Research Journal of Medical Sciences 4 (3): 243-247, 2010
ISSN: 1815-9346

Etiology and Antibiotic Resistance Patterns of Community-Acquired Extended-Spectrum Beta-Lactamase-Producing Gram Negative Isolates in Sanandaj

[1]Rashid Ramazanzadeh, [2]Fariba Farhadifar and [3]Majid Mansouri
[1]Department of Microbiology, [2]Department of Gynecology, [3]Department of Pediatrics,
Faculty of Medicine, Kurdistan University of Medical Science, Sanandaj, Iran

Abstract: The aim of this study was to determine the distribution and antibiotic susceptibility patterns of Extended-Spectrum Beta-Lactamase (ESBL) producing bacterial strains isolated from patients with community acquired infections at Sanandaj two major hospitals in Iran. The study took place at the Faculty of Medicine, Kurdistan University of Meical science, Sanandaj, Iran. About 158 g negative strains were evaluated isolated from various clinical specimens. The double-disk synergy test was performed on the isolates for the detection of ESBL. These genes were confirmed by PCR methods. The majority of community acquired ESBL types belong to CTX-M (10.76%) and SHV (10.76%). Resistance to ceftazidime and cefotaxime were 33.54 and 34.18%, respectively. Multiple resistances antibiotics were often associated with ESBL producing organisms. This is the first report of prevalence of ESBL producing isolates originating from the community in Sanandaj. ESBL producing isolates types especially CTX-M-producing gram negative bacteria are a rapidly developing problem in Iran. A heightened awareness of these organisms by clinicians and enhanced testing by laboratories including molecular surveillance studies is required to reduce treatment failures to limit their introduction into hospitals and to prevent the spread of these emerging pathogens within the community.

Key words: Extended-spectrum beta-lactamase, community-acquired, antibiotic, bacterial stain, infaction, pathogen

INTRODUCTION

Most of resistance to anti bacterial agents associated with admission to hospitals. Resistance to extended spectrum beta lactames in gram negative bacteria are predominantly community-acquired source. Therefore, Community acquired infection are interesting in controlling the spread of resistance to hospitals (Laupland *et al*., 2008).

Extended-Spectrum Beta-Lactamases (ESBLs) are enzymes conferring broad resistance to to beta-lactam antibiotics including third-generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidim. (Apisarnthanarak *et al*., 2007; Paterson, 2006; Sidorenko *et al*., 2004; Apisarnthanarak *et al*., 2008), <150 type of ESBLS have been described and the majority of this enzymes belonging to the TEM and SHV family (Valverde *et al*., 2004; Mendelson *et al*., 2005).

The beta lactamase genes are located on the large plasmids that confer resistance to other classes of antimicrobial agents and are readily transmissible from strain to strain and between different species of enteric Gram-negative bacilli (Paterson, 2006). Extended-spectrum Beta-Lactamases (ESBLs) are found in a variety of members of the family Enterobacteriaceae (Mendelson *et al*., 2005). ESBLs are detected most commonly in *Klebsiella pneumoniae* and *Escherichia coli* (Lin *et al*., 2006). The aim of the present study was to determinate the prevalence and the type of the ESBLs produced by gram negative isolates and antibiotic susceptibility pattern of this isolates among nonrepetitive clinical strains over an 12 months period from January 2007 to January 2008 isolated from outpatient referred to Microbiology laboratory at the University Hospital of Sanandaj, Iran.

MATERIALS AND METHODS

Study population and specimen types: This study was conducted at Faculty of Medicine, Kurdistan University of Meical science, Sanandaj, Iran. Isolates of gram negative bacteria were collected from various specimens of patients who were referred for Toohid sand Beesat Hospitals microbiology lsboratories. Specimens included urine, wound, respiratory tube, blood, cerebrospinal fluid, etc.

