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The Frequency of Extended Spectrum Beta Lactamase (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: A Report from Mashhad, Iran

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ABSTRACT

Background: In recent decades, extended spectrum beta-lactamase (ESBL) producing bacteria have increased worldwide. The most important *causative agents of nosocomial infections* throughout the world, *Escherichia coli* and *Klebsiella pneumoniae* as main ESBL-producing bacteria are so highly regarded. Trends in the treatment of infections by such bacteria have led to a global concern. This study was conducted to evaluate the incidence of ESBL producing *E. coli* and *K. pneumoniae* among inpatients and outpatients referred to the Imam Reza hospital unit in Mashhad during 2007-8.

Methods: This study represents a descriptive cross-sectional study. All 339 samples from hospital and a special clinic of the Imam Reza hospital of Mashhad were collected and cultured in defined media. Identification by morphological and biochemical tests were performed to determine the *Enterobacteriaceae* genera. The secretion of ESBL was studied by the double disc diffusion method. At the end, the data were analyzed by statistical software.

Results: Out of 339 isolates collected from 192 women (56.6%) and 147 men (43.4%), 26.5% of *E. coli* (n = 211) and 43% of *K. pneumoniae* (n = 128) were ESBL positive. Most of the ESBL-positive isolates were related to ICU and the least of them were related to neonatal ward.

Conclusion: The present study indicates the high prevalence of ESBL producing *Enterobacteriaceae* family especially in inpatients. Limiting the spread of such superbugs is of utmost importance.

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Introduction

Certainly, modern medicine is indebted to developments in antibiotics research areas and antibiotics, by treating and elimination of infectious agents play a critical role in survival and increased quality of life (1). Currently there are various kinds of beta-lactam antibiotics such as penicillins, cephalosporins, monobactams and carbapenems for treatment of patients infected with bacterial infections (2, 3). Beta-lactamases according to the type of their substrates are divided into four functional groups: penicillinase, extended spectrum beta-lactamase producing bacteria (ESBL), carbapenemase and cephalosporinase type AmpC (4).

ESBL is found in certain genera of the *Enterobacteriaceae* family including *Escherichia coli* and *Klebsiella pneumoniae* and other bacteria like *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa* (5). Plasmid-encoded ESBL can spread among bacteria. This enzyme hydrolyzes a beta-lactam antibiotic and changes the expression of efflux pump or purines which can cause resistance (6). Until now 200 types of ESBL have been recognized throughout the world. The first was TEM which was discovered in 1963. Other common ESBL enzymes are SHV, CTX, PER and VEB (7-9). These enzymes are inhibited by clavulanic acid, sulbactam and tazobactam (10). Nowadays resistance to antibiotics like fluoroquinolones, aminoglycosides and cotrimoxazole in ESBL producing bacteria has resulted in multidrug resistant genera which have complicated the treatment of infections caused by these bacteria.

Approximately 67% of the bacteria isolated from nosocomial infections are claimed to be capable of producing beta-lactamase (11). Prolonged length of stay in hospitals, use of urinary catheters and unjustified use of antibiotics are the major risk factors to acquire ESBL producing organisms. In order to choose the appropriate antibiotic therapy, certain laboratory methods like double disc diffusion test (DDT), double disc synergy test (DDST), combination disc testing (CPT) and E-test have been developed for identification of ESBL phenotype (12, 13).

Today, there is a concern about the spread of such bacteria from hospital to community (14). Treatment of the infections caused by these organisms is a major challenge for healthcare facilities and preventive strategists. Since ESBL producing gram-negative bacilli, including multidrug resistant bacteria are important causes of infections in the healthcare settings, the aim of this study is to survey of the rate of ESBL producing *E. coli* and *K. pneumoniae* in patients referring to hospital and special clinic of the Imam Reza hospital of Mashhad.

