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Extended Spectrum Beta-Lactamases Among Hospitalized Patients in Surgery Wards, Ilam, Iran

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Abstract

Objectives of this study were to study the molecular epidemiology of ESBL-producing *Klebsiella pneumoniae* in selective hospitals in Iran, to determine the prevalence of TEM, SHV and CTX-M genes responsible for ESBL production among ESBL-producing *K. pneumoniae,* to investigate the susceptibility of *Klebsiellae spp* producing ESBLs towards non-beta-lactam antibiotics, all in different seasons. Clinical isolates of *K. pneumoniae* were identified during Mar. 2007 to Apr. 2008 in Ilam hospitals, the province in west of Iran. All isolates were found in surgery wards. ESBL activity was first evaluated using the standard disc diffusion test for cephalosporins and monobactam, then using the double-disc synergy test between cephalosporins and clavulanate. PCR assay had done for ESBLs genes detection. The results showed sixteen *K.pneumoniae* were identified by chemical methods. No resistance had occurred among *K.pneumoniae* toward non-beta-lactam antibiotics. BlaSHV was dominant gene responsible for ESBLs production while just one blaTEM along with blaSHV were found. BlaCTX-M was not responsible for ESBLs production in our study. 37.5% *K.pneumoniae* producing ESBLs in surgery ward in west of Iran must be considerable and need to further study in different part of hospital in Ilam and in Iran. Strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing these resistances.

Keywords: *K.pneumoniae*, surgery ward, Iran

Introduction

ESBLs are known as extended-spectrum because they are able to hydrolyze a broader spectrum of beta-lactam antibiotics than the simple parent beta-lactamases from which they are derived. Such ESBLs have also the ability to inactivate beta-lactam antibiotics containing an oxyimino-group such as oxyimino-cephalosporins (eg; ceftazidime, ceftriaxone, cefotaxime) as well as oxyimino-monobactam [1,2]. Furthermore, they are not active against cephamycins and carbapenems. Generally, they are inhibited by beta-lactamase-inhibitors such as clavulanate and tazobactam. ESBLs have been found in a wide range of Gram-negative rods. However, the vast majority of strains expressing these enzymes belong to the *Enterobacteriaceae* family [1]. *K.pneumonia* seems to remain as the major ESBL-producer [3]. The strong selective pressure for the use of beta-lactam drugs exerted on ESBL producer strains may lead to the selection of strains that hyperproduce ESBL, to the emergence of strains expressing different types of ESBLs, to the selection of complex mutant enzymes with inhibitor resistant phenotype or porin alteration which lead to the development of resistance to cephamycins and other antimicrobials [4,5,6]. The plasmids that harbor genes encoding ESBLs frequently contain other genes encoding mechanisms of resistance to aminoglycoside and cotrimoxazole [7]. Quinolone resistance is frequently found in ESBL producer strains; although, the mechanism of co resistance is not clear [8]. Objectives of this study were to study the molecular epidemiology of ESBL-producing *Klebsiella pneumoniae* in selective hospitals in Iran, to determine the prevalence of TEM, SHV and CTX-M genes responsible for ESBL production among ESBL-producing *Klebsiellae pneumoniae,* to investigate the susceptibility of *Klebsiellae spp* producing ESBLs towards non-beta-lactam antibiotics, all in different seasons.

Methods

Bacterial isolates

Clinical isolates of *K. pneumoniae* were identified during the period March 2007 to April 2008 in in Ilam hospitals, the province in west of Iran. All isolates were found in surgery wards.

Identification of *Klebsiella pneumoniae*

Gram stained in Modified Preston-Morrel Method, Oxidase and Catalase. Media: SIM, Simon Citrate , MR-VP , Lysine Iron Agar, Kligler Agar, Phenylalanin Agar, Urea Agar, Malonate, Blood Agar, Maccanky Agar [9] were used in our study.

Antibiotic testing

Susceptibilities to antimicrobial agents were determined by the antibiotic disc diffusion method. ESBL activity was first evaluated using the standard disc diffusion test for cephalosporins and monobactam, then using the double-disc synergy test between cephalosporins and clavulanate [10].

Escherichia coli ATCC 25922 was used as an ESBL-negative reference strain, and *K. pneumoniae* ATCC 700603 was used as an ESBL-positive reference strain.

PCR amplification of blaTEM, SHV and CTX-M

DNA extraction was carried out using a rapid alkaline lysis method [3]. The oligonuclotide primers used for the PCR assays were: blaTEM (F: 5-GAGTATCAACATTTCCGTGTC-3, R: 5-TAATCAGTGAG-

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GCACCTTCTC-3), blaSHV (F:5–AAGATCCACTATCGCCCAG-CAG-3,R:5 ATTCAGTTCCGTTTCC CAGCGG-3) [11] and blaCTX-M (F:5-ACGCTGTTGTT AGGAAGTG-3, R:5-TTGAGGCTGGGT-GAAGT-3) [12].

