Journal of Research in Pharmacy Practice

Original Article

Antimicrobial susceptibility pattern of extended-spectrum β-lactamase-producing bacteria causing nosocomial urinary tract infections in an Iranian referral teaching hospital

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Received: August 2013
Accepted: November 2013

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ABSTRACT

Objective: Gram-negative bacilli are the most important cause of nosocomial urinary tract infections (UTIs). The production of extended-spectrum β-lactamase (ESBL) enzymes is a common mechanism of resistance among these bacteria. The aim of this study was to determine the rate of ESBL producing Gram-negative bacteria causing nosocomial UTI in a referral hospital as well as their susceptibility pattern to the most commonly used antibiotics.

Methods: In a prospective cross-sectional study performed over a 6-month period, urinary specimens obtained from hospitalized patients with documented culture-proved nosocomial UTI (age range of 1-87 years). Isolated aerobic Gram-negative bacteria underwent further microbiologic tests for detection of ESBL, as well as antimicrobial susceptibility test using Kirby-Bauer (disk diffusion) and E-test methods.

Findings: During the study period, 213 urine samples were detected to have growth of Gram-negative organism. *Escherichia coli* was the most frequently isolated organism (61%). ESBL was detected in 102 isolates including 38.5% of *E. coli*, 39.5% of *Klebsiella pneumonia*, 88.5% of *Pseudomonas aeruginosa*, and 100% of *Acinetobacter baumannii* strains. Imipenem and meropenem were the most effective antibiotics on *E. coli* and *K. pneumoniae* strains. *P. aeruginosa* and *A. baumannii* strains showed high resistance to all tested antibiotics.

Conclusion: Large numbers of Gram-negative bacteria causing nosocomial UTIs produce ESBL with most being multidrug-resistant. Therefore, routine ESBL detection testing and subsequent antibiogram with disk diffusion method could be useful to determine the best treatment options for UTI.

Keywords: Antimicrobial; extended-spectrum β-lactamase-producing bacteria; Gram-negative; nosocomial urinary tract infection; susceptibility

INTRODUCTION

Nosocomial infection is a significant complication of hospitalization. Urinary tract infections (UTIs) are the most common type of nosocomial infections.[1] Gram-negative bacilli are the most important cause of these infections.[2] These bacteria are showing rising rates of resistance to current therapies. The production of extended-spectrum β-lactamase (ESBL) enzymes is a common mechanism of resistance. ESBLs are enzymes that confer resistance to most beta-lactam antibiotics including penicillins, cephalosporins, and the monobactam aztreonam.[3] These enzymes have been found exclusively in Gram-negative organisms.[4] Although the prevalence of ESBL-producing *Escherichia coli (E. coli)* can vary from country to country, resistance rates to many commonly used therapies have increased throughout the world.[5-9] *E. coli* is the most common cause of UTIs.[10] Cases of UTI caused by ESBL-producing *E. coli* and *Klebsiella pneumonia* as well as *Pseudomonas aeruginosa*, including multidrug-resistant (MDR) strains, are increasing.[11]

Detection of the microbial etiology of infections, including nosocomial UTIs, provides important

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Website: www.jrpp.net

DOI: 10.4103/2279-042X.132703

information in day-to-day decision-making in individual hospitals regarding potential outbreaks, unusual pathogens, antimicrobial resistance, and local trends in the etiology of infections.[12] On the other hand, selection of an appropriate antibiotic for treatment of these infections is dependent to knowledge of both causative pathogens, including drug-resistant organisms, and their antimicrobial susceptibility pattern.

The aim of this study was to determine the rate of ESBL producing Gram-negative bacteria causing nosocomial UTI in our referral teaching hospital as well as their susceptibility pattern to the most commonly used antimicrobials to identify the most appropriate antibiotic treatments for these infections.

METHODS

This was a prospective cross-sectional study performed over a 6-month period from January to July 2013 at Alzahra Hospital, a 950-bed Referral University Hospital in Isfahan, Iran. Urinary specimens were obtained from hospitalized patients (age range of 1-87 years) with documented nosocomial UTI diagnosed using the CDC/NHSN (Centers for Disease Control and Prevention/National Healthcare Safety Network) definition of healthcare-associated infections.[13] The urine samples, collected by midstream clean-catch method, were sent to the hospital's microbiology laboratory and cultured; if microbial growth occurred, differential cultures and tests were performed to identify different bacterial strains. Bacterial species were identified by different microbiologic tests, including growth in eosin-methylene-blue and MacConkey media, Gram stain, urease production, H_2S production in sulfur-indole motility media, indole production, motility, methyl red test, citrate utilization, and decarboxylase production. Isolated aerobic Gram-negative bacteria underwent further microbiologic tests for detection of ESBL as well as antimicrobial susceptibility test.