Material and Methods

In this descriptive, cross-sectional study, 339 clinical samples including urine (n = 260), wound secretions (n = 46), blood (n = 18), eye secretions (n = 7), pleural fluid (n = 6) and CSF (n = 2) were collected from 2007 till 2008 in hospital (n = 200) and special outpatient clinic (n = 139) of the Imam Reza hospital of Mashhad. The samples were evaluated to determine the frequency of

ESBL producing *E. coli* and *K. pneumonia*. In order to diagnose the gram negative bacilli, the samples were cultured on EMB, MacConkey agar and gram staining, oxidase; and catalase test were performed. Other differential tests like TSI, SIM, Simon citrate and urea were used to determine the existence of *E. coli* and *K. pneumoniae*. In order to survey ESBL secretion, DDT method was carried out with the use of discs from Mast Company (United Kingdom). First, suspension with opacity of 0.5 McFarland standards was prepared and then cultured on Müeller-Hinton agar (Merck Company, Germany). Two plates of Müeller-Hinton agar media were separately used for each isolate. In one plate, 30 µg augmentin disc (a combination of amoxicillin and clavulanic acid) and 15 µg aztreonam disc were placed at 25-30 mm from one another. In the other plate, 30 µg ceftazidime was placed with the same distance from ceftazidime-clavulanic acid disc (10 µg clavulanic acid and 30 µg cefotaxime). Then the plates were incubated at 30°C for 18-24 h. If the inhibition zone diameters of ceftazidime / clavulanic acid and cefotaxime / clavulanic acid were increased more than or equal to 5 mm compared to ceftazidime and cefotaxime discs alone, the isolate was considered as ESBL-positive.

Results

Samples were collected from 192 (56.6%) females and 147 (43.4%) males. From 339 isolates, 211 were *E. coli* and 128 were *K. pneumoniae* 56 (26.5%) isolates of *E. coli* and 55 (43%) isolates of *K. pneumoniae* were ESBL producers. Additionally, out of the inpatients and outpatients, 93 (46.5%) and 18 (13%) were ESBL positive, respectively (*Table 1*). Urine was the major source for ESBL-producing bacteria and 68% of ESBL-producing *E. coli* and 71% of ESBL-producing *K. pneumoniae* were isolated from urine samples (*Table 1*). The percentage of frequency of ESBL-producing bacteria in ICU, urology, infectious, burn and neonatal units were 75.7, 52.4, 46.2, 20.6 and 9.1%, respectively (*Table 2*).

Table 1. The frequency of ESBL-secreting bacteria according to type of samples isolated from the patients in the study

	Type of sample	*E. coli* No. (%)	ESBLEc No. (%)	*K. pneumoniae* No. (%)	ESBLKp No. (%)
Inpatient	Urine	77 (36.5)	38 (67.9)	56 (43.8)	39 (70.9)
	Blood	8 (3.8)	1 (1.8)	10 (7.8)	2 (3.6)
	Wound secretions	22 (10.4)	3 (5.4)	15 (11.7)	5 (9.1)
	Eye secretions	2 (0.9)	1 (1.8)	2 (1.6)	0 (0)
Outpatient	pleural fluid	2 (0.9)	1 (1.8)	4 (3.1)	2 (3.6)
	CSF	0 (0)	0 (0)	2 (1.6)	1 (1.8)
	Urine	91 (43.1)	11 (19.6)	36 (28.1)	5 (9.1)
	Wound secretions	8 (3.8)	1 (1.8)	1 (0.8)	0 (0)
	Eye secretions	1 (0.5)	0 (0)	2 (1.6)	1 (1.8)
	Total	211 (100)	56 (100)	128 (100)	55 (100)

ESBLEc: ESBL-Positive *E. coli*; ESBLKp: ESBL-Positive *K. pneumoniae*

Table 2. Frequency of ESBL-secreting bacteria based on the ward (for inpatients)

Ward	*E. coli* (ESBL+/Total) (%)	*K. pneumoniae* (ESBL+/Total) (%)	Total (%)
ICU	12/17 (70.6)	16/20 (80)	28/37 (75.7)
Urology	14/33 (42.4)	19/30 (63.3)	33/63 (52.4)
Infectious	10/25 (40)	8/14 (57.1)	18/39 (46.2)
Burn	3/21 (14.3)	4/13 (30.8)	7/34 (20.6)
Neonatal	1/4 (25)	0/7 (0)	1/11 (9.1)
Other	4/11 (36.4)	2/5 (40)	6/16 (37.5)
Total	44/111 (39.6)	49/89 (55.1)	93/200 (46.5)