Microbiological methods: All samples were routinely cultured on MacConkey and blood agar plates. Blood samples were cultured in Blood culture bottles. Isolates

Corresponding Author: Rashid Ramazanzadeh, Department of Microbiology, Faculty of Medicine, Kurdistan University of Medical Science, Sanandaj, Iran

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

were identified at the species level using standard biochemical tests and microbiological methods. Only one isolate per patient was included in the study.

Antibiotic susceptibility testing: 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 Laboratory Standards Institute (CLSI) (NCCLS Document M2-A7, 2000). The following antimicrobial agents were tested: amikacin (30 μg), ampicillin (10 μg), cefalotin (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), cotrimoxazole (1.25/23.75 μg), gentamicin (10 μg), tetracycline (30 μg), ceftizoxime (30 μg) and norfloxacin (10 μg).

Detection of ESBL production: ESBL production was detected using the Double-Disk Synergy (DDS) test (Jarlier *et al*., 1988). 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).

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 χ^2-test. All differences in which the probability of the null hypothesis was $p<0.05$ were considered significant.

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 shown in (Table 1) (Yao *et al*., 2007).

RESULTS AND DISCUSSION

During the study period, 301 consecutive clinical isolates of gram-negative were isolated. Of these, 158 (52%) were belong to outpatient referred to hospitals microbiology laboratory. As shown in Table 2 most of strains were isolated from urinary tract samples. *Escherichia coli* were most frequent isolates and *Pseudomonas aeruginosa* and other gram negative bacteria were in the next grads. Distributions of isolates in two hospitals were the same.

All isolates of *E. coli* were examined for Extended Spectrum Beta-Lactamase (ESBL) production. Of the 158 *isolates*, 20 (12.66%) were positive for ESBL enzymes. The sources of the ESBL-producing isolates tested are shown in Table 3. CTX-M type was the most detected shown in this study. The most abundant source of ESBL-producing strains was *Escherichia coli*.

The results of antibiotic susceptibility testing are shown in Table 4. In general, resistance rates of *E. coli* isolates were higher than other gram negative bacteria. Amikacin, norfloxacin, ciprofloxacin and third-generation cephalosporins were shown to be the most active antibiotics against these isolates *in vitro*.

Most of research focused on epidemiological studies of ESBLs in hospital settings. However, the epidemiology of these infections outside hospitals had not been studied well in this region. This study confirms the existence of ESBLs as a cause of infection in outpatients referring to major educational hospitals laboratories.

Urinary Tract Infection (UTIs) were the most frequent infection type in outpatients samples and *E. coli* strains were isolated from the most of community-acquired UTIs (Table 2). *Escherichia coli* is frequently found to be responsible for intra abdominal and soft tissue infections (Rodriguez-Bano *et al*., 2004, 2006).

Therefore, resistance to commonly used antimicrobials in *E. coli* represents a problem for the treatment of these infections. As shown in Table 4 and Table 3 *E. coli* causing UTIs was more resistance to antibacterial agents tested *in vitro* and in comparing with other gram negative isolates producing most of ESBL enzymes.

Table 2: Distribution of gram negative isolates according to clinical sources and hospitals

Hospital	Sample source	Microorganisms: Alkaligenes	*Citrobacter freundii*	*E. coli*	*Klebsiella pneumoniae*	*Proteous mirabilis*	*Pseudomonas aeruginosa*	*Salmonella arizona*	*Enterobacter* sp.	Total
		(%)								
Besat H.	Blood	0.00	0.00	0.00	0.63	0.00	0.00	0.00	0.00	0.63
	Urine	0.00	0.00	43.04	3.80	0.00	2.53	0.63	3.80	53.80
	Wound	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.63
Besat H. Total		0.00	0.00	43.04	4.43	0.00	2.53	0.63	4.43	55.06
Toohid H.	Blood	0.63	0.00	1.27	0.00	0.00	0.00	0.00	0.00	1.90
	Lung	0.00	0.00	0.00	0.00	0.00	0.63	0.00	0.00	0.63
	Urine	0.00	0.00	34.81	1.27	0.63	4.43	0.00	0.63	41.77
	Wound	0.00	0.63	0.00	0.00	0.00		0.00	0.00	0.63
Toohid H. Total		0.63	0.63	36.08	1.27	0.63	5.06	0.00	0.63	44.94
Total		0.63	0.63	79.11	5.70	0.63	7.59	0.63	5.06	100.00