Results

Sixteen isolates of *K.pneumoniae* were identified by chemical methods. They were tested against 3rd generation of cephalosporins and aztreonam. Results analyzed by SPSS. The results allocated 37.5% resistance to ceftazidime (Ca) (30µg), Cefteriaxone (Ci) (30µg), cefpodoxime (Cep) (30µg) and Aztreonam (Ao) (30µg). Resistance toward cefotaxime (Ce) (30µg) was 50% (Table 1). *K.pneumoniae* resistant to aztreonam and one or more 3rd generation of cephalosprins were suspected to produce ESBLs. Confirming stage performed by using of Ceftazidime alone (30ug) versus ceftazidime/clavulanic (Cac) (30/10µg), cefotaxime (30µg) alone versus cefotaxime /clavulanic acid (Cec) (30/10µg) and cefpodoxim alone versus cefpodoxim /clavulanic acid (Cepc) (30/10µg). These results showed all *K.pneumoniae* that were suspected to produce ESBLs were confirmed by Cac and Cepc, while didn't observe confirmation by Cec. Regardless of the zone diameters, a>5mm increase in a zone diameter for an antimicrobial agent tested in combination with clavulanic acid versus its zone size when tested alone, indicated a probable ESBL production [13]. Resistance to non-beta-lactam antibiotics including: amikacin (Ak) (30µg), cotrimoxazol (Co) (30µg) ciprofloxacin (Cf) (30µg), imipenem (I) (30µg) didn't observe. BlaSHV was dominant gene responsible for ESBLs production while just one blaTEM along with blaSHV were found. BlaCTX-M was not responsible for ESBLs production in our study (Figure 1).

Discussion

The development of extended-spectrum cephalosporins in the early 1980s was regarded as major addition to our therapeutic armamentarium in the fight against beta-lactamase-mediated bacterial resistance [14]. This data obtained from clinical samples of *K. pneumoniae*, shows high antibiotic resistance. Worldwide resistance to antibiotics has increased in *Klebsiella* [15].

	K.pneumoniae	**Ca**	**Ce**	**Ci**	**Cep**	**Ao**
Spring	3 (18.75%)	1 (33.33%)	0	1 (33.33)	0	0
Summer	4 (25%)	2 (50%)	3 (75%)	1 (25%)	2 (50%)	2 (50%)
Fall	5 (31.25%)	3 (60%)	4 (80%)	2 (40%)	3 (60%)	3 (60%)
Winter	4 (25%)	1 (25%)	1 (25%)	2 (50%)	1 (25%)	1 (25%)
Total	**16 (100%)**	**6 (37.5%)**	**8 (50%)**	**6 (37.5%)**	**6 (37.5%)**	**6 (37.5%)**

Table 1: Screening stage for detection of *K.pnemoniae* producing ESBLs from admitted patients in Surgery wards in Ilam hospitals.

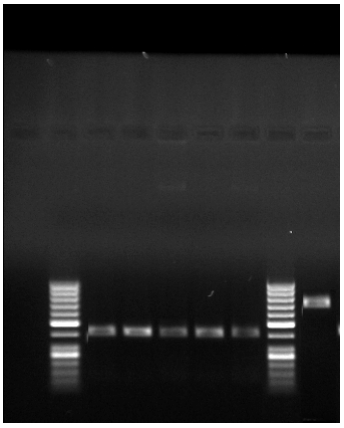

Figure 1: Right to left: Marker=50bp, five blaSHV, Marker=50bp, blaTEM.

Carbapenems are the drugs of choice for many infections caused by gram positive and gram-negative bacteria [16]. Imipenem, cotrimoxazol, ciprofloxacine and amikacin were found to be the most effective antibiotics against *K.pneumoniae* producing ESBLs in this study. Nowadays, ESBLs is considered a problem among the hospitalized patients throughout the world. The prevalence of ESBLs among the clinical isolates, which is rapidly changing over time, varies greatly and geographically worldwide. Patients suffering from infections caused by ESBL- producing organisms are at increasing risks of treatment failures with broad-spectrum beta-lactam antibiotics. In a survey, one hundred and sixty-eight clinical isolates of *K.pneumoniae* were collected during September 2006 to February 2007 from three general hospitals of Tehran in Iran .The most isolates of *K.pneumoniae* were from urine (n= 82), respiratory tract (n= 21) and blood (n= 16) specimens. 69% from One hundred and sixty eight clinical isolates were positive and fifty-one isolates (31%) were negative for ESBLs production [11]. By the early 1990s, 25 to 35% of nosocomially acquired *K.pneumoniae* isolates were ESBL producing in France (Marty et al., 1998). In 1988, isolates of *K. pneumoniae* containing SHV-2 were reported from China (Jacoby et al., 1988). Several outbreaks of infections with ESBL-producing *Klebsiella spp.* have been reported from South Africa Cotton, et al. [17], but no national surveillance figures have been published. However, it has been reported that 36.1% of *K.pneumoniae* isolates collected in a single South African hospital in 1998 and 1999 were ESBL producers (Bell et al., 2002). Different percentage of ESBLs had reported in different parts of the world. Our study showed 37.5% of *K.pneumoniae* had ESBLs in surgery ward in west of Iran. Thus, it must be considerable further study in different part of Iran. Cefteriaxone resistance in fall had the highest percentage of resistant due to 3rd generation of cephalosporins in surgery ward (80%) and *K.pneumoniae* producing ESBLs more occurred in fall, too. The dominant gene responsible for ESBLs production was BlaSHV while the lowest frequency of ESBLs gene had observed for BlaCTX-M. Studies of molecular epidemiology of these resistance genes can also be used for comparison with genes already isolated from other parts in Iran and of the world.

In conclusion, strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing these resistances.

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