

Epidemiology and risk factors for ESBL-producing *Klebsiella pneumoniae*: a case control study

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Abstract

Introduction: Increased production of extended-spectrum β -lactamases (ESBLs) has become an important issue for treatment of severe *Klebsiella pneumoniae* (*K. pneumoniae*) infections. This study aimed to evaluate risk factors of infection from ESBL-producing *K. pneumoniae* (ESBL-KP).

Methodology: Risk factors were evaluated using a retrospective case control design. Fifty-two patients admitted to Firat University Hospital (FUH) with invasive infections from ESBL-KP were employed as cases. Patients admitted to FUH with non-ESBL-producing *K. pneumoniae* invasive infection were chosen as controls. Potential risk factors of the cases and controls were evaluated using hospital charts. Pulsed-field Gel Electrophoresis (PFGE) was used to show the relatedness of ESBL-KP strains.

Results: In univariate analysis, the following factors were found significant for ESBL-KP: pre-infection hospital stay, nosocomial origin, central venous catheterization, surgical intervention, antibiotic use longer than one week, and previous hospitalization. In contrast, stepwise logistic regression analysis showed that two variables, previous antibiotic use ($p = 0.000$) and surgical intervention ($p = 0.006$), remained significantly associated with risk for infection with an ESBL-KP. Molecular epidemiology identified several clusters among the ESBL-producing isolates.

Conclusions: Antibiotic use and surgical intervention were significant associated factors for infections with ESBL-KP.

Key words: ESBL, epidemiology, risk factors, case control study, PFGE typing

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Introduction

The oxyimino beta-lactams (β -lactams) were introduced into the treatment of bacterial infections in 1981. By 1983, extended-spectrum β -lactamase (ESBL)-producing organisms had already been isolated in Germany [1]. *Klebsiella pneumoniae* (*K. pneumoniae*) are the most commonly implicated organisms harboring a diversity of ESBL genotypes [2,3]. In Turkey, a much higher figure was quoted from different hospitals for *K. pneumoniae* isolates, among which the percentage of ESBL production was about forty percent [4,5]. In recent years, the importance of such ESBL-mediated infections has been increasingly recognized [6-8]. The association of ESBL-producing infections with both negative clinical outcomes and increased cost is of great concern [9,10].

Studies from various countries indicate that the major risk factors include severe underlying disease, prior administration of multiple antibiotics, surgical intervention, presence of indwelling catheters, or long stay in hospital [2,9-12]. Intubation and mechanical ventilator assistance in intensive care causes further risks for acquisition. Even though risk factors for the acquisition of ESBL-producing organisms have been reported from some centres, data from Turkey are not sufficient. This study aims to elucidate risk factors for invasive infections with ESBL-producing *K. pneumoniae* (ESBL-KP).

Methodology

Study location

The study was performed at Firat University Hospital (FUH) in Elazig city centre. It is one of the major teaching hospitals in the eastern part of

Turkey. FUH has approximately 850 beds and offers secondary as well as tertiary medical care to patients from Elazig and the surrounding region. It is also the referral centre for the surrounding area, and patients with severe, complicated cases are transferred to FUH from other hospitals. Molecular typing was performed at the Molecular Typing Laboratory of Infectious Diseases Department, Dicle University Hospital, Diyarbakir.

Study population: cases and controls

In a retrospective approach, cases were defined as consecutive patients who were hospitalized between 1 January 2004 and 31 October 2005, and from whom ESBL-KP were isolated from their clinical specimens during hospital stay. Only patients with signs and/or symptoms of active infection were included in the study. Patients were excluded if they had a documented infection with an ESBL-producing organism previous to the study period or if they were judged to have colonization rather than true infection. Controls consisted of the patients admitted to the hospital during the same period and from whom *K. pneumoniae* that were not ESBL-producing were recovered. One patient was selected as a control for each case patient; the following suitable patient after the identification of each case was included as control.

The medical records of the cases and controls were retrieved and reviewed. Information was obtained about basic demographic characteristics (age, sex, pre-infection hospital stay, and nosocomial origin) as well as co-morbid diseases (surgical intervention, renal diseases, respiratory diseases, central nervous diseases, and others), presence of previous antibiotic use, urinary catheters, ICU admission, previous hospitalization, recent surgery, and length of hospital stay. The diagnosis of nosocomial infection was established according to CDC criteria.

Previous hospitalization was defined as hospitalization at FUH or at another hospital within 30 days prior to the current admission. Recent surgery was defined as any surgical procedure performed in the operating room within 30 days of entry in the study. The origin of isolate was accepted as nosocomial if the strain was isolated more than one week after hospitalization. The relationship between ESBL-producing *K. pneumoniae* strains and the use of antibiotic in the previous 30 days was assessed. The antibiotics were grouped as carbapenems, third-generation cephalosprins,

quinolones, and others. Previous antibiotic therapy was defined as any systemic antibiotic given at least seven days within 30 days preceding the isolation of the organism.