ESBL detection

ESBL detection was performed using the criteria suggested by Clinical and Laboratory Standards Institute (CLSI).[14] Mueller-Hinton agar culture medium (Himedia, India) was inoculated by a direct saline suspension of isolated colonies with turbidity of 0.5 McFarland. Then antibiotic disks (Mast, UK), cefotaxime (30 μg) and ceftazidime (30 μg) were placed on the agar surface at a distance of 30 mm (center to center) from each other. After 16-18 h of incubation at 37°C, results were interpreted by measurement of inhibition zone diameter around each disk. According to CLSI criteria, an inhibition zone of ≤27 mm for cefotaxime and ≤22 mm for ceftazidime indicated that the strain probably produced ESBL.

Confirmatory tests for ESBL detection

Combined disk test was done for strains that showed ESBL production by the previous test.[15] Mueller-Hinton agar was used as culture medium. Antibiotic disks containing cefotaxime (30 μg), cefotaxime/clavulanic acid (30 μg/10 μg), ceftazidime (30 μg) and ceftazidime/clavulanic acid (30 μg/10 μg) were used. If the inhibition zone diameter of each antibiotic combined with clavulanic acid was ≥5 mm larger than that of antibiotic alone, the production of ESBL by the strain was confirmed.

For isolated *P. aeruginosa* and *Acinetobacter baumannii* strains, in addition to the above method, double-disk synergy test (DDST) was also used.[16,17] Both tests were done on cloxacillin (250 μg/ml)-containing Mueller-Hinton agar plates to inhibit cephalosporinase activity and enhance the ability of DDST for detection of ESBL.[16] DDST was performed by placing disks of ceftazidime, cefotaxime, aztreonam, and cefepime (30 μg, each) at a distance of 20 mm (center to center) from a disk of amoxicillin/clavulanic acid (20 μg/10 μg). ESBL production was confirmed when the cephalosporin inhibition zone was expanded by the clavulanate.

Antimicrobial susceptibility tests

Isolated ESBL-producing Gram-negative bacteria underwent antimicrobial susceptibility test using Kirby–Bauer (disk diffusion) method followed by E-test method for isolates that showed either intermediate sensitivity or resistance in the first test to determine the reliability of its results. Both tests were performed according to CLSI guidelines and results were interpreted using CLSI breakpoints.[14] The tested antibiotic disks (Mast, UK) included: Ampicillin (10 μg), ampicillin/sulbactam (10 μg/10 μg), ceftazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg), piperacillin/tazobactam (100 μg/10 μg), imipenem (10 μg), meropenem (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), and nitrofurantoin (300 μg). The E-test method was done using the E-test strips (Liofilchem, Italy) of the following antibiotics: Ceftazidime, cefepime, imipenem, amikacin, and ciprofloxacin.

RESULTS

During the study period, 213 urine samples had growth of Gram-negative organism. *E. coli* was the most frequently isolated organism (n = 130, 61%), followed by *K. pneumoniae* (n = 38, 17.8%), *P. aeruginosa* (n = 26, 12.2%), and *A. baumannii* (n = 9, 4.2%). The remaining 10 isolates (4.7%) were from other species.

Of 213 isolated Gram-negative bacteria, 102 isolates were ESBL-positive which included 50 isolates of *E. coli* (38.5%), 15 isolates of *K. pneumoniae* (39.5%),

23 isolates of *P. aeruginosa* (88.5%), all isolates of *A. baumannii* (100%), and 5 isolates of other species (50%).

Table 1 shows the susceptibility pattern of isolated ESBL-producing Gram-negative bacteria determined by disk diffusion test. As shown, imipenem and

Table 1: Antimicrobial susceptibility pattern of isolated ESBL-producing Gram-negative bacteria determined by disk diffusion test

