The prevalence of extended-spectrum beta lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in clinical isolates and risk factors

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ABSTRACT

Objectives: To determine the prevalence of extended-spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae (K. pneumoniae)* and *Escherichia coli (E. coli)*, risk factors of ESBL-producing strains and antimicrobial susceptibility pattern of ESBL-producing and non producing strains.

Methods: The study took place at the Faculty of Medicine, Osmangazi University, Eskisehir, Turkey from March to November 2002. We evaluated 100 *K. pneumoniae* and 100 *E. coli* strains isolated from various clinical specimens, as well as the patients from whom these strains were isolated. The double-disk synergy test was performed on the isolates for the detection of ESBL. We visited the patients with a growth of *E. coli* or *K. pneumoniae* or both from their clinical specimens in their wards if they were hospitalized, while the outpatients with a growth of these microorganisms were evaluated from their hospital records.

Results: The prevalence of ESBL-producing *K. pneumoniae* was 47% and *E. coli* was found as 12%. The ESBL-producing isolate rates were 50% (14/28) in intensive care units, 36.1% (35/97) in wards and 13.3% (10/75) in outpatients. Foley catheter ($p<0.001$), intravenous catheter ($p<0.001$), central venous catheter ($p=0.002$), intubation ($p<0.001$), surgery ($p<0.001$) and mechanical ventilation ($p=0.002$) were found as the risk factors for the acquisition of *E. coli* and *K. pneumoniae* with ESBLs.

Conclusion: In our study, the prevalence of ESBL-producing isolates was high. The results of the study suggest that an antimicrobial policy and early removal of interventional apparatus be of importance for the control of ESBL-producing *K. pneumoniae* and *E. coli*.

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There are many mechanisms of antimicrobial resistance, but ß-lactamase production is the most important mechanism for bacterial resistance to ß-lactam antibiotics. Since *Klebsiella pneumoniae (K. pneumoniae)* with extended-spectrum ß-lactamase (ESBL) was first isolated in Germany in 1983, many outbreaks caused by multi-resistant strains have been reported all over the world.[1,2] In addition, as ESBL-producing *Escherichia coli (E. coli)* and *K. pneumoniae* isolates are frequently resistant to multiple antimicrobial agents, therapeutic options for these infections are severely limited.[3]

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Current guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) recommend screening all *K. pneumoniae*, and *E. coli* isolates for which minimal inhibition concentrations (MIC) of cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone are >2 μg/mL for evidence of an ESBL.[4] Several tests have been developed to confirm the presence of ESBLs, including the double-disk diffusion assay, the ESBL E-test strip, and several automated susceptibility systems. *Enterobacteriaceae* producing both AmpC ß-lactamases and ESBLs have been increasingly reported worldwide. Since high-level expression of AmpC ß-lactamases may mask recognition of ESBLs, it should also be examined whether AmpC exists or not.[5,6]

In this study, we tried to find the following: 1. the prevalence of ESBL-producing *K. pneumoniae* and *E. coli*; 2. risk factor for acquisition of ESBL-producing *K. pneumoniae*, and *E. coli*; 3. antimicrobial susceptibility pattern of ESBL-producing and non producing strains.

Methods. This study was conducted at Osmangazi University Faculty of Medicine, a teaching hospital with 950 beds located in Eskisehir, Turkey. From March to November 2002, all *K. pneumoniae* and *E. coli* strains isolated from clinical specimens by the microbiological laboratory were collected.

Bacterial susceptibility to all antimicrobial agents was determined by Vitek (bioMérieux, Marcy-l' Etoile, France) system or the API 20E (bioMérieux, Marcy-l' Etoile, France) system .

The double-disk synergy test was performed by a standard disk diffusion assay. In this test, organisms were swabbed onto a Mueller- Hinton agar (Oxoid Basingstoke, Hampshire, UK) plate. A susceptibility disk containing amoxicillin/clavulanate (20/10 μg) was placed in the center of the plate, and other disks containing aztreonam, ceftazidime and cefotaxime (30 μg each) were placed at variable distances (20-30 mm) center to center from the amoxicillin/clavulanate disk. Clavulanate enhancement of the diameter of the inhibition zone around either the cephalosporin disk by at least 4 mm was regarded as presumptive evidence for the presence of ESBL. Inducible ß-lactamase was determined with direct induction test in the strains.

