Objectives: Extended-spectrum beta-lactamases (ESBLs) have been detected more frequently in members of the Enterobacteriaceae family, particularly Escherichia coli and Klebsiella pneumoniae. Infections caused by ESBL-producing bacteria are often resistant to treatment with various antibiotic classes and accompanied by increased complication risks, mortality, and costs. In this study, blood culture results were analyzed to determine the change in the ESBL production rate and antibiotic susceptibilities in E. coli and K. pneumoniae isolates over a period of 3 years.

Methods: The results of blood cultures sent to our laboratory between February 2014 and August 2016 were examined retrospectively. Repeat isolates from the same patient were not included when antibiotic susceptibility rates and clinical distributions were calculated. A BD Bactec FX automated blood culture system (Becton Dickinson and Company, Franklin Lakes, NJ, USA) was used to examine the blood cultures. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (Product name?; Bruker Daltonics, Inc., Billerica, MA, USA) was used to identify microorganisms. For antibiotic susceptibility tests (AST) and ESBL detection, the Kirby Bauer disk diffusion method or a Phoenix automated system (Becton Dickinson and Company, Franklin Lakes, NJ, USA) was used. When the AST results were evaluated, Clinical and Laboratory Standards Institute breakpoints were used for 2014 and 2015, and European Committee on Antimicrobial Susceptibility Testing breakpoints were used for 2016.

Results: During the 3-year period, 224 (35%) of 632 E. coli and 137 (31%) of 439 K. pneumoniae isolates were determined to be ESBL-producers. The ESBL-positive isolate percentage for E. coli and K. pneumoniae for 2014, 2015, and 2016 was 23%, 36%, 48% and 23%, 32%, 37%, respectively. The increase in ESBL was statistically significant for both E. coli (p<0.001) and K. pneumoniae (p=0.011).

ESBL-positive E. coli and K. pneumoniae strains were most sensitive to carbapenem-class antibiotics, amikacin, and colistin. While there was no meropenem-resistant strain, 5 (3.3%) ertapenem-resistant and 1 (0.7%) imipenem-resistant ESBL E. coli strains were detected. The ESBL K. pneumoniae strain resistance rate to ertapenem, imipenem, and meropenem was 12%, 11.2%, and 11.1%, respectively. The resistance rates of K. pneumonia strains to ertapenem, imipenem, meropenem, and piperacillin-tazobactam increased significantly over the study period (p<0.001).

Conclusion: Monitoring ESBL rates and the antibiotic susceptibility of E. coli and K. pneumoniae strains of bloodstream infections is of the utmost importance in guiding empiric antibiotic therapies and patient management.

Keywords: Blood culture; extended-spectrum beta-lactamase; resistance.

Despite advances in treatment and supportive care, bloodstream infections (BSIs) continue to be one of the most important causes of morbidity and mortality in hospital patients. BSIs caused by multiple drug-resistant microorganisms are becoming more widespread and have become a serious threat to public health. Monitoring of the resistance profiles of these microorganisms is very important in terms of combating antimicrobial resistance.[1,2] In recent years, there has been an increase in the incidence of BSIs caused by extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* isolates, and when compared to ESBL-negative isolates, BSIs caused by ESBL-positive isolates are associated with an increase in treatment failure and higher mortality rates.[2–4]

ESBLs hydrolyze all cephalosporins, aztreonam, and penicillins, except cephemycins and induce resistance against this group of antibiotics. Although ESBL-producing strains are resistant to antibiotics that do not contain beta-lactam, resistance to carbapenems is rarely seen.[5] On the other hand, use of excessive and inappropriate carbapenem in clinical practice can accelerate the emergence of carbapenem-resistant bacteria. Since the isolates of carbapenem-resistant *Enterobacteriaceae* are also resistant to many other antibiotics and considered virulent pathogens, serious precautions should be taken to prevent the spread of these microorganisms.[6]

The aim of this study was to determine the ESBL rate in *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from blood cultures and to determine the susceptibilities of ESBL-producing strains to various antibiotics, as well as the distribution among clinics.

**Methods**

The results of blood cultures sent between February 2014 and August 2016 to the laboratory were analyzed retrospectively. Repeated isolates from the same patient were not included in the calculation of the rate of antibiotic susceptibility or distribution among clinics. The blood cultures were incubated in a BD Bactec FX automated blood culture system (Becton Dickinson and Company, Franklin Lakes, NJ, USA) for 5 days. During this period, 5% sheep blood agar and chocolate agar were cultivated from the bottles signaling bacterial growth and Gram-stained slides were prepared. Matrix-mediated laser desorption ionization-flight time mass spectrometry (MALDI-TOF MS) (Product name?; Bruker Daltonics, Inc., Billerica, MA, USA) and antibiotic susceptibility tests (AST) were performed using a Phoenix automated system (Becton Dickinson and Company, Franklin Lakes, NJ, USA) or the Kirby Bauer disc diffusion method to identify the bacteria.

