The Prevalence of ESBLs Producing *Klebsiella pneumoniae* Isolates in Some Major Hospitals, Iran

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Abstract: Aims of this study were to investigate on antibiotic resistance and molecular epidemiology of *K.pneumoniae* producing ESBLs isolates of respiratory tract infections in some major hospitals in Iran. *K.pneumonaie* were obtained of patients with RTI. *K. pneumoniae* producing ESBLs detected by screening, confirming and PCR methods. During the 12-month period, a total of one hundred and thirteen of *K.pneumoniae* were found from RTI in three cities in different region of Iran which Sixty seven strains (59.2%) were ESBLs producer. In Ilam hospitals, seventeen strains (43.6%), in Milad hospital, thirty-seven strains (74%) and in Emam Reza hospital, thirteen strains (54.2%) were ESBLs producer. The findings showed that among sixty-seven *K.pneumoniae* producing ESBLs, Sixty-three strains (94%) were positive for *bla*SHV, eleven strains (16.4%) contained blaTEM and sixteen strains (23.9%) harbored *bla*CTX-M. Imipenem was found as an effectiveness antibiotic. In the current study, Majority of the ESBLs production had occurred in Milad hospital in Tehran (74%). In conclusion, spreading ESBL-producing strains is a concern, as it causes limitations to the antimicrobial agents for optimal treatment of patients.

Keywords: ESBLs, *Klebsiella pneumoniae*, blaSHV, blaTEM, blaCTX-M.

INTRODUCTION

Extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* have spread rapidly worldwide and pose a serious threat in healthcare-associated infections [1]. ESBLs have spread threateningly in many regions of the world and now comprise over three hundred variants (*http://www.lahey.org/studies*).

ESBLs are plasmid-mediated enzymes that hydrolyze broad-spectrum beta-lactams and are strongly inhibited by clavulanate. ESBLs are transmitted by plasmids among bacteria. Furthermore, antibiotics such as trimethoprim-sulfamethoxazole, aminoglycosides and fluoroquinolones are often co-transferred on a resistance plasmid, resulting in multiple drug resistance. Thus clinical treatment failure occurs frequently, especially when inappropriate antimicrobial therapy is used to treat infections caused by ESBL- producing organisms. Therefore, if infections with ESBL-producing organisms can be predicted by the clinical characteristics of patients, this may lead to a better selection of antibiotics and may improve the outcome of infections [2].

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This study was done to investigate on antibiotic resistance and molecular epidemiology of *K. pneumoniae* producing ESBLs in patients with respiratory tract infections in some major hospitals in Iran.

MATERIAL & METHODS

Sample collection: one hundred and thirteen clinical isolates of *K.pneumoniae* were identified during Mar. 2007 to Apr. 2008 in five hospitals in three Iranian cities (Ilam in west of Iran, Tabriz in west north of Iran, and Tehran in center and capital of Iran). *K. pneumoniae* isolates were obtained of sputum, tracheal aspirates, bronchial washing and bronchoalveolar lavage. Collection of multiple samples of the same patient was, however, avoided in the database.

Screening Stage

Kirby-Bauer disk diffusion test by using Mueller-Hinton agar diminished zones of inhibition around 3rd generation beta-lactam disks were considered suggestive of ESBL production. According to NCCLS (2005) the following antibiotics were used to indicate ESBL production: cefpodoxime (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefteriaxone (30 μg) and aztreonam (30 μg) [3].

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Clavulanic Acid Association Test

For the combined disk method, disks containing cefpodoxime (30 μg), ceftazidime (30 μg) and cefotaxime (30 μg) with and without clavulanic acid (10 μg), were used. The resulting inhibition zones were compared. The test was considered positive when the difference of zone diameters between the beta-lactam disk and disk containing antibiotic associated with clavulanic acid was > 5mm [4].

Effect of non Beta-Lactame Antibiotics Against *Klebsiella pneumoniae*

Amikacin (Ak) (30ug), cotrimoxazol (Co) (30ug) ciprofloxacin (Cf) (30ug), imipenem (I) (30ug) were used among *K.pneumoniae* producing ESBLs toward non beta-lactam antibiotics [5]. All antibiotic disks were obtained in HiMedia Company in India.

Molecular Methods

DNA Extraction

K.pneumoniae Producing ESBLs were cultured in LB broth at 37°C overnight, and then DNA was extracted by using the DNA extraction kit (fermenrtase).