The patients with a growth of *E. coli* or *K. pneumoniae* or both from clinical specimens that were obtained from them were visited on their wards if they were hospitalized. The outpatients with a growth of these microorganisms were evaluated from their hospital records.

The first isolate was used in patients where multiple isolation were found in the same patient. The names, hospital identification numbers, age, gender and departments of the patients were recorded. Previous or ongoing intensive care unit admissions were also recorded for all patients. The isolation sites of the bacteria were recorded as blood, urine, sputum, tracheal aspirate, wound specimen, catheter and others. The isolation time was recorded as days between the hospitalization day of the patient and the day the culture specimen was taken. The isolations were classified as colonization, community acquired infection and nosocomial infection according to clinical findings of infection and definitions of Center for Diseases Control and Prevention (CDC) for nosocomial infections. The diagnoses of the infections were classified as bloodstream infection, urinary tract infection, surgical site infection, catheter related infection, lower respiratory tract infection and other infections. Urinary catheter, intravenous (IV) catheter, central venous catheter, arterial catheter, intubation, tracheotomy, mechanical ventilation applications and surgical procedures prior to isolation of the microorganisms were evaluated and recorded for all patients. The underlying diseases, immune status, chemotherapy applications of the patients were also recorded. The antimicrobial therapy administered within the 30 days before specimen collection was recorded. The test results of antimicrobial susceptibility of all isolates were recorded.

All statistical analysis was performed using the Statistical Package for Social Science version 11.5. Contingency table analysis was carried out by x^2 test, Fisher's exact test or continuity correction test if appropriate. Continuous or interval variable analysis was completed with t-test or Mann-Whitney test. Multivariate analysis was applied to all the key variables, which were significantly associated with the outcome in the univariate analysis, by using a logistic regression model to identify the risk factors in the acquisition of ESBL-producing *K. pneumoniae* and *E. coli*. A p value <0.05 was considered statistically significant.

Results. During the study period, 100 isolates of *K. pneumoniae* and 100 isolates of *E. coli* were collected. Only first isolates of all patients were collected. There were 59 patients from whom *E. coli* or *K. pneumoniae* infection ESBL-producing were detected. Of these isolates, 47 were *K. pneumoniae* and 12 were *E. coli*. The prevalence of ESBL-producing strains was 29.5% (59/200). Inducible ß-lactamase was not determined in all strains.

The 5.1% of the ESBL-producing isolates were found sensitive to ceftazidime, cefoperazone and ceftriaxone. The distribution of ESBL-producing isolates to isolation sites was as follows: 28 isolates

from urine, 12 isolates from blood, 9 isolates from wound, 7 isolates from bronchial aspirate, 2 isolates from sputum and 1 isolates from a central venous catheter tip.

In this study, the mean age was 39.7 years in ESBL-producing group and 37 years in ESBL-non-producing group. Male to female ratios were 32/27 in ESBL-producing group and 47/94 in ESBL-non-producing group. Mean internal between date of isolation and admission day was 11.5 days in ESBL-producing group and 4.9 days in ESBL-non-producing group. Twenty-eight of patients were from ICU, 97 of them from wards and 75 of them were outpatients. The ESBL-producing isolate rates were 50% (14/28) in intensive care units, 36.1% (35/97) in wards and 13.3 % (10/75) in outpatients. In intensive care units, ESBL-producing isolate rate was significantly higher than wards and outpatient setting ($p<0.001$). In outpatient, group ESBL-producing isolate rate was found significantly lower than wards ($p<0.001$). The interval between the date of admission and isolation of the bacteria was found longer in ESBL-producing isolates ($p<0.001$) (**Table 1**).

The risk factors found to be significantly associated with the acquisition of ESBL-producing isolates on univariate analysis were insertion of urinary catheter ($p<0.001$), insertion of IV catheter ($p<0.001$), insertion of central venous catheter ($p=0.002$), intubation ($p<0.001$), application of surgery ($p<0.001$) and application of mechanical ventilation ($p=0.002$) (**Table 2**).

The third generation cephalosporin ($p=0.001$) and aminoglycoside ($p<0.001$) usage was found to be higher in ESBL-producing group (**Table 3**). The antimicrobial susceptibility test results of ESBL-producing and non-producing isolates were evaluated (**Table 4**).

