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Full Length Research Paper

Prevalence and characterization of extended-spectrum beta-lactamase production in clinical isolates of *Klebsiella* spp.

Rashid Ramazanzadeh

Microbiology Department, Faculty of Medicine, Kurdistan University of Medical Science, Sanandaj, Iran.
E-mail: rashid@muk.ac.ir or atrop_t51@yahoo.com.

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Extended-spectrum beta-lactamases (ESBLs) are most prevalent in *Klebsiella pneumoniae*. This organism is frequently isolated from clinical specimens and can cause septicemia, pneumonia or urinary tract infection. We investigated a spread of *Klebsiella* spp. isolates producing ESBL in a university hospital of Sanandaj-Iran. Over one year period, a total of 48 *K. pneumoniae* isolates, were examined by double disk tests and PCR methods. Ten isolates were defined as ESBLs. The ESBL producer isolates was more resistant to selected antibiotics than ESBL negative isolates. The most frequent ESBL type was CTX-M. This is the first report of *Klebsiella* spp. isolates producing ESBL in Sanandaj hospitals. Production of ESBLs by *K. pneumoniae* is a widespread nosocomial problem. Knowledge about their prevalence is essential to guide towards appropriate infection control and antibiotic management strategies.

Key words: Extended-spectrum β-lactamase (ESBL), hospital, *Klebsiella* spp.

INTRODUCTION

Multidrug resistant gram negative bacilli belonging to the family Enterobacteriaceae have been increasingly responsible for infections among the neonates admitted to the hospitals in many countries and *Klebsiella* spp. Constitutes a majority of these pathogens (Baby et al., 2008; Liu et al., 2008; Usha et al., 2008; Vinue et al., 2008). Resistance of *Klebsiella pneumoniae* to extended-spectrum β-lactams antibiotics is commonly mediated by β-lactamases. Indeed, *K. pneumoniae* is a major host of plasmid-located extended-spectrum beta-lactamases (ESBL). ESBLs are clavulanate-susceptible enzymes capable of hydrolyzing oxyimino-cephalosporins and monobactams, but not cephamycins and carbapenems. Infections due to ESBL-producing bacteria present a major therapeutic dilemma since the choice of antibiotics is restricted. Nosocomial outbreaks are often caused by ESBL-producing isolates, particularly in intensive care, they result from the clonally transmission of epidemic isolate and/or the horizontal transfer of resistance genes Messai et al. (2008).

Many and regular studies on ESBL-producing bacteria are conducted in numerous countries, whereas very few information on this issue is available in Iran. We performed this study in clinical isolates of *Klebsiella* spp., collected from two hospitals in Snanadaj, to investigate the prevalence of ESBLs.

MATERIALS AND METHODS

Study population and specimen types

This study was conducted at Faculty of Medicine, Kurdistan University of Medical science, Sanandaj, Iran. From January 2007 to January 2008, 48 consecutive, non-duplicate isolates of *Klebsiella* spp. were collected from various specimens (absences, blood, lung, trachea and urine) of patients who were referred to Toohid and Beesat Hospitals.

Microbiological methods

All samples were routinely cultured on MacConkey and blood agar plates. Blood samples were cultured in Blood culture bottles. Isolates were identified at the species level using standard biochemical tests and microbiological methods. Only one isolate per patient was included in the study.

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

Table 2. Distribution according to clinical sources of *Klebsiella* spp. isolates producing extended-spectrum beta-lactamases (ESBL).

Source	Number of ESBL-producing isolation	
	Tested	Positive (%)
Abseces	1	0 (0)
Blood	17	3 (6.25)
Lung	4	2 (4.17)
Trachea	6	2 (4.17)
Urine	20	3 (6.25)
Total	48	10 (20.83)

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 and Laboratory Standards Institute (CLSI) guidelines Clinical and laboratory standards institute (2009). The following antimicrobial agents were tested: amikacin (30 µg), cefalotin (30 µg), cefotaxime (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 (30µg), 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 $\chi 2$ 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 listed in (Table 1) Yao et al. (2007).

RESULTS

A total of 48 clinical isolates of *Klebsiella* spp. collected from two general hospitals in Sanandaj, Iran were studied. The sources of the ESBL-producing *Klebsiella* spp. isolates tested are shown in (Table 2). Urinary tract infections were the most abundant source of ESBL-producing *Klebsiella* spp. strains (6.25%; $p < 0.001$).

Ten (20.83%) isolates were defined as ESBLs producers according to the results of disc-diffusion and PCR methods. The antimicrobial resistance pattern of *Klebsiella* spp. isolated from the Sanandaj two general hospitals are depicted in (Table 3). The ESBL producer isolates was more resistant to selected antibiotics than ESBL negative isolates.

The pattern extended spectrum beta-lactamase types based on hospitals is shown in (Table 4).The most frequent ESBL type was CTX-M in this study.