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was carried out by following primers: *bla*TEM (Forward 5-GAGTATCAACA TTTCCGTGTC-3, Reverse primer 5-TAATCAGTGAGG CAC CTTCTC-3), *bla*SHV (5'–AAGATCCACTATCGCCCA GCAG-3, Reverse 5–AAGATCCA CTATCGCCCAGCAG-3) [6] and *bla*CTX-M, (forward 5-ACGCTGTTGTTAGGAA GTG-3, reverse 5-TTGAGGCTGGGTGAAGT-3) [7].

RESULTS

During the 12-month period, a total of one hundred and thirteen of *K.pneumoniae* isolates were obtained from respiratory tract infection in three cities in different part of Iran. While total of sixty-seven strains (59.2%) produced ESBLs, numbers of forty-six strains (40.8%) were non ESBLs producer. Generally, amongst ESBLs producer *K.pneumoniae*, all the strains were resistant to aztreonam and cefpodoxime. Cefotaxime (68.6%) allocated the lowest resistant among third generation of cephalosporin. Imipenem were found as an effectiveness antibiotic while resistance to cotrimoxazol (35.8%) was more than the others (Table **1**). In non ESBLs *K.pneumoniae* strains, no resistance occurred among aztreonam, while the highest resistance observed in cefotaxime and ceftazidime (50%). In this study, all the non ESBLs *K.pneomoniae* were susceptible to the whole non-beta lactam antibiotics (Table **2**). In Ilam city in the west of Iran, thirty-nine *K.pneumoniae* were found that seventeen strains (43.6%) were ESBLs producer and twenty-two strains (56.4%) were negative for ESBLs production; in Tehran city in capital of Iran (Milad hospital), of fifty *K.pneumoniae*, thirty-seven strains (74%) were positive for producing ESBLs and thirteen strains (26%) were negative on behalf of producing ESBLs and in Tabriz city in the west north of Iran (Emam Reza hospital), twenty-four *K. pneumoniae* obtained which thirteen strains (54.2%) were ESBLs producer and eleven strains (45.8%) were negative.

Among *K.pneumoniae* producing ESBLs, 25.4% (n=17), 55.2% (n=37) and 19.4% (n=13) strains were isolated in Ilam, Milad and Emam Reza hospitals, respectively (Table **1**).

Results revealed that among sixty-seven *K.pneumoniae* producing ESBLs, Sixty-three strains (94%) were positive for *bla*SHV, eleven strains (16.4%) contained *bla*TEM and sixteen strains (23.9%) harbored *bla*CTX-M. *bla*CTX-M and *bla*SHV together were present in fourteen strains (20.9%), nine strains (13.4%) carried both *bla*SHV and blaTEM, four isolates (6%) were positive for *bla*TEM and *bla*CTX-M and finaly, four isolates (6%) carried all three *bla*SHV, *bla*TEM and *bla*CTX-M.

In Milad hospital, all *K.pneumoniae* producing ESBLs were positive for blaSHV, eight strains (21.6%) contained

Table 1. Antibiotic Panel of *K.pneumoniae* Producing ESBLs Strains

Antibiotics	ESBLs Positive *K.pneumoniae* In Ilam Hospital N=17	ESBLs Positive *K.pneumoniae* In Emam Reza Hospital N=13	ESBLs Positive *K.pneumoniae* In Milad Hospital N=37	ESBLs Positive *K.pneumoniae* Total N=67
	Resistance % Sensitivity%	Resistance % Sensitivity%	Resistance % Sensitivity%	Resistance % Sensitivity%
Ca	14 (82%) 3 (18%)	13 (100%) 0	35 (94.6%) 2 (5.4%)	62 (92.5%) 5 (7.5%)
Ce	6 (35.2%) 11 (64.8%)	12(92.3%) 1 (7.7%)	28(75.7%) 9 (24.3%)	46 (68.6%) 21(31.3%)
Ci	17 (100%) 0	13 (100%) 0	32 (86.5%) 5 (13.5%)	62 (92.5%) 5 (7.5%)
Cep	17 (100%) 0	13 (100%) 0	37 (100%) 0	67 (100%) 0
Ao	17 (100%) 0	13 (100%) 0	37 (100%) 0	67 (100%) 0
Ak	3 (18%) 14 (82%)	3 (23.1%) 10 (76.9%)	13 (35.1%) 24 (64.9%)	19 (28.3%) 48(71.6%)
Cf	2 (12%) 15 (88%)	1 (7.7%) 12 (92.3%)	8 (21.6%) 29 (78.4%)	11 (16.4%) 56(83.6%)
Co	7 (41%) 10 (59%)	3 (23.1%) 10 (76.9%)	14 (37.9%) 23 (62.1%)	24 (35.8%) 43(64.1%)
I	0 17 (100%)	0 13 (100%)	0 37 (100%)	0 67(100%)

