

Cross-sectional study of extended spectrum beta-lactamase producing gram-negative bacilli from clinical cases in Khorramabad, Iran

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ABSTRACT

Introduction: Antibiotic resistance among bacteria in particular those producing Extended Spectrum Beta lactamases (ESBLs) has a very significant role in hospital acquired infections. Some of the gram negative bacilli including *Klebsiella pneumoniae* and *Escherichia coli* are known to be ESBL producers which cause uncontrollable infections because they are also often resistant to other antimicrobial agents. This study was designed to assess the ESBL producing gram-negative bacilli among clinical isolates of inpatients at Shohada-ye Ashayer Hospital, Khorramabad, Iran.

Materials and Methods: Samples were processed with routine laboratory methods and gram-negative bacilli were identified by standard tests. ESBL producing gram-negative bacilli were screened by MacConkey Agar containing 4 mg/liter ceftazidime and confirmed with the double disk synergy method.

Results: Fifty-three cases (23.6%) of 225 total isolated gram-negative bacilli were positive in terms of ESBL production. *Klebsiella pneumoniae* comprising 20 cases (8.9%) was the dominant organism producing ESBLs followed by *Escherichia coli* (10 cases; 4.4%) and *Pseudomonas aeruginosa* (10 cases; 4.4%). The most ESBL producing organisms were found in urine samples (21 cases; 39.6%). Ten cases (18.9%) of isolates were from samples collected with sterile bronchoscopy.

Conclusion: Results of the study indicated that ESBL producing gram negative bacilli are frequently isolated from Shohada-ye Ashayer Hospital. Regarding the high resistance of these strains against many of the antibiotics and even against carbapenems, health care professionals need to plan policies to fight the induction and spread of such strains.

Keywords: Hospital acquired infection, *Escherichia coli*, *Klebsiella pneumoniae*, ESBL, Khorramabad, Iran.

INTRODUCTION

In recent decades, resistance of bacteria to antimicrobials has been a problem in the world. Some types of resistance, in particular resistance to beta-lactams, are very important in hospital acquired infections. Resistance to expanded spectrum beta-lactams has been found among the strains of *K. pneumoniae* and *E. coli*. Isolates that produce extended spectrum beta-lactamase (ESBLs) are resistant to penicillins, extended spectrum cephalosporins, and monobactams (aztreonam). ESBL producing strains have created unresolved problems for clinical microbiologists and professional experts of in-

fection control (1, 2).

There are different classifications of beta-lactamases. One of the more extensively used methods was invented by Bush, Jacoby and Medeiros in which beta-lactamases are divided into four groups on the basis of substrate type and physical characteristics such as molecular weight and isoelectric point (3, 4). One of these groups is that of ESBLs that appeared after large scale production and consumption of extended spectrum cephalosporins. First, they were reported in the early 1980s in Europe and now they are being reported all over the world (1, 5). ESBLs are variants of primary enzymes TEM-1, TEM-2 and SHV-1. This variation is special because of changes in one or more amino acids (1).

ESBL producing strains have not been studied in many of the Asian countries like Iran extensively. The epidemiologic information has been acquired accidentally from the report of research centers that

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study special patient groups or from indirect studies and evaluation of special antibiotics resistance. In most of the studies, resistance against cephalosporins and other extended spectrum drugs has been assessed. These studies have focused on the resistance of gram-negative bacilli against extended spectrum drugs such as ceftazidime, ciprofloxacin, ceftizoxim, and the third generation cephalosporins which have been isolated from many hospital environments (6-7).

ESBL producing strain isolates have been reported from Iran (8-11) and most Asian countries. Based on estimates, 7-9 % of *E. coli* and 27-38 % of *Klebsiella* species in Malaysia produce ESBLs (12). Considering the above mentioned issues and because of the important role of ESBL producing strains in hospital acquired infections, this research was designed to study the presence of these strains in different parts of the Shohada-ye Ashayer Hospital, Khorramabad, Iran .

MATERIALS AND METHODS

This is a cross-sectional study performed in a 6-months period at Shohada-ye Ashayer Hospital, Khorramabad Iran. Ceftazidime powder used for screening of ESBLs was received from Exir Pharmaceutical company (Exir, Borujerd, Iran). Augmentin (Aug 30C), ceftazidime (CAZ 30C), cefpodoxime (CPD 10C), cefotaxime (CTX 30C), ceftriaxone (CRO 30C) and Ceftazidime, ceftazidime/clavulanic acid disks were purchased from Mast (Mast groups Ltd. Merseyside, UK). Ceftazidime and ceftazidime/clavulanic acid disks were used for phenotypic confirmation of strains for ESBL production.

ESBL Screening. Gram negative bacilli isolated from different sections of the hospital were screened in McConkey Agar containing 4 mg/l of ceftazidime. Any light growth was considered ESBL production with MIC of 4 µg/ml of ceftazidime.