Table 3: Frequency of extended spectrum beta-lactamase enzymes in gram negative isolates

TEM	Alkaligenes	Microorganisms: *Citrobacter freundii*	*E. coli*	*Klebsiella pneumoniae*	*Proteous mirabilis*	*Pseudomonas aeruginosa*	*Salmonella arizona*	*Enterobacter* sp.	Total
		(%)							
Negative	0.63	0.63	72.15	4.43	0.00	6.96	0.63	5.06	90.51
Positive	0.00	-	6.96	1.27	0.63	0.63	0.00	0.00	9.49
CTX-M									
Negative	0.63	0.63	70.25	3.80	0.63	7.59	0.63	5.06	89.24
Positive	0.00	0.00	8.86	1.90	0.00	0.00	0.00	0.00	10.76
SHV									
Negative	0.63	0.63	70.89	3.80	0.63	6.96	0.63	5.06	89.24
Positive	0.00	0.00	8.23	1.90	0.00	0.63	0.00	0.00	10.76
OXA-1									
Negative	0.63	0.63	73.42	5.70	0.00	6.96	0.63	5.06	93.04
Positive	0.00	0.00	5.70	0.00	0.63	0.63	0.00	0.00	6.96
OXA-2									
Negative	0.63	0.63	76.58	5.06	0.63	6.96	0.63	5.06	96.20
Positive	0.00	0.00	2.53	0.63	0.00	0.63	0.00	0.00	3.80

Table 4: Antibiotic resistance rate (%) of gram negative bacteria isolated from outpatients

CTX	Alkaligenes	Microorganisms: *Citrobacter freundii*	*E. coli*	*Klebsiella pneumoniae*	*Proteous mirabilis*	*Pseudomonas aeruginosa*	*Salmonella arizona*	*Enterobacter* sp.	Total
		(%)							
Resistance	0.00	0.00	23.42	1.90	0.63	4.43	0.00	3.16	33.54
CAZ									
Resistance	0.00	0.00	24.05	1.90	0.63	4.43	0.00	3.16	34.18
SXT									
Resistance	0.63	0.00	36.08	1.90	0.00	4.43	0.63	3.16	46.84
GM									
Resistance	0.00	0.00	14.56	0.63	0.00	2.53	0.00	2.53	20.25
AN									
Resistance	0.00	0.00	4.43	0.63	0.00	0.63	0.00	1.27	6.96
AM									
Resistance	0.63	0.63	38.61	1.90	0.63	3.80	0.00	3.16	49.37
CF									
Resistance	0.63	0.63	17.72	1.90	0.63	4.43	0.00	3.16	29.11
CRO									
Resistance	0.63	0.00	20.89	0.00	0.63	4.43	0.00	2.53	29.11
CP									
Resistance	0.00	0.00	16.46	0.63	0.00	3.80	0.00	1.27	22.15
NOR									
Resistance	0.00	0.00	15.19	0.63	0.00	3.80	0.00	1.27	20.89
TE									
Resistance	0.63	0.00	39.24	2.53	0.00	5.70	0.63	3.16	51.90
NA									
Resistance	0.63	0.00	20.25	2.53	0.00	4.43	0.00	1.27	29.11

AM-Ampicillin, CF-Cefalotin, CRO-Ceftriaxone, CTX-Cefotaxim, CAZ-Ceftazidim, GM-Gentamicin, AN- Amikacin, CP-Ciprofloxacin, NOR- Norfloxacin, TE-Tetracyclin, NA-Nalidixic acid, SXT-Trimethoprim sulfamethoxazole

