Original Article

Antibiotic coresistance among extended-spectrum beta lactamase-producing urinary isolates in a tertiary medical center: A prospective study

Abstract

Aim: Urinary tract infections are among the most common infections encountered in clinical practice. Study was conducted to detect extended-spectrum beta lactamase (ESBL) type of resistance in urinary isolates in North Eastern Region of India. **Materials and Methods:** Midstream urine sample was collected from 200 patients clinically suspected to be suffering from urinary tract infections and attending Outpatients Departments and different wards in Assam Medical College and Hospital Dibrugarh, a tertiary teaching hospital from September 2007 to August 2008. The patients who did not have a course of antibiotic before 15 days of study were included, whereas patients with known history of diabetes, thyroid disorders, renal disease, and hypertension were excluded from the study group. Urine samples were cultured as per guidelines and ESBL detected by double disc diffusion tests. **Statistical Analysis:** Test of proportion and two-tailed *Z* test were used for data analysis. **Results:** In all, 171 isolates of Gram-negative bacilli were detected of which 42 isolates produced ESBL. So the detection rate of ESBL in the study was 24.56%. The ESBL-producing isolates were 19 (28.78%) in males and 23 (21.9%) in females, and this difference was not found to be significant ($P > 0.05$). In 97.61% of isolates, associated resistance was observed for ampicillin and cotrimoxazole. Ciprofloxacin and gatifloxacin showed coresistance of 69.04% and 71.42%, respectively. Associated resistance for amoxycillin/clavulanic acid and piperacillin/tazobactum was 38.09% and 35.71%. All the isolates of Enterobacteriaceae producing ESBL were 100% sensitive to imipenem. **Conclusion:** These data provided the much needed information on the prevalence of antimicrobial resistance among pathogens causing urinary tract infections. Results seem helpful in providing useful guidelines in choosing an effective antibiotic in cases with urinary tract infection and also initiating therapy in antimicrobial-resistant strains.

Key words:
Antibiotic coresistance, extended-spectrum beta lactamase, urinary isolates

Introduction

Urinary tract infections are among the most common infections encountered in clinical practice. The infections range from a single acute symptomatic infection, with a susceptible organism such as *Escherichia coli* which may develop a spontaneous cure, to a more serious recurring infection such as chronic pyelonephritis which may be caused by resistant, and often difficult to treat, organisms.[1]

Beta lactam antibiotics are used extensively in treating urinary tract infections. In recent years, bacterial resistance to beta lactam antibiotics has risen dramatically with ESBL contributing to this increase. These enzymes hydrolyze extended-spectrum cepholosporins such as ceftazidime, cephotaxime, and monobactum such as aztreonam.[2] They arise by mutations in genes for common plasmid-mediated

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beta lactamases. Some ESBLs confer high level resistance to all oxymino beta lactums, but for other ESBL resistance is only slightly increased or increased selectively for particular beta lactums. This creates a problem because organisms producing less active ESBL can show sensitivity to an antibiotic and cause significant disease.[3] In many cases, the plasmids that encode ESBL also encode other antimicrobial genes. Therefore, it is common for organisms expressing an ESBL to express coresistance to aminoglycoside, trimethoprim sulphamethoxazole, and tetracyclines.[4] This study was conducted in the Department of Microbiology, Assam Medical College and Hospital Dibrugarh, to detect ESBLs in isolates of patients suffering from urinary tract infections and the coresistance among the ESBL isolates to other antimicrobials.