Multivariate analysis was applied to all key variables by a logistic regression model to identify the risk factors, which were significantly associated with acquisition of *K. pneumoniae* and *E. coli* with ESBLs. Only insertion of endotracheal tube (odds ratio, 4.335; 95% CI, 2.068 ± 9.086; $p<0.001$) and aminoglycosides usage (odds ratio, 3.725; 95% CI, 1.725 ± 8.041; $p=0.001$) remained as risk factors.

Discussion. The *E. coli* and *K. pneumoniae* are leading causes of serious infections. After the initial description of ESBL production by *K. pneumoniae* strains in 1983[1] and *E. coli* strains in 1987, resistance to broad-spectrum cephalosporins was increasingly reported.[7]

The main goal of this study was to assess the impact of risk factors on the ESBL-producing *E. coli* and *K. pneumoniae* strains isolated from clinical specimens. Prevalence of ESBL-producing isolates among 100 *E. coli* (12%) and 100 *K. pneumoniae* (47%) strains in our hospital was 29.5%.

In this study, the prevalence of ESBL-producing isolates was found very high in hospitalized patients than non-hospitalized. These rate were 35/97 (36.1%) in hospitalized group and 10/75 (13.3%) in non-hospitalized. The rate of ESBL-producing isolates was found higher in ICU than wards and not admitted group, which was 14/28 (50%) ($p<0.001$). In previous reports, ESBL-producing isolates were found to be particularly common in high-risk patients in ICUs, whose infection control was difficult and who were under high antibiotic pressure. ICUs are considered to be source from which resistant organisms disseminate to other hospital areas.[8,9]

The process of colonization or infection by *E. coli* and *K. pneumoniae* with ESBLs often begins following contact with colonized patients, staff or contaminated objects. Invasive manipulations may allow direct transmission of pathogens. Previous studies suggested that Foley catheter, IV catheter, arterial catheter, central venous catheter, endotracheal tube, surgery, tracheotomy and mechanical ventilation could be related to colonization or infection with ESBL-producing *Enterobacteriaceae*.[3,10-14] We found that Foley catheter ($p<0.001$), IV catheter ($p<0.001$), central venous catheter ($p=0.002$), intubation ($p<0.001$), surgery ($p<0.001$) and mechanical ventilation ($p=0.002$) were risk factors in the acquisition of *E. coli* and *K. pneumoniae* with ESBLs by univariate analysis. Using a logistic regression, endotracheal tube and previous antimicrobial therapy with aminoglycosides were found to be risk factors.

Lin at al[12] reported 4 isolates of *K. pneumoniae* with ESBLs to be sensitive to ceftazidime, one isolate sensitive to ceftriaxone at NCCLS break points. In our study, all 7 isolates of *E. coli* and *K. pneumoniae* with ESBL were found sensitive to ceftazidime, cefotaxime, cefoperazone; 2 of them sensitive to ceftazidime and cefotaxime, 3 of them sensitive to cefoperazone, one of them sensitive to ceftazidime, one of them sensitive to cefotaxime at NCCLS break points. Such microbiology laboratory reports can mislead physicians as they may choose inappropriate antibiotics to treat infections due to ESBL-producing bacteria. In fact, only few therapeutic choices for infections due to ESBL-producing bacteria are available. Carbapenems appears to be the drug of choice for serious infections due to *K. pneumoniae* and *E. coli* with ESBLs. Other alternatives for treatment may have limitations. It is reported that 30% of *Klebsiella* species with ESBLs was resistant

Table 1 - Demographic data of the patients and ICU, ward, outpatient distribution of the patients.

Variable	ESBL(+) N=59	(%)	ESBL (-) N=141	(%)	*P*-value
Mean age (years)	39.7		37		0.531*
Male/female	32/27		47/94		0.009†
Mean interval between date of isolation and admission day	11.5		4.9		<0.001‡
ICU	14/28	(50)	14/28	(50)	
Wards	35/97	(36.1)	62/97	(63.9)	<0.001§
Outpatient	10/75	(13.3)	65/75	(86.7)	

*T-test, †Continuity correction, ‡Mann-Whitney test, §Pearson chi-square (The difference between ICU, wards and outpatients)
ESBL - extended spectrum beta-lactamase, ICU - intensive care unit

Table 2 - The interventions during hospitalization and relationship with ESBL-producing strains.