In this study, isolates for TEM, SHV, OXA and CTXM-type genes were tested. Distribution of ESBL types varies widely by geographic region (Bradford, 2001). The experimental approach for the detection of multiple-lactamase-encoding genes in the same isolate allowed precise assessment of the contents of the strains. By this procedure, several of the strains investigated in this study produced combination of ESBL types and such a combination was found in 12.66% of the strains tested. The majority of community-acquired ESBL types belong to CTX-M (10.76%) and SHV (10.76%). This ESBL have previously been described in Iran but the rate of prevalence was differe fron this study results. In (Mehrgan and Rahbar, 2008) studies of the 135 ESBL-positive isolates, 22 (16.3%) appeared to be of the CTX-M type based on a phenotypic determination method and also (Hosseini-Mazinani *et al*., 2007) reported that 60% carried the TEM gene and 26% of the organisms harbored SHV type enzymes. In other studies the prevalence of bla (SHV) and bla (TEM) were 69.6 and 32.1%, respectively (Shahcheraghi *et al*., 2007).

CTX-M-producing strains of *E. coli* have emerged as a rapidly developing problem and have been reported from most part of the world (Woodford *et al*., 2004). For example (Valverde *et al*., 2004) reported that nearly 70% of patients colonized with CTX-M ESBL types were not hospitalized, demonstrating that the community compartment is essential for the maintenance of these enzymes. Moreover, the community can be a reservoir of ESBLs not yet detected in clinical isolates (Valverde *et al*., 2004). Therefore the rate of CTX-M b-lactamases may be high. The low number of CTX-M producing organisms identified may be a result of insufficient laboratory screening practices for CTX-M enzyme, the lack of routine use of PCR to identify CTXM type genes and the lack of screening for the presence of ESBL-producing organisms from community based sites.

Organisms that express an ESBL are frequently resistant to other antimicrobial agents as many of these additional resistance genes are encoded on the ESBL-associated plasmid (Jacoby and Sutton, 1991).

In the study, high rates of resistance were detected against tetracycline, trimethoprim-sulfamethoxazole and the ampicillin. Resistance to ceftazidime and cefotaxime were 33.54 and 34.18%, respectively. These results were in agreement with Mehrgan and Rahbar (2008); Hosseini-Mazinani *et al*. (2007) results. The resistance prevalence in the study may reflect significant antibiotic pressure.

CONCLUSION

In this study ESBL-producing gram negative bacteria are emerging multiple resistance organisms. Patients with these strains need prolonged treatment duration that increase costs of treatment and may be increased mortality rate. Physicians should be aware of the occurrence of ESBL-producing bacteria among community and target initial appropriate empiric antimicrobial therapy to reduce the spread of this isolate to hospital settings. When treatment protocols are designed, the prevalence of ESBL isolates must be taken into consideration and on this basis, a rational choice of empirical antibiotic therapy can then be recommended. The data describe the first occurrence of ESBL-producing gram negative bacteria in outpatients referring to Sanandaj hospitals and highlight the need for additional clinical and molecular epidemiologic studies along with intervention trials to help minimize the emergence of endogenous and exogenous ESBL producing microorganisms.

ACKNOWLEDEGMENTS

The researchers kindly thank the Research deputy of Kurdistan university of Medical science for financial support. We acknowledge Mr. Ghafari, Mrs. Amjadi, Mrs. Giyasi amd Mrs. Bahmani the co-operative colleagues in the microbiology laboratory in Toohid and Beasat Hospitals and Faculty of Medicine for their technical assistance.

REFERENCES

Apisarnthanarak, A., P. Kiratisin and L.M. Mundy, 2008. Predictors of mortality from community-onset bloodstream infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. Infect. Control Hospital Epidemiol., 29: 671-674.

