedia, Mumbai) was exposed to discs of cefotaxime (30 µg), ceftazidime (30 µg), and the disc of amoxiclav (augmentin) (20ug amoxicillin / 10ug clavulanic acid). The cefotaxime and ceftazidime disc were placed 20mm center to center from amoxiclav disc. The test isolate was considered to produce ESBL, if the zone size around the cefotaxime and ceftazidime disc increased towards the augmentin disc.

2) *CLSI phenotypic confirmatory test (PCT)* (Wayne, 2001): Ceftazidime and cefotaxime disc alone and in combination with clavulanic acid were placed on Mueller Hinton agar plates containing lawn culture of test strain. A ≥ 5mm increase in the zone diameter of third generation cephalosporins, tested in combination with clavulanic acid versus its zone when tested alone was considered indicative of ESBL production.

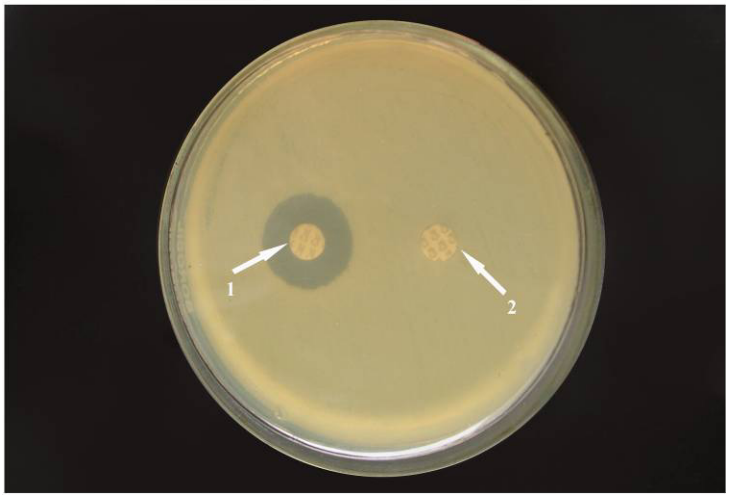
E.coli ATCC 25922 was used as ESBL negative control and *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control.

Results

A total of 350 uropathogens were isolated from symptomatic UTI patients. From MSU specimens 282 uropathogens were isolated, 65 isolates were obtained from catheterized urine samples and 03 from suprapubic aspirate. *E.coli* was the predominant isolate (57.7%) followed by *K.pneumoniae* (28.3%). Other isolates are shown in Table No. 1.UTI was more common in female patients as compared to males (Table No.2). Antibiotic resistance pattern showed *E.coli* to be maximum resistant to amino penicillin followed by cephalexin. Most effective antibiotic against *E.coli* was nitrofurantoin. For *K.pneumoniae*, gentamicin, amikacin and cotrimoxazole were found to be effective. By routine disc diffusion susceptibility tests, 166 out of 341 (48.7%) gram negative isolates showed resistance to cefotaxime whereas 164 (48.1%) were resistant to ceftazidime(Table No.3).

Number of gram negative bacilli found to be potential ESBL producers according to CLSI guidelines were 187 (54.84%) out of a total of 341 tested (Table No. 4). ESBL production was confirmed in 41(21.93%) isolates of the 187 tested. ESBL producers isolated from MSU specimens were 29(10.28%) whereas 12(18.46%) ESBL producers were isolated from catheterized urine samples. ESBL production was detected in 29(15.51%) isolates by modified DDST whereas; additional 12 ESBL producers were detected by CLSI PCT (21.93%). Maximum ESBL production was seen in *K.pneumoniae* isolated from IPD patients (22.22%) followed by *E.coli* (13.76%). PCT was found to be better than modified DDST for detection of ESBL production (Table No. 5).

Photograph 1



Photograph 2

Photograph 1-Positive Modified Double Disc Synergy Test-DDST (1- Ceftazidime disc, 2-Amoxicillin-Clavulanic acid disc, 3- Cefotaxime disc)

Photograph 2- Positive CLSI Phenotypic Confirmatory Test CLSI-PCT (1-Ceftazidime-Clavulanic acid disc, 2-Ceftazidime disc)

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Table No 1- Bacterial isolates in symptomatic UTI cases

S.No	Bacterial isolates	No.	%
1	*E. coli*	202	57.7
2	*Klebsiella pneumoniae*	99	28.3
3	*Pseudomonas aeruginosa*	30	8.6
4	*Enterococcus faecalis*	08	2.3
5	*Proteus mirabilis*	08	2.3
6	*Acinetobacter* spp	02	0.57
7	*Staphylococcus aureus*	01	0.28
	Total	350	

Table No 2- Age and sex wise distribution of patients with culture proven UTI

S.No	Bacterial isolates	No.	%
1	*E. coli*	202	57.7
2	*Klebsiella pneumoniae*	99	28.3
3	*Pseudomonas aeruginosa*	30	8.6
4	*Enterococcus faecalis*	08	2.3
5	*Proteus mirabilis*	08	2.3
6	*Acinetobacter* spp	02	0.57
7	*Staphylococcus aureus*	01	0.28
	Total	350	

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Table No.3- Antibiotic resistance pattern of urinary isolates from symptomatic UTI cases

