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Original Article

Evaluation of Extended Spectrum Betalactamase(ESBL) Positive Strains of *Klebsiella pneumoniae* And *Escherichia coli* in Bacterial Cultures

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ABSTRACT

Background and Objective: **To evaluate extended spectrum betalactamase (ESBL) positive strains of *Klebsiella pneumonia* and *Escherichia coli* in positive bacterial cultures.**

Materials and Methods: **In this analytical cross-sectional study, between March 2006 and March 2007, 170 bacterial isolates including 133 cases of *E. coli* and 37cases of *K. pneumonia* were examined. All cases underwent double disk diffusion for ESBL. Demographic data were assessed and all data analyzed accordingly.**

Results: **Patients' mean age was 55±26.63 yr. Ninety six cases (56.5%) were female and 74 cases (43.5%) were male. Clinical presentation of infection were 118 cases UTI (96.4%), 15 cases septicemia (8.8%), 16cases wound infection (9.4%), 7 cases pneumonia (4.1%), 1 case meningitis (0.6%) and 13 cases other presentations (7.6%). Frequency of ESBL positive in *E. coli* isolates was 38 cases (28.6%) and in *K. pneumonia* isolates was 10 cases (27%). There was no significant correlation between ESBL positivity and age, gender, ward or clinical presentation of infection.**

Conclusion: **Incidence of ESBL positive isolates of *E. coli* and *K. pneumonia* was high. These results should be considered in administration of broad-spectrum antibiotics by clinicians.**

Keywords: *E. coli*, *Klebsiella pneumonia*, Broad spectrum betalactamase (ESBL)

Introduction

Entrobacteriacae group is the main cause of bacterial infection in the world. *E. coli* and *Klebsiella pneumonia* are the most prevalent causes of nosocomial infection, in this family (1, 2).

It is believed that antibiotic resistance is the most important cause of failure in infection treatment, especially in enterobacteriacae nosocomial infections. Betalactamases are the main mechanism of betalactam group resistance in gram-negative bacteria. During the last decade, acquiring resistance to 3rd generation of cephalosporins and extended spectrum betalactamases (ESBLs) among enterobacteriacae group increased significantly (3, 4).

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In enterobacteriacae group, the main betalactamase enzymes are TEM and SHV. Plasmid mediated mutation in TEM and SHV genes may cause ESBL. *E. coli* and *K. pneumonia* are the most prevalent ESBL producing isolates (5, 6).

It is believed that wide and abundant administration of 3rd generation of cephalosporins is the main cause of ESBL incidence (5). Rate of nosocomial infections varies in different hospitals and depends on many factors, like pathogens and antibiotic resistance of hospital (3, 5, 7). Evaluating ESBL producing pathogens of a hospital can help clinicians in empirical treatment of high-risk patients with serious nosocomial infections (5).

The main aim of this study was to determine the incidence of ESBL producing *K. pneumonia* and *E. coli* to reduce the antibiotic therapy failure in hospitals.

Materials and Methods

In this analytical cross-sectional study, 37 isolates of *K. pneumonia* and 133 isolates of *E. coli* in Microbiology Ward of Hazrat Rasul Hospital, Tehran, Iran during April and November of 2008 were included.

Sensitivity to specific antibiotics was evaluated by paper diffusion disks. A suspension of isolates in sterile normal saline is prepared and the absorbance of solution was 0.5 Mcfarland on 12-x10CFU/ml cell. A full surface-covering culture on Muller-Hinton agar gel (4mm) plates were produced with a sterile swab.

After 15min delay for absorption of microbe suspension in agar, antibiotic disks (Mast group company Ltd., Merseyside, U.K.) were placed on agar surface with an applicator.

Then plates were incubated for 16-18 hrs, as recommended by Clinical and Laboratory Standards Institute (CLSI). After incubation, microbe-free zones around disks were evaluated with a ruler and recorded in millimeter scale.

Based on CLSI criteria, if there was a suspicion of being the isolate ESBL producing, double disk diffusion was done (Table 1).