Table 2. Antibiotic Panel of *K.pneumoniae* non-ESBLs Strains

Antibiotics	ESBLs Negative *K.pneumoniae* In Ilam Hospital N=22	ESBLs Negative *K.pneumoniae* In Emam Reza Hospital N=11	ESBLs Negativ *K.pneumoniae* In Milad Hospital N=13	ESBLs Negative *K.pneumoniae* Total N=46
	Resistance % Sensitivity%	Resistance % Sensitivity%	Resistance % Sensitivity%	Resistance % Sensitivity%
Ca	11 (50%) 11 (50%)	6 (54.5%) 5 (45.6%)	6 (46.2%) 7 (53.8%)	23 (50%) 23 (50%)
Ce	9 (40.9%) 13 (59.1%)	4 (36.3%) 7 (63.7%)	10(76.9%) 3(23.1%)	23 (50%) 23(50%)
Ci	12 (54.5%) 10 (45.6%)	5 (45.5%) 6 (54.5%)	5 (38.5%) 8 (61.5%)	22 (47.9%) 24 (52.1%)
Cep	1 (4.5%) 21 (94.5%)	1 (9%) 10 (90.9%)	0 13(100%)	2 (4.34%) 44(95.6%)
Ao	0 22 (100%)	0 11 (100%)	0 13(100%)	0 46(100%)
Ak	0 22 (100%)	0 11 (100%)	0 13(100%)	0 46(100%)
Cf	0 22 (100%)	0 11 (100%)	0 13(100%)	0 46(100%)
Co	0 22 (100%)	0 11 (100%)	0 13(100%)	0 46(100%)
I	0 22 (100%)	0 11 (100%)	0 13(100%)	0 46(100%)

*bla*TEM and ten isolates (27%) carried *bla*CTX-M. *bla*SHV and *bla*CTX-M presented in ten (27%) of strains. Eight strains (21.6%) carried both *bla*SHV and *bla*TEM. Four isolates were positive for both *bla*TEM and *bla*CTX-M and all three genes were found in four isolates (10.8%).

In Ilam hospital, among seventeen *K.pneumoniae* producing ESBLs, blaSHV was found in thirteen strains (76.5%), two strains (11.8%) were positive for *bla*TEM, and *bla*CTX-M presented in five strains (29.4%). Three strains (17.7%) carried both *bla*SHV and *bla*CTX-M.

In Emam Reza hospital, all strains were positive for blaSHV, while one strain carried *bla*TEM and one isolate was positive for *bla*CTX-M and one for both *bla*SHV and *bla*CTX-M, as well.

DISCUSSION

The high rate of ESBLs among hospitalized patients is a global problem. It is generally thought that patients infected by an ESBL-producing organism are at an increased risk of treatment failure with an expanded-spectrum beta-lactam. The prevalence of ESBL producing isolates of *K. pneumoniae* varies in different countries [8].

Countries with a high rate of prevalence include Turkey (60%), Latin America (45.4%), Western Pacific (24.6%), and Europe (22.6%) [9].

In this study, ESBLs production was variable from 43.6% in Ilam hospital to 74% in Milad hospital that showed different frequency of ESBLs production in different region in Iran. Our study showed significantly high ESBLs production.

The prevalence of respiratory isolates of *K. pneumoniae* with ESBL phenotype has been reported from less than 1% in Japan [10] to 83.3% in China [11].

We showed that the percentage of respiratory isolates with *K. pneumoniae* was high. Milad hospital contributed to more of the *K.pneumoniae* isolates. The lowest *K.pneumoniae* producing ESBLs had observed in Ilam hospital. Non-beta-lactam antibiotic resistance in Milad hospital was more than the others. Imipenem was found as an effectiveness antibiotic.

In this study, susceptibility testing of *K.pneumonie* strains producing ESBLs showed that the highest resistance rate among 3rd generation of cephalosporins and aztreonam were cefpodoxime and aztreoname in all hospitals, cefteriaxone in Emam Reza and Ilam hospitals and ceftazidime in Emam Reza hospital. The highest non-beta lactam antibiotic resistance occurred in cotrimoxazol in Ilam hospital. Imipenem was found as effectiveness antibiotic among ESBLs producing *K.pneumoniae* strains.