Materials and Methods

Midstream urine sample was collected from 200 patients clinically suspected to be suffering from urinary tract infections and attending Outpatients Departments and wards of Medicine, Surgery, Gynecology and Obstetrics and Pediatrics Departments in Assam Medical College and Hospital Dibrugarh a tertiary teaching hospital from September 2007 to August 2008. The patients who did not have a course of antibiotic before 15 days of study were included, whereas patients with known history of diabetes, thyroid disorders, renal disease, and hypertension were excluded from the study group. In catheterized patients, urine specimen was obtained by sterilized syringe and needle from catheter port after disinfecting catheter with 70% alcohol.[5] Urine sample was transported to the Department of Microbiology and processed immediately. In case of delay, samples were refrigerated at 4°C. Urine was cultured in MacConkey agar and Blood agar and the colonies detected after incubation were characterized. The Gram-negative bacilli thus isolated were characterized on the basis of gram staining, motility, and standard biochemical tests.[6] Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method as per NCCLS guidelines.[7] The antibiotic discs used were ampicillin (10 μg), cotrimoxazole (25 μg), cephoxitin (30 μg), cephotaxime (30 μg), amikacin (10 μg), ciprofoxacin (30 μg), gatifloxacin (5 μg), nitrofurantoin (300 μg), amoxycillin/clavulanic acid (20/10 μg), piperacillin/tazobactum (100/10 μg), and imipenem (10 μg). These were procured from Himedia, Mumbai; the reference strains used as control for disc diffusion testing were *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853.

The isolates of Gram-negative bacilli were tested for ESBL production by double disc diffusion test (DDDT).[8] A lawn culture of test strain on Mueller-Hinton Agar (Himedia) was exposed to disc of cephotaxime (30 μg) and a disc of amoxyclav (20 μg amoxicillin/10 μg clavulanic acid) arranged in pairs. The discs were arranged so that the distance between them was approximately twice the radius of the inhibition zone produced by cephotaxime disc tested on its own. The test isolate was considered to produce ESBL if zone size around antibiotic disc increased toward amoxyclav disc [Figure 1].[8] A positive control *Klebsiella pneumonia* ATCC 700603 and a negative control *E. coli* ATCC 25922 was put up along with the test.

All collected data were later on statistically analyzed and presented. test of proportion and two-tailed *Z* test were used for data analysis. Statistical analysis was carried out using SPSS 14 (SPSS Inc., Chicago, IL, USA).

Results

In all, 171 isolates of Gram-negative bacilli were detected from 200 patients suffering from urinary tract infection; 42 of these isolates produced ESBL. So the detection rate of ESBL in the study was 24.56% [Table 1]. Number of *E. coli* detected was 116, of which 16 produced ESBL (13.9%). *Klebsiella* spp. detected was 30, of which 10 produced ESBL (33.33%). Five isolates out of 7 (71.42%) of *Proteus mirabilis* produced the enzyme. Maximum ESBL producers were detected in *Proteus vulgaris*, *Enterobacter* spp., and

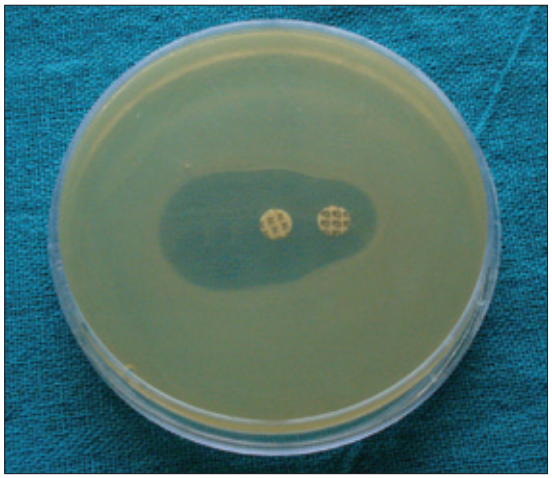

Figure 1: ESBL detected if inhibition zone around cephalosporin disc extended on the side nearest amoxyclav disc

Table 1: Distribution of ESBL strains among the isolated uropathogens

Organisms detected	Number detected	No. of ESBL detected	Percentage
Escherichia coli	116	16	13.79
Klebsiella spp.	30	10	33.33
Proteus mirabilis	7	5	71.42
Proteus vulgaris	1	1	100
Providencia spp.	2	1	50
Pseudomonas aeruginosa	8	5	62.5
Enterobacter spp.	1	1	100
Citrobacter spp.	5	2	40
Acinetobacter spp.	1	1	100
Total	171	42	24.56

ESBL – Extended-spectrum beta lactamase

Acinetobacter spp. (100%). *P. aeruginosa* detected was 8, out of which 5 (62.5%) produced ESBL. Number of *Providencia* spp. detected was 2, out of which 1 produced ESBL; 2 isolates out of 5 isolates (40%) of *Citrobacter* spp. produced ESBL.

