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Original Article

Etiologic agents and extended-spectrum beta-lactamase production in urinary tract infections in Sanandaj, Iran

Rashid Ramazanzadeh*

Department of Microbiology, Faculty of Medicine, Kurdistan University of Medical Science, Sanandaj- Iran

Abstract. Extended-spectrum β-lactamases has emerged as an important mechanism of resistance in Gram-negative bacteria. In this study, we aimed to determine the frequency of extended spectrum beta lactamase producing isolates in (urinary tract infections). ESBL-production was tested by the double-disc synergy method and more confirmed by PCR amplification. Out of 188 isolated microorganisms Escherichia coli (80%) was the most frequent. Production was determined in 14.5% of clinically gram negative isolates. CTX-M type was the most prevalent type (12.7%) and SHV and TEM type were in the next ranks. Our data indicate the spread of these enzymes in clinically gram negative isolates in urinary tract samples collected from two general hospitals of Sanandaj.

Key words: Urinary tract infections; extended-spectrum βeta-lactamase; *Escherichia coli*

1. Introduction

Urinary tract infection is among the most common nosocomial and community acquired infections. Information on prevailing levels of antimicrobial resistance among common pathogens associated with urinary tract infection is useful in making an appropriate choice of empiric therapy (1). Resistance to antibiotic treatment in patients with urinary tract infections (UTIs) is a representative example of the increasing problem of antimicrobial resistance (2).

Extended-spectrum β-lactamases (ESBLs) has emerged as an important mechanism of resistance in Gram-negative bacteria. More than 300 variants of ESBL have been described and the majority of these belong to the CTX-M, TEM and SHV family (3). Unfortunately, ESBL-producing organisms often also have resistance determinants to other important antibiotic groups, such as aminoglycosides and fluoroquinolones, leaving an extremely limited range of effective agents.

Delay in appropriate therapy for infections with ESBL producing bacteria not only prolongs hospital stay, but is also associated with increased mortality (4-8).

*Correspondence: Corresponding author, e-mail: rashid@muk.ac.ir or atrop_t@yahoo.com
Address: Microbiology Department, Faculty of Medicine, Kurdistan University of Medical Science, Pasdaran street, Post cod. 66177-13446, Sanandaj- Iran, phon:+989143104424, Fax: +98(871)6664674

ESBLs has been reported from other sample site from Iran (3,9-14). *Escherichia coli* accounts for most uncomplicated UTIs. However, recent data indicate that urinary tract infections (UTIs) caused by ESBL-producing *E. coli* may be an emerging problem in various parts of the world (4-7,15,16).

The aim of this study was to characterize ESBL-producing gram negative bacteria isolated from the urine of patients based on their susceptibility to antimicrobial agents.

2. Materials and methods

2.1. Study population and specimen types

This study was conducted at Faculty of Medicine, Kurdistan University of Medical science, Sanandaj, Iran from September 2007 to September 2008; isolates were collected from urinary tract of 188 patients who were referred for Toohid and Beesat Hospitals. All samples were routinely cultured on MacConkey and blood agar plates. Isolates were identified at the species level using standard biochemical tests and microbiological methods. Only one isolate per patient was included in the study.

2.2. Antibiotic susceptibility testing

Antibiograms were carried out with Kirby-Bauer method. Disk-diffusion tests were carried out with antibiotic-containing disks on Mueller-Hinton agar plate (Merck).

The results were expressed as susceptible or resistant according to the criteria recommended by the 'Clinical and Laboratory Standards Institute (CLSI) (17). The following antimicrobial agents were tested: amikacin (30 µg), ampicillin (10 µg), cefalotin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), co-trimoxazole (1.25/23.75 µg), gentamicin (10 µg), nalidixic acid (30 µg), tetracycline (30 µg), ceftizoxime (30 µg), and norfloxacin (10 µg).

2.3. Detection of ESBL production

ESBL production was detected using the double-disk synergy (DDS) test [18]. ESBL presence was assayed using the following antibiotic disks (MAST, UK): cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime (30 µg), and ceftazidime/clavulanic acid (30/10 µg).

2.4. Statistical analysis

Data were entered into a database using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). Differences between proportions were analyzed using the $\chi 2$ test. All differences in which the probability of the null hypothesis was $p < 0.05$ were considered significant.