Table 1. Screening test for extended spectrum betalactamases (1)

Antibiotic	disk & zone break point
Cefpodoxime	10mcg:<=17mm
Ceftazidime	30mcg:<=22mm
Cefotaxime	30mcg: <=27mm
Ceftriaxone	30mcg:<=25mm

Double Disk Diffusion technique:

Isolated microbe was emulsified with a wire loop in 0.9% saline. The suspension was spread by a cotton head swab on the surface of Muller-Hinton agar.

Co-Amoxiclav disk was placed in the center of plate. Then cefotaxime, ceftriaxone, ceftazidime and cefopodoxime disks placed around the Co-Amoxiclav disk. The gap between satellite disks and the central disk was defined around 30mm.

Results were evaluated and recorded after 24 h. If growth-free zones of satellite disks spread to the central disk microbe-free zone, the isolate was ESBL producing (Fig. 1). If there was not any spread of satellite zones to central zone, the test was repeated again. If the test was negative for 2 times, the isolate was determined as NOT ESBL producing (7).

All data were analyzed with SPSS12 software. *P* values under 0.05 assumed significant.

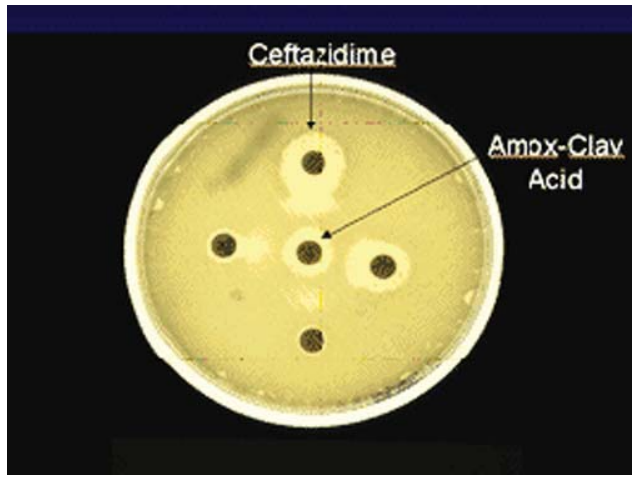

Fig 1. Double disk diffusion technique. Growth-free zone of ceftazidime disk (short arrow) extended to growth-free zone of Co-Amoxiclav disk(long arrow).

Results

Mean age of patients was 55.00±26.63 yr. Ninety six cases (56.5%) were female and 74 cases (43.5%) were male.

Clinical presentation was UTI in 118 cases (96.4%), septicemia in 15 cases (8.8%), wound infection in 16 cases (9.4%), pneumonia in 7 cases (4.1%), meningitis in 1 case (0.6%) and other infections in 13 cases (7.6%).

Specimen type was as follows: 118 cases (96.4%) urine, 15 cases (8.8%) blood, 7 cases (4.1%) tracheal tube washing fluid, 5 cases (2.9%) swab, 6 cases (3.5%) wound exudates, 5 cases (2.9%) eye swab, 13 cases (7.6%) other types and 1case (0.6%) CSF. ESBL producing isolates incidence was in 48 cases (28.2%); 38 cases (28.6%) of *E. coli* and 10 cases (27%) of *K. pneumonia*.

The frequency of ESBL producing isolates in each ward of hospital and based on clinical presentation of infection and specimen type is shown in Tables 2-4.

Table 2. ESBL positive isolates based on ward of admission

WARD			BACTERIA		Total
			K. pneumonia	E. coli	
Outpatient	ESBL	negative	3	8	11
		Positive	0	5	5
		Total	3	13	16
Pediatric	ESBL	negative	2	4	6
		positive	0	2	2
		Total	2	6	8
Internal	ESBL	negative	8	66	74
		positive	1	18	19
		Total	9	84	93
Surgery	ESBL	negative	10	8	18
		positive	5	8	13
		Total	15	16	31
ICU	ESBL	negative	0	5	5
		positive	2	0	2
		Total	2	5	7
Ob.Gyn	ESBL	negative	2	3	5
		positive	0	3	3
	Total	Total	2	6	8
NICU	ESBL	Negative	2	1	3
		positive	2	2	4
		Total	4	3	7