Double disk synergy/Disk approximation method. Screened strains were cultured overnight in nutrient broth and were diluted according to 0.5 McFarland turbidity using normal saline. Using cotton swabs, these strains were plated on Mueller-Hinton agar. Subsequently, co-amoxiclav disk was placed in the center, and each of the ceftazidime, cefotaxime, ceftriaxone and cefpodoxime disks was placed, in the periphery, 2.5-3 cms from the center. Plates were incubated 24 hours in 35°C and inhibition zones between and around the disks were observed. Any extension of inhibition zones between central coamoxiclav and environmental

disks was considered a presumptive ESBL producing strain which was confirmed by the phenotypic confirmatory method (13, 14).

Phenotypic confirmation of ESBL production.

Positive strains in the screening steps were confirmed by the following method: Mueller-Hinton agar plates were cultured with 0.5 McFarland turbidity of presumptive bacteria. Then, ceftazidime and ceftazidime/clavulanic acid disks were placed on the plates. After 24 hours of incubation at 35°C, regardless of the zone diameters, a more than 5mm increase in the zone diameter for ceftazidime/clavulanic acid versus ceftazidime zone size alone, was used to confirm ESBL production.

RESULTS

From the 225 isolated gram-negative bacilli, 103 demonstrated growth on McConkey Agar containing 4 mg/l ceftazidime (MIC= 4 µg/ml). Fifty-three of 103 strains were detected as ESBL producing with confirmatory tests. *K. pneumoniae*, observed in 20 cases, was the most isolated ESBL producing strain among studied bacteria. Other isolated species are shown in Table 1.

DISCUSSION

Drug resistant strains have emerged after extensive use of antibiotics. ESBL producing strains are special kinds of drug resistance that were first reported in 1980 (1). At the present time, these strains are being reported from different countries of the world. So, ESBL strains are one of the emerging infectious threats for health care professionals and community. For example, David *et al.* reported that until 2001/2002 most ESBL isolates in the UK were *Klebsiella* species many of which were from specialists' units (<http://www.hpa.org.uk/cdr/archive04/news2704.html>). Shen *et al.* studied 14 isolates of *Klebsiella* in China using disk diffusion and the E-test. In addition to their resistance against cephalosporins and beta-lactamase production, these strains were also resistant against aminoglycosides, fluoroquinolones, tetracyclines and cotrimoxazole (14). Gulay *et al.*, after studying 44 *K. pneumonia* isolates from hospital acquired infections, showed that all of them were resistant to amoxicillin, while 26 strains were resistant when examined simultaneously with clavulanic acid. By using the double disk synergy, they showed that 84% of strains were ESBL producers (15). In another study, Christian *et al.*, by using the double disk method, reported 114 (82%) out of 139 *K. pneumonia* strains isolated from

Table 1. The number & percentage of bacterial species isolated from patients.

	Total isolates	Growth on McConkey (4 mg/1 ceftazidime)	ESBL positive
<i>k. pneumoniae</i>	50(22.2%)	32(14.2%)	20(8.9%)
<i>E. coli</i>	87(38.7%)	24(10.7%)	10(4.4%)
<i>P. aeruginosa</i>	27(12%)	19(8.4%)	10(4.4%)
<i>Enterobacter</i> . spp	19(8.4%)	9(4%)	4(1.8%)
<i>Providencia</i> . spp	7(3.1%)	4(1.8%)	2(0.9%)
<i>Citrobacter</i> . spp	9(4%)	5(2.2%)	2(0.9%)
<i>Acinetobacter</i> . spp	9(4%)	3(1.3%)	2(0.9%)
<i>Proteus</i> . spp	12(5.3%)	4(1.8%)	1(0.4%)
<i>Aeromonas</i> . spp	5(2.2%)	3(1.3%)	1(0.4%)
Total count	225(100%)	103(45.8%)	53(23.5%)

19 laboratories in USA were ESBL producing (16). Another study carried out in USA on 906 isolates of enterobacteriaceae indicated that 83 (9%) were ESBL producing (17).

Most of the studies focused on resistance against third generation cephalosporins and other extended spectrum antibiotics. For example, in a study conducted by Ghana'at and Sadeghian, susceptibility of 1,261 pathogenic strains against ciprofloxacin, ceftazidime, and ceftizoxime was evaluated (6). They reported good efficacy for these drugs against isolates of *K. pneumoniae*. On the other hand, Irajian *et al.*, reported that ESBL production rate of 18.1% among *K. pneumoniae* and *E. coli* strains in a cross-sectional study (18). In a recent study, Cheong *et al.*, reported resistance patterns against ceftazidime of 36,343 bacterial isolates from six general Malaysian hospitals to be 5.5%, 16.6% and 6.8% in *E. coli*, *Klebsiella* spp. and *P. aeruginosa*, respectively (12).

Studies on infected or colonized patients with ESBL producing bacteria have shown that all patients have some general predisposing factors including surgeries, indwelling catheters, use of extended spectrum antibiotics, nursing history and long term hospitalization. So, physicians visiting patients with these mentioned risk factors should remember the possibility of ESBL related resistant infections. In such conditions, health care professionals should consider the management, treatment and control precautions.

In conclusion, the increasing rate of ESBL producing gram-negative infections and their high resistance against most of the extended spectrum antibiotics makes detection of ESBL producing strains neces-

sary and this detection should be included in routine programs of microbiology laboratories. Moreover, an antibiotic prescription committee should be constituted and it should supervise antibiotic prescription seriously in hospitals to prevent the emergence of such strains.

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