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. pneumoniae* 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 *bla*TEM 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*, *bla*SHV, *bla*TEM, *bla*CTX-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 clavulenate. 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].

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].
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) (30μg), cotrimoxazol (Co) (30μg) ciprofloxacin (Ci) (30μg), imipenem (I) (30μg) 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 (fermentase).

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was carried out by following primers: blaTEM (Forward 5-GAGTATCAACATTCCCAGTCGGGTACC-3, Reverse 5-CTCAGTTGAGG-3), blaSHV (5’–AAGATCCACTATCGCCCAACGAG-3, Reverse 5–AAGATCCACTATCGCCCAACGAG-3) [6] and blaCTX-M, (forward 5-ACGCTGTTGTTAGGAAATGTG-3, reverse 5-TTGGAGCGCTGGAAGTCT-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.pneumoniae 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 blaSHV, eleven strains (16.4%) contained blaTEM and sixteen strains (23.9%) harbored blaCTX-M. blaCTX-M and blaSHV together were present in fourteen strains (20.9%), nine strains (13.4%) carried both blaSHV and blaTEM, four isolates (6%) were positive for blaTEM and blaCTX-M and finally, four isolates (6%) carried all three blaSHV, blaTEM and blaCTX-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=37 | ESBLs Positive K.pneumoniae In Milad Hospital N=67 | ESBLs Positive K.pneumoniae Total N=67 |
|-------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 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%)                                   |
The Prevalence of ESBLs producing Klebsiella pneumoniae Solates

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 Negative 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%)               |
| Cl          | 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 (95.6%)                               | 13 (100%)                                       | 4 (3.4%) 44 (96.6%)            |
| Ao          | 0 11 (100%)                                    | 0 11 (100%)                                    | 0 13(100%)                                      | 0 46(100%)                     |
| Ak          | 0 11 (100%)                                    | 0 11 (100%)                                    | 0 13(100%)                                      | 0 46(100%)                     |
| Ct          | 0 11 (100%)                                    | 0 11 (100%)                                    | 0 13(100%)                                      | 0 46(100%)                     |
| Co          | 0 11 (100%)                                    | 0 11 (100%)                                    | 0 13(100%)                                      | 0 46(100%)                     |
| I           | 1 (4.5%) 22 (95.5%)                             | 0 11 (100%)                                    | 0 13(100%)                                      | 0 46(100%)                     |

blaTEM and ten isolates (27%) carried blaCTX-M. blaSHV and blaCTX-M presented in ten (27%) of strains. Eight strains (21.6%) carried both blaSHV and blaTEM. Four isolates were positive for both blaTEM and blaCTX-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 blaTEM, and blaCTX-M presented in five strains (29.4%). Three strains (17.7%) carried both blaSHV and blaCTX-M.

In Emam Reza hospital, all strains were positive for blaSHV, while one strain carried blaTEM and one isolate was positive for blaCTX-M and one for both blaSHV and blaCTX-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. pneumoniae 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 recurrent 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 blaSHV. In Ilam Hospital, frequency of blaCTX-M was more than blaTEM.

In Milad Hospital, Frequency of blaCTX-M was more than blaTEM.

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

The prevalence of blaSHV,blaTEM 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 blaTEM, blaSHV, blaCTX-M-I and blaCTX-M-III among these isolates were 54%, 67.4%, 46.51% and
29%, respectively (12). Our results revealed high prevalence of blaSHV and low frequency of blaTEM and blaCTX-M. Significantly, blaSHV 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].