Microorganism	Antibiotic	*n*	Susceptibility, *n* (%)		
			Sensitive	Intermediate	Resistant
Escherichia coli	Ampicillin	50	0	0	50 (100)
	Ampicillin/sulbactam	50	15 (30)	8 (16)	27 (54)
	Ceftazidime	50	0	0	50 (100)
	Cefotaxime	50	0	0	50 (100)
	Cefepime	50	1 (2)	0	49 (98)
	Piperacillin/tazobactam	50	42 (84)	0	8 (16)
	Imipenem	50	48 (96)	2 (4)	0
	Meropenem	50	50 (100)	0	0
	Amikacin	50	35 (70)	0	15 (30)
	Ciprofloxacin	50	9 (18)	0	41 (82)
	Levofloxacin	50	6 (12)	13 (26)	31 (62)
	Nitrofurantoin	50	43 (86)	0	7 (14)
Pseudomonas aeruginosa	Ampicillin	23	0	0	23 (100)
	Ampicillin/sulbactam	23	0	0	23 (100)
	Ceftazidime	23	0	0	23 (100)
	Cefotaxime	23	0	0	23 (100)
	Cefepime	23	1 (4.3)	0	22 (95.7)
	Piperacillin/tazobactam	23	0	0	23 (100)
	Imipenem	23	0	1 (4.3)	22 (95.7)
	Meropenem	23	0	0	23 (100)
	Amikacin	23	1 (4.3)	1 (4.3)	21 (91.4)
	Ciprofloxacin	23	0	0	23 (100)
	Levofloxacin	23	0	0	23 (100)
	Nitrofurantoin	23	0	0	23 (100)
Klebsiella pneumoniae	Ampicillin	15	0	0	15 (100)
	Ampicillin/sulbactam	15	6 (40)	4 (26.7)	5 (33.3)
	Ceftazidime	15	0	0	15 (100)
	Cefotaxime	15	0	0	15 (100)
	Cefepime	15	0	0	15 (100)
	Piperacillin/tazobactam	15	11 (73.3)	0	4 (26.7)
	Imipenem	15	15 (100)	0	0
	Meropenem	15	15 (100)	0	0
	Amikacin	15	7 (46.7)	0	8 (53.3)
	Ciprofloxacin	15	3 (20)	0	12 (80)
	Levofloxacin	15	3 (20)	6 (40)	6 (40)
	Nitrofurantoin	15	5 (33.3)	0	10 (66.7)
Acinetobacter baumannii	Ampicillin	9	0	0	9 (100)
	Ampicillin/sulbactam	9	0	0	9 (100)
	Ceftazidime	9	1 (11.1)	0	8 (88.9)
	Cefotaxime	9	0	0	9 (100)
	Cefepime	9	0	0	9 (100)
	Piperacillin/tazobactam	9	0	0	9 (100)
	Imipenem	9	0	0	9 (100)
	Meropenem	9	0	0	9 (100)
	Amikacin	9	0	1 (11.1)	8 (88.9)
	Ciprofloxacin	9	0	0	9 (100)
	Levofloxacin	9	0	0	9 (100)
	Nitrofurantoin	9	0	0	9 (100)

ESBL=Extended-spectrum β-lactamase

meropenem was the most effective antibiotics on *E. coli* and *K. pneumoniae* strains. On the other hand, *P. aeruginosa* and *A. baumannii* strains showed high resistance to all tested antibiotics.

Table 2 presents the results of E-test susceptibility testing performed for five agents against isolates resistant/intermediately resistant to antibiotic disks. As shown, good consistency was seen for results of the two methods. The most frequent inconsistency of results was observed for amikacin against the isolates of *E. coli* and *P. aeruginosa*.

DISCUSSION

This study was conducted to detect the most frequent Gram-negative bacterial pathogens including ESBL producers causing nosocomial UTIs in our referral hospital and to determine their antibiotic susceptibility pattern. In our research, *E. coli* was the most frequently isolated bacteria followed by *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*. This is consistent with the results of several other studies in Iran[18,19] and other countries[2,20,21] which reported either *E. coli* or *Klebsiella* spp. as the most frequent pathogens causing nosocomial UTI. However, the prevalence of other microorganisms is different in various centers. In a study on nosocomial UTIs in 228 hospitals from 29 European countries, the six most commonly isolated microorganisms were, in decreasing order: *E. coli* (35.6%), *Enterococci* (15.8%), *Candida* (9.4%), *Klebsiella* (8.3%), *Proteus* (7.9%), and *P. aeruginosa* (6.9%).[20] However, we did not evaluate the organisms other than Gram-negative bacteria such as Enterococci.

In our study, 47.9% (n = 102) of isolated Gram-negative bacilli were ESBL-positive with *E. coli* strains being the most frequent agents followed by *Pseudomonas*, *Klebsiella*, and *Acinetobacter*. Similarly, in a study performed in India, 48.3% of isolated uropathogens were found to be ESBL producers.[21] In contrast to our results, in the study of Hosain Zadegan *et al*. in an Iranian hospital, 23.5% of isolated Gram-negative microorganisms (53 of 222 isolates) were ESBL producers with the most frequent isolates being *K. pneumoniae* (8.9%), *E. coli* (4.4%), and *P. aeruginosa* (4.4%); also, of nine isolated *Acinetobacter* spp. strains, 2 (0.9%) were ESBL-positive.[22] In another Iranian study conducted by Irajian *et al*. on different clinical specimens, ESBL was detected among 18.1% of all isolated *E. coli* and *K. pneumoniae* strains. Frequency of ESBL production was 17.45% and 19.6% for these two organisms, respectively.[23] These values are very lower than the rates in our study. This may be due to the fact that our study was performed only on urine samples as in the above-mentioned works, the most ESBL producing organisms were found in