Risk factors	ESBL (+) N=59	(%)	ESBL (-) N=141	(%)	Odds ratio	*P*-value
Urinary catheter	33	(55.9)	43	(30.5)	2.893	<0.001*
Intravenous catheter	41	(69.5)	57	(40.4)	1.755	<0.001†
Surgery	21	(35.6)	15	(10.6)	4.642	<0.001*
Intubation	32	(54.2)	22	(15.6)	6.411	<0.001*
Arterial catheter	10	(16.9)	11	(7.8)	2.412	0.095*
Central venous catheter	20	(33.9)	19	(13.5)	3.293	0.002*
Tracheotomy	3	(5.1)	2	(1.4)	3.723	0.154‡
Mechanical ventilation	16	(27.1)	13	(9.2)	3.664	0.002*
Immune compromised	1	(1.7)	4	(2.8)	-	1‡

*Continuity correction, †Pearson Chi-square, ‡Fisher's Exact Test
ESBL - extended spectrum beta-lactamase

Table 3 - The antimicrobials used within 30 days before specimen collection.

Antimicrobials	ESBL (+) N=59 (%)	ESBL (-) N=141 (%)	Odds ratio	*P*-value
Aminoglycosides	29 (49.2)	22 (15.6)	5.229	<0.001*
Penicillin group	17 (28.8)	24 (17)	-	>0.05*
First-generation cephalosporins	4 (6.8)	3 (2.1)	-	>0.05†
Second-generation cephalosporins	3 (5.1)	4 (2.8)	-	>0.05†
Third-generation cephalosporins	23 (39)	22 (15.6)	3.456	0.001*
Forth-generation cephalosporins	5 (8.5)	5 (3.5)	-	>0.05†
Quinolones	9 (15.3)	12 (8.6)	-	>0.05

*Continuity correction, †Fisher's Exact Test
ESBL - extended spectrum beta-lactamase

Table 4 - The antimicrobial susceptibility patterns of ESBL-producing and non-producing strains to various antimicrobials.

Antimicrobials	ESBL (+) N=59	(%)	ESBL (-) N=141	(%)	Total N	(%)	*P*-value*
Ampicillin	2	(3.4)	53	(37.6)	55	(27.5)	<0.001
Amoxicillin/ clavulanate	7	(11.9)	93	(66)	100	(50)	<0.001
Cefazolin	3	(5.1)	92	(65.2)	95	(47.5)	<0.001
Cefoxitin	50	(84.7)	131	(92.9)	181	(90.5)	0.113
Cefotaxime	3	(5.1)	133	(94.3)	136	(68)	<0.001
Ceftazidime	3	(5.1)	135	(95.7)	138	(69)	<0.001
Cefoperazone	3	(5.1)	123	(87.2)	126	(63)	<0.001
Gentamicin	26	(44.1)	131	(92.9)	157	(78.5)	<0.001
Tobramycin	19	(32.2)	129	(91.5)	148	(74)	<0.001
Aztreonam	0	(0)	126	(89.4)	126	(63)	<0.001
Ciprofloxacin	36	(61)	128	(90.8)	164	(82)	<0.001
Ofloxacin	37	(62.7)	130	(92.2)	167	(83.5)	<0.001
Trimethoprim/ sulfamethoxazole	28	(47.5)	102	(72.3)	130	(65)	0.001
Imipenem	59	(100)	141	(100)	200	(100)	-

*2 proportions test, ESBL - extended spectrum beta-lactamase

to piperacillin-tazobactam in Europe.[15] In addition, ceftazidime resistance in *K. pneumoniae* seems to be an important clinical problem especially in ICU. As the relationship between over use of antimicrobials and the increased risk of drug-resistant pathogens, the appropriate use of these agents seems to be a corner stone. The laboratory reports must alert the clinicians for ESBL-producing strains as some of the ESBL-producing strains can be found susceptible to penicillins and cephalosporins but can not be used for treatment (**Table 4**). It is reported that restriction of oxyimino-beta-lactam use in the management and successful control of a nosocomial ESBL-producing *K. pneumoniae* outbreak was effective.[13]

Evaluation the risk factors for ESBL-producing *E. coli* and *K. pneumoniae* infections altogether could be a limitation for this study. In conclusion, our results were suggesting Foley catheter, IV catheter, central venous catheter, intubation, surgery and mechanical ventilation to be risk factors for ESBL-producing *E. coli* and *K. pneumoniae*. The appropriate antimicrobial use and early removal of unnecessary interventional apparatus is of importance for the control and decreasing the prevalence of ESBL-producing *K. pneumoniae* and *E. coli*.

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