Out of the 171 Gram-negative bacteria isolated, 66 were detected from urinary isolates of male patients, whereas 105 were detected from isolates of female patients. The ESBL-producing isolates were 19 (28.78%) in males and 23 (21.9%) in females [Table 2]. Applying the test of significance using the two-tailed *Z* test with confidence interval (CI) 95%, $P>0.05$.

The ESBL-producing isolates of *E. coli* showed coresistance of 93.7% against ampicillin and cotrimoxazole. The isolates of *Klebsiella*-producing ESBL showed 100% coresistance against ampicillin and cotrimoxazole. The five ESBL isolates of *Proteus mirabilis* showed 100% coresistance against ampicillin, amikacin, gatifloxacin, and cotrimoxazole. *Proteus vulgaris* ESBL isolate showed coresistance to ciprofloxacin, gatifloxacin, and cotrimoxazole. The ESBL-producing isolate of *Providencia* spp. showed coresistance against ampicillin, amikacin, ciprofloxacin, gatifloxacin, cotrimoxazole, nitrofurantoin, and piperacillin/tazobactum. The five isolates of *P. aeruginosa*-producing ESBL showed 100% coresistance toward ampicillin and cotrimoxazole and 80% coresistance toward amikacin and nitrofurantoin. The two ESBL-producing isolates of *Citrobacter* spp. showed 100% coresistance to ampicillin, amikacin, and cotrimoxazole. One isolate of ESBL-producing *Enterobacter* spp. showed 100% coresistance with ampicillin, amikacin, and cotrimoxazole. The ESBL-producing single isolate of *Acinetobacter* spp. showed 100% coresistance toward ampicillin, amikacin, ciprofloxacin, gatifloxacin, and cotrimoxazole. Among the 42 isolates of *Enterobacteriaceae*-producing ESBL, 97.61% of associated resistance was observed for ampicillin and cotrimoxazole. Ciprofloxacin and gatifloxacin showed coresistance of 69.04% and 71.42%, respectively. Associated resistance for amoxycillin/clavulanic acid and piperacillin/tazobactum was 38.09% and 35.71%. All the isolates of *Enterobacteriaceae*-producing ESBL was 100% sensitive to imipenem [Table 3].

Table 2: Distribution of ESBL strains among male and female patients

Sex	Gram-negative bacteria detected	ESBL isolates (%)
Male	66	19 (28.78)
Female	105	23 (21.9)
Total	171	42

Using two-tailed *Z* test with CI 95% $P>0.05$; ESBL – Extended-spectrum beta lactamase

Discussion

In this study, the detection rate of ESBL-producing isolates among the Gram-negative organism was 24.56%. A study from North India on ESBL production in uropathogens showed 26.6% ESBL producers which belonged to *Klebsiella, E. coli, Enterobacter*, *Proteus*, and *Citrobacter* species.[9] Tankhiwala *et al*. detected 48.3% urinary isolates to be ESBL producers, *E. coli*, *Klebsiella*, and *Acinetobacter* species being predominant.[10] In a study from South India, 41% of *E. coli* and 40% of *Klebsiella* species were found to be ESBL producers in isolates of patients suffering from urinary tract infection.[11] A study from Manipal showed 32% *E. coli*, 37% *Klebsiella* species, and 20% *Citrobacter* species among the urinary pathogen produced ESBLs.[12] Akram *et al*. detected 34.42% of ESBL producers among *E. coli* in urinary isolates. [13] It can be seen from earlier studies that there is a wide range of variation in detection rate of ESBL producers in urinary isolates. In this study, 13.9% of *E. coli* isolates produced ESBL and 33.3% of *Klebsiella* isolates were ESBL producers. Among *P. mirabilis* isolates, 71.4% produced ESBL 62.5% of *P. aeruginosa* strains, 50% of *Providencia*