Majority of the ESBLs production recurred in Milad hospital (74%). The highest resistance toward non-beta-lactam antibiotic was observed in cotrimoxazol (41%) in Ilam hospital. The best antibiotics were Imipenem (100%) in all hospitals and following ciprofloxacine (92.3%) in Emam Reza Hospital. In non-ESBLs, *K.pneumoniae* Resistance toward cefotaxime (76.9%) was more than the other antibiotics. The most genes responsible for ESBLs production were found in *bla*SHV. In Ilam Hospital, frequency of *bla*CTX-M was more than *bla*TEM.

In Milad Hospital, Frequency of *bla*CTX-M was more than *bla*TEM.

Our finding in Emam Reza hospital had showed frequency of blaTEM and blaCTX-M were as an equal.

The prevalence of *bla*SHV,*bla*TEM and blaCTX-M genes in this study was 94%, 16% and 23.9%, respectively.

In Iran, Feizabadi *et al.*, in 2009 showed that 69.7% of *K. pneumoniae* isolates in Tehran were ESBL producers and the prevalence of *bla*TEM, *bla*SHV, *bla*CTX-M-I and *bla*CTX-M-III among these isolates were 54%, 67.4%, 46.51% and

29%, respectively (12). Our results revealed high prevalence of *bla*SHV and low frequency of *bla*TEM and *bla*CTX-M. Significantly, *bla*SHV was more responsible for ESBLs production [12].

In the study a tertiary care hospital in Tehran 77% of *K.pneumoniae* were ESBLs producer [13] while in our study in Ilam and Emam Reza hospitals frequency of ESBLs production were lower while the results of Milad hospital (74%) was near to the study of mehregan *et al*., [13]. In a survey by bazzaz *et al*., in 2007 in general hospital in Iran 59.2% of isolates were positive for ESBLs production and all isolates were susceptible to imipenem [14], our finding showed all strains were susceptible to imipenem. Our findings in Milad hospital revealed higher ESBLs production as comparison to bazzaz *et al*., [14].

Shahcheragi *et al*., [6] showed that ESBLs production observed in 33% of isolates while in our study the lowest ESBLs production was presented in Ilam hospital (43.6%). All isolates in survey of shahcheraghi *et al*., [6] were susceptible to imipenem and ciprofloxacine resistance was observed in 32% of *K.pneumoniae*. our finding revealed all strain were susceptible to imipenem but the highest rate of resistant to ciprofloxacine was observed in Milad hospital and that was 21.6%. In the study of shahcheraghi *et al*., 69.6% of strains carried *bla*SHV and 32.1% of *K.pneumonie* producing ESBLs harbored *bla*TEM [6] while our results showed all strains in Emam Reza and Milad hospitals carried *bla*SHV and this was 76.5% in Ilam hospital (94%). Frequency of *bla*TEM was lower (16.4%) than survey of shahcheraghi *et al*., in this study we found different ESBLs production in different regions of Iran that one of the reason may related to population in different city and also Using of antibiotic in Iran is uncontrollable, use of antibiotics and injectable formulations was high in the Islamic Republic of Iran. The high number of prescriptions for antibiotics (58% on average) may be because in the majority of the provinces, the data collected only covered a period of 1 month. And different prescriptions may have result of different resistance to antibiotics [15]. In conclusion, spreading ESBL-producing strains is a concern, as it causes limitations to the antimicrobial agents for optimal treatment of patients. The most reliable and effective antimicrobial treatment for infections caused by this organism is imipenem [16] and also had shown in this study. *Bla*SHV was found as a predominant gene responsible for ESBLs production and future study need to determine type of *bla* genes responsible for ESBLs producing strains in Iran and more study in different part of Iran.

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ABBREVIATIONS

Ak	=	Amikacin
Ao	=	Aztreonam
Ca	=	Ceftazidim
Cac	=	Ceftazidime/clavulanic
Ce	=	Cefotaxime
Cec	=	Cefotaxime /clavulanic
Cep	=	Cefpodoxim
Cepc	=	Cefpodoxim /clavulanic acid
Cf	=	Ciprofloxacin
Ci	=	Cefteriaxon
Co	=	Cotrimoxazol
ESBL	=	Extended spectrum beta-lactamases
I	=	Imipenem
K. pneumoniae	=	*Klebsiella pneumoniae*
RTI	=	Respiratory tract infection

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