Apisarnthanarak, A., P. Kiratisin, P. Saifon, R. Kitphati, S. Dejsirilert and L.M. Mundy, 2007. Clinical and molecular epidemiology of community-onset, extended-spectrum beta-lactamase-producing *Escherichia coli* infections in Thailand: A case-case-control study. Am. J. Infect. Control, 35: 606-612.

Bradford, P.A., 2001. Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology and detection of this important resistance threat. Clin. Microbiol. Rev., 14: 933-951.

Hosseini-Mazinani, S.M., F. Eftekhar, M. Milani and S. Ghandili, 2007. Characterization of beta-lactamases from urinary isolates of *Escherichia coli* in Tehran. Iran Biomed. J., 11: 95-99.

Jacoby, G.A. and L. Sutton, 1991. Properties of plasmids responsible for production of extended-spectrum beta-lactamases. Antimicrob. Agents Chemother., 35: 164-169.

Jarlier, V., M.H. Nicolas, G. Fournier and A. Philippon, 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis., 10: 867-878.

Laupland, K.B., D.L. Church, J. Vidakovich, M. Mucenski and J.D. Pitout, 2008. Community-onset extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli*: Importance of international travel. J. Infect., 57: 441-448.

Lin, T.L., S.I. Tang, C.T. Fang, P.R. Hsueh, S.C. Chang and J.T. Wang, 2006. Extended-spectrum beta-lactamase genes of *Klebsiella pneumoniae* strains in Taiwan: Recharacterization of shv-27, shv-41 and tem-116. Microbial Drug Resistance, 12: 12-15.

Mehrgan, H. and M. Rahbar, 2008. Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* in a tertiary care hospital in Tehran, Iran. Int. J. Antimicrob. Agents, 31: 147-151.

Mendelson, G., V. Hait, J. Ben-Israel, D. Gronich, E. Granot and R. Raz, 2005. Prevalence and risk factors of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in an Israeli long-term care facility. Eur. J. Clin. Microbiol. Infect. Dis., 24: 17-22.

NCCLS Document M2-A7, 2000. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. 7th Edn., National Committee for Clinical Laboratory Standards, Wayne, PA.

Paterson, D.L., 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. Am. J. Infect. Control, 34: S20-S28.

Rodriguez-Bano, J., M.D. Navarro, L. Romero, M.A. Muniain and M.D. Cueto *et al*., 2006. Bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli* in the CTX-M era: A new clinical challenge. Clin. Infect. Dis., 43: 1407-1414.

Rodriguez-Bano, J., M.D. Navarro, L. Romero, L. Martínez-Martínez and M.A. Muniain *et al*., 2004. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. J. Clin. Microbiol., 42: 1089-1094.

Shahcheraghi, F., H. Moezi and M.M. Feizabadi, 2007. Distribution of TEM and SHV beta-lactamase genes among *Klebsiella pneumoniae* strains isolated from patients in Tehran. Med. Sci. Monit., 13: BR247-BR250.

Sidorenko, S.V., A.G. Berezin and D.V. Ivanov, 2004. [Molecular mechanisms of cephalosporin resistance in Gram-negative bacteria of the Enterobacteriaceae family]. Antibiot Khimioter, 49: 6-16.

Valverde, A., T.M. Coque, M.P. Sanchez-Moreno, A. Rollan, F. Baquero and R. Canton, 2004. Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain. J. Clin. Microbiol., 42: 4769-4775.

Woodford, N., M.E. Ward, M.E. Kaufmann, J. Turton and E.J. Faqan *et al*., 2004. Community and hospital spread of *Escherichia coli* producing CTX-M extended spectrum beta-lactamases in the UK. J. Antimicrob. Chemother., 54: 735-743.

Yao, F., Y. Qian, S. Chen, P. Wang and Y. Huang, 2007. Incidence of extended-spectrum beta-lactamases and characterization of integrons in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolated in Shantou, China. Acta. Biochim. Biophys. Sin (Shanghai), 39: 527-532.