Table 3: Associated resistance to other antibiotics among ESBL isolates

Antimicrobial agent	Number of isolates									
	16	10	5	1	1	5	1	2	1	42
	E. coli (%)	*Klebsiella* spp. (%)	*P. mirabilis* (%)	*P. vulgaris* (%)	*Providencia* spp. (%)	*P. aeruginosa* (%)	*Enterobacter* spp. (%)	*Citrobacter* spp. (%)	*Acinetobacter* spp. (%)	Total (%)
Ampicillin	15 (93.7)	10 (100)	5 (100)	1 (100)	1 (100)	5 (100)	1 (100)	2 (100)	1 (100)	41 (97.61)
Cotrimoxazole	15 (93.7)	10 (100)	5 (100)	1 (100)	1 (100)	5 (100)	1 (100)	2 (100)	1 (100)	41 (97.61)
Amikacin	12 (75)	8 (80)	5 (100)	–	1 (100)	4 (80)	–	2 (100)	1 (100)	33 (78.57)
Ciprofloxacin	12 (75)	6 (60)	4 (80)	1 (100)	1 (100)	2 (40)	1 (100)	1 (50)	1 (100)	29 (69.04)
Gatifloxacin	12 (75)	6 (60)	5 (100)	1 (100)	1 (100)	2 (40)	1 (100)	1 (50)	1 (100)	30 (71.42)
Nitrofurantoin	10 (62.5)	7 (70)	3 (60)	–	1 (100)	4 (80)	–	1 (50)	–	26 (61.9)
Amoxycillin + clavulanate	6 (37.5)	3 (30)	2 (40)	–	-	3 (60)	1 (100)	1 (50)	–	16 (38.09)
Piperacillin + tazobactum	6 (37.5)	4 (40)	2 (40)	–	1 (100)	1 (20)	–	1 (50)	–	15 (35.71)
Imipenem	–	–	–	–	–	–	–	–	–	–

Figures indicate total number of isolates, number of isolates sensitive to the drugs, and their percentages, respectively; ESBL – Extended-spectrum beta lactamase

spp. strains, and 40% of *Citrobacter* spp. strains were ESBL producers. The sole strain of *P. vulgaris*, *Enterobacter spp*, and *Acinetobacter* species were detected to produce ESBL.

Urinary tract infection was found to be more prevalent in females than males according to studies by Pfau *et al*.[14] and Wong *et al*.[15] In this study, ESBL isolates detected in males were 28.78% and in females were 21.9%. Applying the two-tailed *Z* test with CI 95%, this difference was not found to be significant ($P>0.05$).

ESBL-producing organisms are reported to be more resistant to commonly used antibiotics. Moreover, they are resistant to oxyiminocephalosporins and aztreonam resulting in treatment failure.[16] A study by Schwaber *et al*.[17] have reported the ESBL isolates to be 25% susceptible to gentamicin, 30% to cotrimoxazole, 41% to ciprofloxacin, and 60% susceptible to piperacillin/tazobactum. Morosini *et al*.[18] in their study have also stated coresistance of ESBL isolates to different antibiotics. This study has showed resistance of ESBL isolates to ciprofloxacin, amikacin, and sulphonamides.

In this study, 97.61% ESBL-producing organisms were resistant toward ampicillin and cotrimoxazole. There was 69.04% coresistance toward gatifloxacin and ciprofloxacin. But all the strains were sensitive to imipenem, which is considered as the treatment of choice for organisms producing ESBL Paterson[19] Antibiotic intake causes colonization of the urinary tract with organism having different mechanisms of resistance toward antimicrobials. Hence, the judicial use of antibiotics is essential in preventing infection by antibiotic resistant strains.

In conclusion, these data provided the much needed information on the prevalence of antimicrobial resistance among pathogens causing urinary tract infections. The rise of antibiotic resistance in urinary isolates emphasizes the importance of sound hospital infection control, rational prescribing policies, and the need of new antimicrobial drugs. These results seem helpful in providing useful guidelines in choosing an effective antibiotic in cases with urinary tract infection and also initiating therapy in antimicrobial-resistant strains.

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