2.5. ESBL- PCR

Template DNA was prepared as follows: a cell pellet from 1.5 ml of overnight culture was resuspended in 500 µl of TE (10 mM Tris, 1 mM EDTA, pH 8.0) after centrifugation and boiling for 10 min. After centrifugation, the supernatant was used for PCR. The primers and conditions for PCR are listed in Table 1 (19).

Table 1. Primers and conditions of polymerase chain reaction used in this study

Primer	PCR primers (5′→3′)	Expected size (bp)	PCR conditions	PCR product
SHV-F	**GGGTTATTCTTATTTGTCGC**	928	94 ºC, 5 min; 35	SHV-1, -2, -5, -7, -
SHV-R	**TTAGCGTTGCCAGTGCTC**		cycles of 94 ºC, 1	11, -12, -18, -26, -32,
			min, 58 ºC, 1 min,	-33, -38, -44, -46, -49
			72 ºC, 1 min	
TEM-F	**ATAAAATTCTTGAAGACGAAA**	1080	94 ºC, 5 min; 35	TEM-1, -52, -71, -
TEM-R	**GACAGTTACCAATGCTTAATCA**		cycles of 94 ºC, 1	104, -105, -138, -151,
			min, 58 ºC, 1 min,	-152
			72 ºC, 1 min	
CTX-M-F	**ACGCTGTTGTTAGGAAGTG**	759	94 ºC, 5 min; 35	CTX-M-1, -3, -12, -
CTX-M-R	**TTGAGGCTGGGTGAAGT**		cycles of 94 ºC, 45	15, -22, -30, -32, -33,
			s, 58 ºC, 45 s, 72 ºC,	-38, -52, -57, -58, -
			1 min	60, -61
OXA-1-F	**ACACAATACATATCAACTTCGC**	813	94 ºC, 5 min; 35	OXA-1, -4, -30, -31, -
OXA-1-R	**AGTGTGTTTAGAATGGTGATC**		cycles of 94 ºC, 1	47
			min, 58 ºC, 1 min,	
			72 ºC, 1 min	
OXA-2-F	**TTCAAGCCAAAGGCACGATAG**	814	94 ºC, 5 min; 35	OXA-2, -3, -15, -21, -
OXA-2-R	**TCCGAGTTGACTGCCGGGTTG**		cycles of 94 ºC, 45	32
			s, 61 ºC, 45 s, 72 ºC,	
			1 min	

3. Results

We received and examined 188 urine specimens during the study period. The species distribution included *Escherichia coli* 150(80%), *Klebsiella pneumoniae* 10(5.3%), *Pseudomonas aeruginosa* 14(7.5%), *Enterobacter aerugenes* 7(3.8%), and *Proteus mirabilis* 1(0.5%) and *Citrobacter freundii* 1 (0.5%). The highest resistance rate was (51%) to SXT and the lowest (14.4%) to amikacin. Table 2 summarizes the percentage of resistance of gram negative bacilli isolated from inpatients and outpatients.

Table 2. The pattern of antimicrobial resistance of gram-negative bacteria isolated from patients

Bacteria		Antibiotic resistance (%)												
	No.	CTX	CAZ	SXT	GM	AN	AM	CF	CRO	CP	CT	NOR	TE	NA
C. freundii	1	0.5	0.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. coli	150	22.9	22.9	39.4	13.8	5.9	38.3	17.6	20.2	17.6	10.6	14.9	35.1	19.1
E. aerogenes	7	2.1	2.1	2.1	1.6	2.1	1.6	1.1	1.1	1.1	1.1	1.1	1.6	1.6
P.agglomerans	10	1.6	1.6	2.1	0.5	2.1	1.6	1.1	1.6	1.1	1.6	0.5	1.1	2.1
P.oryzihabitans	3	1.1	1.1	1.1	1.1	1.6	1.6	0.5	0.5	0.5	0.5	0.5	1.6	1.6
K. pneumoniae	1	0.5	0.5	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. mirabilis	14	3.7	3.7	5.3	2.7	2.7	1.6	1.6	1.6	1.6	1.6	1.6	1.6	2.7
P. aeruginosa	1	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
S. arizona	1	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	188	32.4	32.4	51.1	19.7	14.4	45.7	21.8	25.0	21.8	15.4	18.6	41.0	27.7

CTX: Cefotaxime, CAZ: Ceftazidime, SXT: co-trimoxazole, GM: Gentamicin, AN: Amikacin, AM: Ampicillin, CF:Cefalotin, CRO: Ceftriaxone, CP: Ciprofloxacin, CT: Ceftizoxim, NOR: Norfloxacin, TE: Tetracyclin, NA: Nalidixic acid

Out of 188 Gram-negative isolates, 27 isolates were positive for ESBL Table 3. CTX-M type was most prevalent type (12.7%) and among the isolated bacteria *E. coli* was high rate of ESBL production in this study.