Microbiologic testing

Identification of microorganisms was performed according to standard procedures [13]. According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and based on disk diffusion susceptibility testing, *K. pneumoniae* were initially accepted as ESBL-producing if they were resistant or intermediately susceptible to ceftazidime (30 µg; diameter ≤ 17 mm), cefotaxime (30 µg; diameter ≤ 17 µm), and aztreonam (30 mg; diameter ≤ 21 mm), and susceptible to cefoxitin (30 µg; diameter ≥ 15 mm) and imipenem (10 µg; diameter ≥ 16 mm) [13].

Molecular typing

Available isolates were evaluated for genetic relatedness by PFGE that was done according to Chu *et al* with *XbaI* digestion of genomic DNA with some modifications [14]. A CHEF Mapper apparatus (Bio-Rad, Hercules, CA, USA) was used for electrophoresis with an initial switch time of 5 seconds and final switch time of 35 seconds, with a total run time of 21 hours. Each gel contained two standard lanes of lambda Ladder PFGE Marker (New England Biolabs, Massachusetts, USA). Using BioNumerics software (GelCompar II, Sint-Martens-Latem, Belgium), computer analyses of the gels were performed. The Dice coefficient and unweighted pair group method with arithmetic averages were used to produce dendrograms for *K. pneumoniae* and clusters were identified. Strains with PFGE profiles of ≥ 85% similarity were considered to be the same strain.

Statistical analysis

The relation between ESBL-producing *K. pneumoniae* strains and possible risk factors was evaluated. Data were entered into a database using SPSS 10.0 for Windows (SPSS Inc, Chicago, USA). The X^2 test and the independent samples *t* test were used for categorical and continuous variables, respectively. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated from 2-by-2 contingency tables. A stepwise multivariate logistic regression was conducted to examine the association of risk factors controlling for potential confounders. The logistic model included all variables for which a *P* value of < 0.1 was obtained in the multivariate

analysis. A *p* value of <0.05 was considered significant. In addition, the relation between ESBL-PK and the use of antibiotic groups use was tested using the X^2 test.

Results

Study sample

During the study period, a total of 77 clinical culture specimens growing ESBL-producing *K. pneumoniae* were obtained from hospitalized patients. Twelve specimens were disregarded because they represented duplicate cultures. Of the remaining 65 patients, 52 had medical records available for review. These patients were included in the study as cases. Controls were identified consecutively on a 1:1 ratio to the cases and were matched on the date of isolation of *K. pneumoniae*; therefore, the final cohort consisted of 104 patients (52 cases and 52 controls). The duration of hospitalization was 16.4 (\pm 12.9) days in all patients. The duration of hospitalization in the case group was 19.8 (\pm 15.6) days and 13.0 (\pm 8.3) days (*P* = 0.001) in controls.

Epidemiological characteristics

Main epidemiological characteristics of cases and controls are shown in Table 1. The mean age of cases was similar to that of controls. There was no significant difference between the two groups with respect to primary diseases and co-morbid conditions. A significant proportion of infections with ESBL-

producing organisms were considered to be hospital-acquired when compared to infections with ESBL-non-producing organisms. In both groups, the most common site of infection was the urinary tract, followed by the respiratory tract, which yielded growth mostly in controls (Table 1).

Risk factors

Several factors were found to be significantly associated with an increased risk for infection with ESBL-producing organisms. Longer than seven days pre-infection hospital stay, previous antibiotic use, catheterization, previous hospitalization, and nosocomial origin of the microorganisms were found significant for ESBL-producing *K. pneumoniae* (Table 1).

On multivariate analysis, antibiotic use (OR = 95.21; CI = 15.8-573.3; *P* = 0.000) and surgical intervention (OR = 10.35; CI = 1.9-55.6; *P* = 0.006) remained significantly associated with an increased risk for infection with an ESBL-producing organism. When the antibiotic groups were compared using the Chi-Square test, the most significant antibiotic group for ESBL-production was quinolones (Table 2).

Molecular epidemiology

All but 11 of the 52 isolates investigated gave banding patterns. Dendrograms produced from these banding patterns identified several clusters of *K. pneumoniae* among these isolates.

Table 1. Characteristics and potential risk factors for ESBL-KP in univariate analyses.

Variables	Cases (%)	Controls (%)	Unadjusted OR (95% CI)	<i>P</i> value
Demographics				
Age in years (mean \pm SD)	53.2 \pm 22.5	53.8 \pm 23.4	-	0.898
Gender (Male) (%)	30 (57.7)	22 (42.3)	1.36 (0.92-2.02)	0.117
Preinfection hospital stay (>7 days)	27 (51.9)	10 (19.2)	4.54 (1.89-10.92)	<0.001
Nosocomial origin	39 (74.7)	29 (56.6)	2.38 (1.04-5.47)	0.039
Primary disease				
Renal diseases	20 (38.5)	12 (23.1)	2.8 (0.89-4.89)	0.089
Respiratory diseases	5 (9.6)	9 (17.3)	0.51 (0.16-1.64)	0.250
Surgery	5 (9.6)	3 (6.0)	1.74 (0.39-7.68)	0.462
CNS Diseases	13 (25.0)	13 (25.0)	1.00 (0.41-2.43)	1.000
Others	9 (17.3)	15 (27.2)	0.52 (0.20-1.32)	0.163
Antibiotic use and hospitalization				
Previous antibiotic use (>7 days)	25 (48.1)	9 (17.3)	4.42 (1.80-10.89)	0.001
Recent surgery	38 (73.1)	18 (34.6)	5.13 (2.22-11.85)	<0.001
Urinary catheter	15 (27.2)	13 (25.0)	1.22 (0.51-2.90)	0.658
Admission to ICU	2 (3.9)	6 (11.5)	3.26 (0.63-16.97)	0.141
Previous hospitalization	33 (63.5)	22 (42.3)	1.58 (1.03-2.42)	0.031