DISCUSSION

ESBLs were found in 10 (20.83%) of 48 clinical isolates. The incidence of ESBL-producing isolates varies according to countries, regions or even hospitals. The ESBLs prevalence rate in the present study was much lower than those reported from Iran Feizabadi et al. (2006); Shahcheraghi et al. (2007) and Turkeydemir et al. (2008) but was in accordance with that reported from Italy Spanu et al. (2002), Algeria et al. (2008) and KoreaKo et al. (2007). The presence of ESBL-producing isolates is indicative of a selection pressure, the new betalactams, such as cefotaxime, are extensively used in hospital

Table 3. Antimicrobial Resistance Pattern of *Klebsiella* spp. Isolates (in percentage) from Sanadaj hospitals.

Isolates	A	Cf	Ct	Ci	G	An	Cp	Nor	Te	Na	Sxt
ESBL positive	80	90	90	90	70	40	60	60	33.33	11.11	70
ESBL negative	42.11	39.47	39.47	18.42	28.95	28.95	13.16	2.63	33.33	15.38	34.21

A- Ampicillin, Cf- Cefalotin, Ct- Ceftizoxim, Ci- Ceftriaxone, G- Gentamicin, An- Amikacin, Cp- Ciprofloxacin, Nor- Norfloxacin, Te- Tetracycline, Na- Nalidixic acid, Sxt- Trimethoprim sulfamethoxazole

Table 4. Distribution of extended spectrum beta-lactamase types in Sanandaj two general hospitals

ESBL type	Hospital		
	Besat H. (%)	Yo ohid H. (%)	Total (%)
CTX-M	12.50	8.33	20.83
SHV	10.42	8.33	18.75
TEM	8.33	6.25	14.85
OXA-1	10.42	4.17	14.58
OXA-2	0.00	4.17	4.17

practice in our country.

Most of ESBL-positive *Klebsiella* spp. has been isolated from urinary tract infection and blood stream infection (6.25%). Therefore, the high rate of ESBL-positive isolate with this infection might be determining the nosocomial spread of this enzyme.

We found ciprofloxacin resistance in 60% in ESBL positive and 13.16% in ESBL negative of *Klebsiella* bacteria. There were marked geographic differences in the occurrence of ciprofloxacin resistance, resistance rates were in range of 33 to 60% of ESBL producing isolates. Ciprofloxacin resistance in *K. pneumoniae* is closely associated with ESBLs. This association is of grave concern since ESBL-producing isolates are usually resistant to penicillins, cephalosporins, aminoglycosides and TMP-SMZ. Therefore, ciprofloxacin resistance severely limits already restricted treatment Paterson et al. (2000); Paterson et al. (2004).

CTX-M type ESBLs have become widely dispersed in many parts of the world and these enzymes confer higher levels of resistance to cefotaxime than to ceftazidime Bonnet (2004). The prevalence of bla_{CTX-M} was 20.83% for ESBL-producing *Klebsiella* strains. ESBL-producing bacteria carried bla_{SHV} at 18.75%. In comparism with Thailand studies Kiratisin et al. (2008), the rate of bla_{CTX-M} and bla_{SHV} were very low. In Indian studies Baby et al. (2008) also, the majority of isolates (19/23) belonged to CTX-M type ESBLs and one isolate was positive for both CTX-M and SHV gene. SHV-type and CTX-M ESBLs have appeared in many Canadian isolates Bush (2008). TEM gene was detected in 41 (65.1%) and 19 (46.3%), whereas SHV gene in 18 (28.6%) and 20 (48.8%) of E. coli and *K. pneumoniae* strains, respectively. SHV enzymes are common plasmid-mediated β-lactamase, which are chromosomally encoded in the majority of isolates of *K. pneumoniae* Arlet et al. (1999), Mahrouki et al. (2008), Messai et al. (2008) and Vinue et al. (2008). *Bla* genes encoding TEM, OXA-1 and OXA-2 were found in ESBL-producing strains 14.85, 14.58 and 4.17%, respectively. TEM gene was detected in 41 (65.1%) and 19 (46.3%), whereas SHV gene was 18 (28.6%) and 20 (48.8%) in E. coli and K. pneumoniae strains, respectively. TEM was originally isolated from blood culture of a patient named Temoniera in Greece, in the early 1960s. TEM being plasmid and transposon mediated has facilitated its spread to other species of bacteria Bradford (2001); Baby et al. (2008); Usha et al. (2008); Vinue et al. (2008). As reported from most part of the world, quite marked differences have since been seen in the pattern of ESBL genes. ESBL production rates are now very high compared with Europe Hawkey (2008) and USA Canton et al. (2008). However, spread of mobile genetic elements, mainly epidemic plasmids and the dispersion of specific clones have been responsible for the increase in ESBL-producing isolates.

In conclusion, this study documents the presence of beta-lactamase among *Klebsiella* isolates in two Sanandaj general hospitals. The prevalence of ESBL producers at our study was lower in comparison to the prevalence reported from other studies. Routine detection of ESBL-producing microorganisms is required to be done by each laboratory by the standard detection methods so as to control the spread of these infections and also to institute proper therapeutic strategies.

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