Table 3. The rate of extended spectrum beta-lactamase types in gram-negative bacteria isolated from urinary tract infections

Microorganisms		ESBL type				
	No.	CTX-M	TEM	SHV	OXA-1	OXA-2
Citrobacter freundii	1	0.5	0.5	0.5	0.0	0.0
Escherichia coli	150	9.6	6.9	8.5	5.9	2.1
Enterobacter aerogenes	7	0.0	0.0	0.0	0.0	0.0
Pantoea agglomerans	10	1.6	1.1	1.6	0.0	0.5
Pseudomonas oryzihabitans	3	0.5	0.5	0.5	0.5	0.0
Klebsiella pneumoniae	1	0.0	0.5	0.0	0.5	0.0
Proteous mirabilis	14	0.5	0.5	0.5	0.5	0.5
Pseudomonas aeruginosa	1	0.0	0.0	0.0	0.0	0.0
Salmonella arizona	1	0.0	0.0	0.0	0.0	0.0
Total	188	12.8	10.1	11.7	7.4	3.2

4. Discussion

Urinary tract infections (UTIs) are one of the most frequently encountered conditions in clinical medical practice requiring antimicrobial therapeutic intervention. To date, *E. coli* has been the most common isolated pathogen causing UT Is (20). In this study, 80% of isolates belonged to *E. coli* strain, followed by *Klebsiella pneumoniae* (5.3%), *Pseudomonas aeruginosa* (7.5%), *Enterobacter aerugenes* (5.4%), *Proteus mirabilis* (0.5%), and *Citrobacter freundii* (0.5%). The prevalence of other gram negative bacteria was in accordance with other reviewed studies (21-28).

Trimethoprim–sulfamethoxazole has been successfully used for treatment of urinary tract infections (20). However, the prevalence of resistance to SXT among clinical isolates is increasing; which is shown in the present study (resistance rates, 51%). Due to the reduced activity of SXT against E. coli isolates, fluoroquinolones such as ciprofloxacin are being used frequently as 1st-line treatment of UTIs. Resistance to norfloxacin and ciprofloxacin was 18.6% and 21.8%, respectively. In other reviewed studies from Iran the resistance rate to floroquinolons was higher than this study (29,30). The results of this study confirmed reduced activity of ciprofloxacin against gram negative isolates. This finding suggests probable limitation of the use of fluoroquinolones for the treatment of these infections as first line choice. Increased resistances to the other agents reflect their wider use in urinary tract infection.

ESBL-producing among clinically gram negative isolates have also become a serious problem in the clinical setting (31). In this study, overall ESBL production rate among gram negative isolates was 14.5%. ESBL-producing *E. coli* isolates including 10% of them. Since prior studies have reported that about 82.9% of clinical isolates were ESBL producers in Tehran (32). Although the prevalence of ESBL-producing *E. coli* isolates in Asian are variable (33). In considering that CTX-M-type ESBLs, the most widely spread enzymes among non-TEM and non-SHV plasmid-mediated ESBLs, the rate of this enzyme was 12.77% in this study. SHV and TEM type were in the next ranks. The CTX-M enzymes have originated from *Kluyvera* spp. and recently gained prominence in Enterobacteriaceae with reports from various parts of the world (6,34-36). Further molecular characterization of the ESBL types are needed to determine the clonal transmission of these enzymes in order to control the drug resistance in this province.

5. Conclusion

In conclusion, our data indicate the spread of ESBL type in clinically gram negative isolates in two general hospitals of Sanandaj. The most bacterial isolate was *E. coli* and CTX-M was the most prevalent ESBL type. Further clinical study is required to monitor the molecular epidemiology and transmission of ESBL types in order to control the spread of drug resistance among gram negative bacteria in Community and hospitals settings.

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