Antibiotics	*E.coli*		*K.pneumoniae*		*P.aeruginosa*		*E.faecalis*		*P.mirabilis*		*Acinetobacter* spp		*S.aureus*	
	OPD (74)	IPD (128)	OPD (14)	IPD (85)	OPD (04)	IPD (26)	OPD (02)	IPD (06)	OPD (02)	IPD (06)	OPD -	IPD (02)	OPD -	IPD (01)
Amikacin	24	52	04	41	-	04	-	01	01	01	-	01	-	01
Amino penicillin	52	120	09	62	ND	ND	01	04	-	03	-	02	-	01
Cefotaxime	29	65	06	52	-	13	01	03	-	-	-	01	-	-
Ceftazidime	31	67	04	48	01	11	01	02	-	01	-	01	-	-
Cephalexin	36	74	06	58	ND	ND	01	04	-	04	-	02	-	-
Cotrimoxazole	29	64	07	39	ND	ND	ND	ND	01	04	-	ND	-	ND
Gentamicin	36	52	04	40	02	14	01	03	-	03	-	01	-	01
Nalidixic acid	37	72	06	47	ND	ND	ND	ND	01	03	-	02	-	ND
Nitrofurantoin	19	05	05	45	ND	ND	ND	ND	-	04	-	02	-	ND
Norfloxacin	34	69	05	43	01	07	01	01	-	01	-	01	-	ND
Piperacillin	ND	ND	ND	ND	-	03	ND	ND	ND	ND	-	ND	-	ND
Vancomycin	ND	ND	ND	ND	ND	ND	-	-	ND	ND	-	ND	-	-

Figures in parenthesis indicate number of isolates

N D-Not detected

Table No. 4-Gram negative bacilli positive for probable ESBL production by CLSI Standard disc diffusion technique

Gram negative bacilli	Ceftazidime (≤ 22 mm)	Cefotaxime (≤ 27 mm)
E.coli (n=202)	109	97
K.pneumoniae (n=99)	55	61
P.aeruginosa(n=30)	12	13
P.mirabilis(n=08)	01	-
Acinetobacter spp.(n=02)	01	01
Total (n=341)	178	172

n = Number of isolates

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Table No. 5- Comparison of Modified DDST and PCT for ESBL detection in gram negative bacilli

Bacterial isolates	No. of probable ESBL producers (CLSI guidelines)	Modified DDST			PCT		
		OPD	IPD	Total	OPD	IPD	Total
E. coli	109	05	10	15	06	15	21
K. pneumoniae	63	04	09	13	05	14	19
P. aeruginosa	13	-	01	01	-	01	01
P.mirabilis	01	-	-	-	-	-	-
Acinetobacter spp	01	-	-	-	-	-	-
Total	187			29 (15.51%)			41 (21.93%)

Discussion

Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population. In the present study *E.coli* was the predominant isolate followed by *K.pneumoniae*. This tally with the studies of other workers like Varma *et al*, 1972; Gupta *et al*, 2002. Akram *et al*, 2007; Roopa and Sudha, 2010 have also reported *E.coli* followed by *Klebsiella* species as the most prominent organisms isolated from cases of UTI.Our findings, however contrast with the study of Bajaj *et al*, 1999; where *Klebsiella* spp predominated *E.coli*. Both host and bacterial factors have been associated with the pathogenesis of UTI. 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 defenses to allow invasion of normally sterile urinary tract (Johnson, 1997; Kunin, 1987). Female patients presenting with symptoms of UTI were more as compared to male patients. In general, rates of UTIs are higher among women than among men, with cystitis being the most prevalent UTI. In our study CLSI PCT detected additional 12 ESBL producers as compared to modified DDST. 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 (Moland and Thompson, 1994). 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 *K. pneumoniae* isolates from indoor patients. Moyo *et al*, 2010 has also reported higher incidence of ESBL production among isolates from inpatients. High prevalence rate of ESBL producing strains have been reported in *Klebsiella* spp by Gupta *et al*, 2002 and Akata *et al*, 2003. Iqbal *et al*, 2002 have reported ESBL production in *E.coli* ranging

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between 21 to 34%. One isolate of *P. aeruginosa* was found to produce ESBL in our study. Overall incidence of ESBL production in uropathogens is less (21.93%) in our study which is comparable with the study of Lee *et al*, 2010 (13%). Taneja *et al*,2008 has reported 36.5% of uropathogens as ESBL producers. Tankhiwale *et al*, 2004 have reported higher incidence of ESBL production among urinary isolates (48.3%). Higher percentage of ESBL producers were isolated from catheterized urine samples in this study. Lee *et al*, 2010 have also reported the risk of emergence of ESBL –producing bacteria to be higher in patients with Foley catheterization. Therefore, to prevent the emergence of ESBL producing bacteria, unnecessary catheterization should be avoided in patients with UTI.

ESBL producing strains are resistant to a wide variety of commonly used antimicrobials. Their proliferation poses a serious global health concern that has complicated strategies for a growing number of hospitalized patients. Irrational prescription of antimicrobials, available over the counter in India, has contributed to this situation. Hence routine ESBL testing for uropathogens along with conventional antibiogram would be useful for all cases of UTI.

Conclusion

The predominant uropathogen in this study, isolated from symptomatic UTI cases was E.coli. Maximum ESBL production was seen in K.pneumoniae isolated from IPD patients. Production of ESBLs is the most common mechanism of resistance to third generation cephalosporins seen in gram negative bacilli. Therefore a clinical microbiology laboratory, along with conventional antibiotic susceptibility testing should also perform more accurate methods of detecting ESBLs.

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