Discussion

The increasing trend of ESBL-producing bacteria which could be a warning for public health and patients of ICU has imposed additional cost and significant mortality (15). In the present study, 26.5% of *E. coli* and 43% of *K. pneumoniae* were ESBL encoding enzyme. In other studies which carried out in Iran, the relative frequency of ESBL producing *E. coli* was different in percentage 17.4%-89% and for *K. pneumoniae* it was 19.6%-61% (16-22). In the two other recent studies that was conducted in Kashan and Tehran, ESBL producing *E. coli* frequency was 46.6% and 89%, respectively (17, 18). In Tehran, Alipourfard et al (2011) reported that 60% of *E. coli* and 40% of *K. pneumoniae* were ESBL positive and the percentage of the latter was similar to our study (19). In another study in Mashhad, Bazzaz et al, 57.7% of *E. coli* and 61% of *K. pneumoniae* were ESBL positive which were both more than the expectation (20).

In studies performed throughout the world, the frequency of ESBL positive *E. coli* was from 0.2% to 95.4% and ESBL positive *K. pneumoniae* was from 17% to 66.7% (23-31). For example, in United Arab Emirates (2008), out of 130 samples, 39% of *E. coli* and 42% of *K. pneumoniae* were ESBL positive (27). In Turkey (2006), 12% of *E. coli* and 47% of *K. pneumoniae* were ESBL positive and the percentage of *K. pneumoniae* was similar to our study (30). In Tanzania (2009), 24.4% of *E. coli* and 63% of *K. pneumoniae* were ESBL positive and the percentage of *E. coli* was similar to our study (28). In Skopje (2009), 11.8% of *E. coli* and 24.3% of *K. pneumoniae* were ESBL positive (23). In India (2007), out of 2655 samples, 14.4% of *E. coli* and 26.6% of *K. pneumoniae* were ESBL positive and this percentage for both bacteria was lower than our study which can be due to differences in test method, the type of samples and the study population (31).

In this study, ESBL accounted for 40.5% and 12% of *E. coli* and 55% and 4.15% of *K. pneumoniae* of inpatients and outpatients, respectively. The studies carried out in two cities of Iran (2007), from 219 clinical specimens in Tehran, ESBL-positive *E. coli* and ESBL-positive *K. pneumoniae* was present in 6.1% and 31.4% of inpatients and 1.7% and 12.2% of outpatients, respectively. In Tabriz, from 194 clinical specimens, ESBL-positive *E. coli* and ESBL-positive *K. pneumoniae* was present in 4.6% and 21.4% of inpatients and 1.1% and 1% of outpatients, respectively (32). The percentage of ESBL-producing *E. coli* in other countries has been reported 52-93% and 16-51% among

inpatients and outpatients, respectively (24, 28, 33-35).

Intensive care unit is usually the major source of ESBL-producing bacteria in hospitals and there is a great chance for spread of these bacteria among inpatients and nurses through infected hands or hospital instruments (9, 36). In a study carried out in Lebanon (2003), out of 6532 clinical samples, 2% of *E. coli* and 20% of *K. pneumoniae* were ESBL positive (26). In ICU, 28.1% of *E. coli* and 8.34% of *K. pneumoniae* were ESBL positive which is similar to the present study in that ESBL-producing bacteria in ICU were more prevalent than other wards. Urine was the major test sample for ESBL positive (83%) and the next ones were wound and blood. In some other similar studies most of the ESBL producing bacteria were found in urine samples and the percentage varied from 49.5% to 72.8% between studies (24, 37, 38).

Conclusion

Finally, the present study showed high prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in our region. Appropriate use of antibiotics and following of CLSI guidelines for screening beta-lactam resistance by using DDT test is recommended.

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Conflict of Interest

None declared conflicts of interest.

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