ESBL-KP: Extended Spectrum β -lactamase producing *Klebsiella pneumoniae*, OR: Odd Ratios, CI: Confidence Interval, CNS: Central Nervous System, ICU: Intensive Care Unit

Table 2. Prior exposure to various classes of antimicrobial agents among cases and controls.

Antibiotics	Cases (%)	Controls	Unadjusted OR (95% CI)	P value
Carbapenems	2/52 (3.8)	0/52 (0)	1.04 (0.99)	0.153
Third-Generation CPs	15/52 (28.9)	6/52 (11.5)	3.11 (1.02)	0.028
Quinolones	11/52 (21.2)	1/52 (1.9)	13.68 (1.70)	0.002
Others	5/52 (9.6)	3/52 (5.8)	1.74 (0.39)	0.462

CPs: Cephalosporins, OR: Odd Ratios, CI: Confidence Interval

Discussion

Similar to many other countries in the world, the problem of ESBL-producing organisms is increasing in Turkey. Many countries have a remarkable ESBL-producing *K. pneumoniae* problem despite many preventive efforts. Turkey has a special situation for ESBL-producing Gram negatives because of exaggerated antibiotic use and insufficient infection control measures [4,5,15]. Revealing risk factors is an important issue for management and control of hospital-acquired infections. The risk factors for infections with *K. pneumoniae* and *E. coli*-producing ESBL were evaluated together in some studies [2,11]. In the present study, we evaluated only infections with *K. pneumoniae* at a university hospital. Previously, the prevalence of ESBL positivity was reported higher among *K. pneumoniae* than among *E. coli* strains [5].

The role of antibiotics use has been emphasized as the leading risk factor associated with ESBL-producing organisms [2,11,16,17]. Although some studies did not maintain that association, recent data supports the hypothesis that previous use of antibiotics is one of the significant factors. Additionally, the discussion continues about which antibiotic classes are most significant with regard to the increased number of cases of ESBL-producing organisms [2,6,9,18]. The use of third-generation cephalosporins has been reported as the most important class in some studies [2,18,19]. In addition to third-generation cephalosporins, quinolones, aminoglycosides, and carbapenems have been also accused [20-22]. Our results support the hypothesis that there is a strong independent association of recent antibiotic use longer than seven days with infections caused by ESBL-producing organisms. In our study, only third-generation cephalosporins and quinolones were found significant among the

antibiotic classes, whereas the use of carbapenems was not found significant.

Our study discovered that recent surgery had an independent association with ESBL-producing *Klebsiella* species infections. Surgical intervention has previously been reported as a risk for ESBL-producing organisms [23]. The importance of these factors relates to different epidemiological aspects, such as the effectiveness of infection control measures at the hospital. However, in our study, patient-to-patient transmission was not supported with presence of a common shared clone in the molecular fingerprinting study.

The placement of central venous and urinary catheters and other invasive procedures has also been reported as a risk factor in previous studies [2,17,20]. Catheter usage may be considered the most common practice at hospitals; therefore, the quality of catheter use should be reviewed and improved. The unnecessary use of urinary catheters could be prevented by surveillance and educational intervention. In our study, the use of central venous catheters and urinary catheters were not found to be significant factors for ESBL-KP.

Molecular typing with PFGE has been approved to be a valuable molecular technique for strain typing of *K. pneumoniae* [11]. Molecular typing should be supported with conventional epidemiology. In our study, PFGE showed the circulation of many different clones of *K. pneumoniae* in our studied setting. It also showed that ESBL-producing organisms are very common and harbored at the hospital. PFGE also helps to highlight areas of particular concern with regard to transmission. This situation may be related to unjustified antibiotic use at the hospital.

There are some limitations of the current study. The patients were not categorized using Sequential Organ Failure Assessment or Acute Physiology and

Chronic Health Evaluation scores. We did not show the ESBL types, such as SHV and TEM in the study. Identification of ESBL types could be useful for understanding the resistance patterns at the hospital.

Conclusion

We have demonstrated that antibiotic exposure and recent surgery are major independent risk factors for acquiring infections with ESBL-producing organisms especially ESBL producing *K. pneumoniae*. The molecular epidemiology of the ESBL-producing isolates showed that several clones are endemic and circulating at FUH. Finally, there is an urgent need to restrict the unjustified use of broad-spectrum antibiotics, both in the hospital and in the community, and to establish an effective infection control program for stopping the spread of these multidrug-resistant organisms at the hospital and in the country.

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