Table 3. ESBL positive isolates based on clinical presentation of infection

Infection type			BACTERIA		Total
			K. pneumonia	*E. coli*	
UTI	ESBL	negative	13	78	91
		positive	3	24	27
		Total	16	102	118
Septicemia	ESBL	negative	2	6	8
		positive	4	3	7
		Total	6	9	15
Wound infection	ESBL	negative	5	3	8
		positive	2	6	8
		Total	7	9	16
Others	ESBL	negative	2	7	9
		positive	0	4	4
		Total	2	11	13
Pneumonia	ESBL	negative	5	1	6
		positive	1	0	1
		Total	6	1	7
Meningitis	ESBL	positive	0	1	1
		Total	0	1	1

Table 4. ESBL positive isolates based on specimen type

Specimen			BACTERIA		Total
			K. pneumonia	*E. coli*	
Urine	ESBL	negative	13	78	91
		positive	3	24	27
		Total	16	102	118
Wound	ESBL	negative	2	2	4
		positive	0	2	2
		Total	2	4	6
Respiratory	ESBL	negative	5	1	6
		positive	1	0	1
		Total	6	1	7
Blood	ESBL	negative	2	6	8
		positive	4	3	7
		Total	6	9	15
CSF	ESBL	positive	0	1	1
		Total	0	1	1
Eye	ESBL	negative	3	1	4
		positive	1	0	1
		Total	4	1	5
Swab	ESBL	positive	1	4	5
		Total	1	4	5
Others	ESBL	negative	2	7	9
		positive	0	4	4
		Total	2	11	13

There was no correlation between ESBL producing isolates frequency and age, gender, ward, specimen or clinical presentation.

Discussion

This study was performed to determine ESBL producing isolates of *E. coli* and *K. pneumonia* in a general hospital in Tehran.

Nowadays, many studies have been done to evaluate antibacterial resistance of bacterial pathogens and load of pathogen resistance produce nosocomial and community-acquired infections (1).

Enterobacteriacae family, specifically *E. coli* and *K. pneumonia* are responsible for a broad spectrum of clinical infections in immune competent or immune compromised people. Also, those pathogens have a key role in epidemics of nosocomial infections in many hospitals (8, 9).

In recent years, the incidence of ESBL producing isolates of *E. coli* and *K. pneumonia* increased (4, 7, 10). Many researchers and clinicians work together to inspect and report the microbial resistance (innate and acquired resistance), these researches not only necessitate production of new antibiotics, but also inform clinicians and health managers to make efficient strategies to repel this danger (11).

Resistance incidence is high in undeveloped and developing countries like African countries, Saudi Arabia, Turkey and Iran; it is above 40 percent (11-20); but this measure is lower in developed countries and is below 10% (21-24).

As shown in results, ESBL producing isolates of *E. coli* and *K. pneumonia* in this study were about 28%; this measure is lower than other studies done in Iran (11, 12, 15). Incidence of ESBL producing isolates in those mentioned researches was about 50%; this shows that the rate of ESBL positive isolates is lower and hence better in our hospital.

Rate of ESBL positive isolates between *K. pneumonia* and *E. coli* was not significantly different in our study; this result is the same with Bazzaz and Aminzadeh studies (11,13).

UTI was the most frequent type of clinical infection in this study; based on the results of previous studies (10-13, 20) and pathogenic profile of those two bacteria this result is satisfactory.

In this study, there was not any relationship between frequency of ESBL positive isolates and age, gender and ward; this measure is slightly higher in surgical, Ob.Gyn. and ICU wards, although it is not significant. This result is correlated with Angel Diaz study (21).

In the present study, ESBL positive isolates rate was not different between outpatient and inpatient cases; In Mshana, Lehner and Jabeen studies, ESBL positive isolates rate were higher in inpatient cases (13,22,16), but this measure was not different in Angel Diaz study (21).

Conclusion

Incidence of ESBL positive isolates of *K. pneumonia* and *E. coli* was higher than results of studies done in developed countries, but it was lower than developing countries and was as same as studies had been done in Iran. Physicians should notice to these measures, when they administer antibiotics, empirically. At last, administration of new antibiotics should be under observation of infection control committee of hospital; this will reduce the rate of unauthorized use of modern antibiotic and as a result, it will reduce the rate of bacterial resistance.

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