Table 2: Results of E-test method for isolated ESBL-producing Gram-negative bacteria resistant/ intermediately resistant to antibiotics in disk diffusion test

Microorganism	Antibiotic	Disk susceptibility	*n*	E-test, *n* (%)		
				Sensitive	Intermediate	Resistant
Escherichia coli	Ceftazidime	Resistant	50	0	0	50 (100)
	Cefepime	Resistant	49	2 (4.1)	3 (6.1)	44 (89.8)
	Imipenem	Intermediate	2	0	2 (100)	0
	Amikacin	Resistant	15	6 (40)	1 (6.7)	8 (53.3)
	Ciprofloxacin	Resistant	41	0	0	41 (100)
Pseudomonas aeruginosa	Ceftazidime	Resistant	23	0	0	23 (100)
	Cefepime	Resistant	22	0	0	22 (100)
	Imipenem	Resistant	22	0	0	22 (100)
		Intermediate	1	0	1 (100)	0
	Amikacin	Resistant	21	1 (4.8)	7 (33.3)	13 (61.9)
		Intermediate	1	0	1 (100)	0
	Ciprofloxacin	Resistant	23	0	0	23 (100)
Klebsiella pneumonia	Ceftazidime	Resistant	15	0	0	15 (100)
	Cefepime	Resistant	15	0	2 (13.3)	13 (86.7)
	Amikacin	Resistant	8	1 (12.5)	0	7 (87.5)
	Ciprofloxacin	Resistant	12	0	0	12 (100)
Acinetobacter baumannii	Ceftazidime	Resistant	8	0	0	8 (100)
	Cefepime	Resistant	9	0	0	9 (100)
	Imipenem	Resistant	9	0	0	9 (100)
	Amikacin	Resistant	8	0	0	8 (100)
		Intermediate	1	1 (100)	0	0
	Ciprofloxacin	Resistant	9	0	0	9 (100)

ESBL=Extended-spectrum β-lactamase

urine samples (39.6% and 88.4%, respectively).[22,23] Also, difference in the origin of isolated pathogens (nosocomial versus nonnosocomial) may be another contributing factor. Other studies have reported higher rates of ESBL production in *K. pneumoniae* isolates.[24,25]

Our study showed very high levels of resistance to antibiotics among ESBL-producing Gram-negative bacteria causing nosocomial UTIs. Carbapenems (imipenem and meropenem) and piperacillin/tazobactam were the most effective agents against ESBL-positive *E. coli* and *K. pneumoniae* strains. Furthermore, amikacin and nitrofurantoin had acceptable activity only on *E. coli* isolates. Our results show that high numbers of ESBL-producing Gram-negative bacilli are resistant not only to extended-spectrum cephalosporins including cefepime, but also to aminoglycosides (e.g., amikacin) and quinolones (e.g., ciprofloxacin and levofloxacin). The susceptibility pattern of these bacteria to various antimicrobials depends on ESBL genotype.[26] Consistent with our results, in the study of Tankhiwale *et al*. in India, multi-drug resistance was found to be significantly more in ESBL producing isolates (90.5%) than non ESBL-producers (68.9%).[21]

Finally, our study showed good consistency between the results of disk diffusion and E-test methods for antimicrobial susceptibility testing of ESBL-producing Gram-negative bacilli. Most inconsistencies were observed for amikacin against *E. coli* and *P. aeruginosa* strains. Therefore, as also shown in similar comparative studies,[27-31] it seems that the agreement level for these two methods depends on both antibiotic and microorganism tested.

Our study showed that large numbers of Gram-negative bacteria causing nosocomial UTIs produce ESBL with most being multi-drug resistant (MDR). Therefore, routine ESBL detection testing and subsequent antibiogram with disk diffusion method could be useful to determine the best treatments for UTI.

AUTHORS' CONTRIBUTION

All authors contributed to the research idea and study design. Rasool Soltani prepared the manuscript.

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How to cite this article: Soltani R, Ehsanpoor M, Khorvash F, Shokri D. Antimicrobial susceptibility pattern of extended-spectrum β-lactamase-producing bacteria causing nosocomial urinary tract infections in an Iranian referral teaching hospital. J Res Pharm Pract 2014;3:6-11.

Source of Support: Nil, **Conflict of Interest:** None declared.