Shahcheraghi 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 ciprofloxacin resistance was observed in 32% of K. pneumoniae. our finding revealed all strain were susceptible to imipenem but the highest rate of resistant to ciprofloxacin was observed in Milad hospital and that was 21.6%. In the study of shahcheraghi et al., 69.6% of strains carried blaSHV and 32.1% of K. pneumoniae producing ESBLs harbored blaTEM [6] while our results showed all strains in Emam Reza and Milad hospitals carried blaSHV and this was 76.5% in Ilam hospital (94%). Frequency of blaTEM was lower (16.4%) then 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. BlaSHV 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

- Cac = Ceftazidime/clavulanic
- Ce = Cefotaxime
- Cec = Cefotaxime /clavulanic
- Cep = Cefpodoxim
- Cepe = Cefpodoxim /clavulanic acid
- Cf = Ciprofloxacin
- Ci = Cefteriaxon
- Co = Cotrimoxazol
- ESBL = Extended spectrum beta-lactamases
- I = Imipenem
- K. pneumoniae = Klebsiella pneumoniae
- RTI = Respiratory tract infection

REFERENCES

[1] Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P. Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother 2008; 52:2818-24.
[2] Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferrable plasmid. Lancet 1998; 351: 797-9.
[3] NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS - NCCLS. M100-S13. Performance Standards for Antimicrobial Susceptibility Testing; Thirteenth Informational Supplement. Wayne, Pennsylvania, USA: NCCLS documents, 2005.
[4] Jarlier V, Nicolas MH, Fournier G, Philippin A. Extended broad-spectrum Beta-lactamases conferring transferrable resistance to newer Beta-lactam agents in Enterobacteriaceae hospital prevalence and susceptibility patterns. Rev Infect Dis Chigaco, 1998; v.10, p.867-878.
[5] Paterson DL, Mulazimoglu L, Casellas J M, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in Klebsiella pneumoniae isolates causing bacteremia. Clin Infect Dis 2000; 30: 473-8.
[6] Shahcheraghi F, Mozzi H, Feizabadi M. Distribution of TEM and SHV Beta-lactamase genes among Klebsiella pneumoniae strains isolated from patients in Tehran. Med Sci Moint 2007; 13:BR247-250.
[7] Mansouri M, Ramazanazdeh R. Spread of extended spectrum beta-lactamases producing E.coli clinical isolates in sanandaj hospital. J Biol Sci 2009; 9; 362-36.
[8] Shah AA, Hasan F, Ahmed S, Hameed A. Characteristics, epidemiology and clinical importance of emerging strains of Gram-negative bacilli producing extended-spectrum beta-lactamases. Res Microbiol 2004; 155: 409-21.
[9] Gonlugur U, Bakici MZ, Akkurt I, Efeoglu T. Antibiotic susceptibility patterns among respiratory isolates of Gramnegative bacilli in a Turkish university hospital. BMC Microbiol 2004; 4; 32.
[10] Yum JH, Kim S, Lee H, et al. Emergence and wide dissemination of CTX-M-type ESBLs, and CMY-2- and DHA-1-type AmpC beta-lactamases in Korean respiratory isolates of Klebsiella pneumoniae. J Korean Med Sci 2005; 20: 961-5.
[11] Shi J, Li Y, Li C, Cai X, Li H, Peng S. Drug resistance and genotyping of Klebsiella pneumoniae in lower respiratory tract infection. Zhonghua Jie He He Hu Xi Za Zhi 2002; 25: 607-9.
[12] Feizabadi MM, Delfani S, Razi N, et al. Distribution of bla(TEM), bla(SHV), bla(CTX-M) genes among clinical isolates of Klebsiella pneumoniae at Llabafinejad hospital, Tehran, Iran. Microb Drug Resist 2010; 16:49-53.
[13] Mehrgan H, Rahbar M, Arab-Halvaii Z. High prevalence of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae
in a tertiary care hospital in Tehran, Iran. J Infect Dev Ctries 2010; 29; 4: 132-8.

[14] Bazzaz BS, Naderinasab M, Mohamadpoor AH, Farshadzadeh Z, Ahmadi S, Yousefi F. The prevalence of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae among clinical isolates from a general hospital in Iran. Acta Microbiol Immunol Hung 2009; 56:89-99.

[15] Cheraghi AM, Nikfar S, Behmanesh Y. Evaluation of availability, accessibility and prescribing pattern of medicines in the Islamic Republic of Iran. East Mediterr Health J 2004; 10(3): 406-5.

[16] Essack SY. Treatment options for extended-spectrum betalactamase-producers. FEMS Microbiol Lett 2000; 190:181-4.