Amikacin, gentamicin, cefotixin, cefazidine, cefepim, ceftriaxone, piperacillin-tazobactam, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, ciprofloxacin, imipenem, meropenem, and ertapenem susceptibilities were evaluated. The presence of ESBL was determined by the Phoenix device or a double disc synergy test. Resistance to carbapenems was confirmed using the Etest (BioMerieux SA, Marcy-l’Etoile, France) with medium-sensitive/resistant isolates. AST results for 2014 and 2015 were evaluated according to Clinical and Laboratory Standards Institute breakpoints, and European Committee on Antimicrobial Susceptibility Testing breakpoints were used to assess the results of 2016.[7,8]

SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Descriptive statistics of number and percentage were calculated for categorical variables. The trend in the rate of the categorical variables over the years studied was tested using Mantel-Haenszel linear-by-linear association. Statistical significance was accepted as p<0.05.

**Results**

A total of 632 *E. coli* and 439 K. pneumoniae strains were isolated, and 34% of these isolates were ESBL-positive. In all, 224 (35%) *E. coli* isolates and 137 (31%) *K. pneumoniae* isolates produced ESBL. The distribution of ESBL-positive *E. coli* and *K. pneumoniae* strains was determined to be 23%, 36%, 48% and 23%, 32%, 37% for the years 2014, 2015, and 2016, respectively. The increase over time was statistically significant for both *E. coli* (p<0.001) and *K. pneumoniae* (p=0.011).

In the ESBL-producing strains, AST results were calculated after recurrent strains were eliminated. Among the antibiotics tested during a period of 3 years, ESBL-positive *E. coli* and *K. pneumoniae* isolates were most susceptible to amikacin, meropenem, imipenem, and ertapenem, respectively, while the highest resistance rate was to ceftriaxone, cefepime, trimethoprim-sulfamethoxazole, and ciprofloxacin (Table 1).

Evaluation of carbapenem-resistance revealed that one of the ESBL-positive *E. coli* strains (0.7%) was resistant to imipenem, while 5 (3.3%) of these strains were resistant to meropenem. No resistance to meropenem was detected. However, the resistance rate of ESBL-positive *K. pneumoniae* to imipenem, ertapenem, and meropenem was 11.2%, 12%, and 11.1%, respectively. When the change in antibiotic susceptibilities over the study period was examined, it was found that there was no significant difference in the resistance rates of ESBL-positive *E. coli* strains to any antibiotics evaluated. Resistance rates of *K. pneumoniae* strains to imipenem, meropenem, ertapenem, and piperacillin tazobactam were significantly increased (p<0.001).
The distribution of ESBL-producing strains was examined by clinic, it was observed that ESBL-producing strains were most often identified in emergency services (28.1%), followed by the intensive care unit (ICU) (26.5%), pediatric clinics (19.4%), and adult internal medicine clinics (16.2%) (Table 2).

**Discussion**

Multiple drug-resistant bacteria are increasingly being isolated from BSIs. Data on the resistance profiles of resistant microorganisms are very important to help clinicians choose the appropriate treatment and to combat antimicrobial resistance, which is an important public health problem. Infections caused by ESBL-producing strains increase mortality, hospital stay, and costs. In our study, 35% of *E. coli* and 31% of *K. pneumoniae* strains isolated from blood cultures sent from various clinics of our hospital to our laboratory were identified as ESBL-positive strains. The reported rates vary according to the country and region. In a multicenter study conducted in our country, ESBL rates were found to be 42% in hospital-acquired *E. coli* and 41.4% in *K. pneumoniae* isolates. In various studies conducted with blood culture isolates in our country, the reported rate was 26.2% to 44% for *E. coli*, and 41.4% in *K. pneumoniae* isolates.

When the distribution of ESBL-producing strains was examined by clinic, it was observed that ESBL-producing strains were most often identified in emergency services (28.1%), followed by the intensive care unit (ICU) (26.5%), pediatric clinics (19.4%), and adult internal medicine clinics (16.2%) (Table 2).
In our study, although ESBL-positive isolates were not de-
from patients hospitalized in ICUs.

ESBL-positive strains.

ESBL-producing strains.

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26.1% of them from ICUs, and 45.8% from inpatients in oth-
isolated from patients hospitalized in emergency services,
fined separately as nosocomial or community-acquired in-
strains isolated from BSIs was statistically significant.

ESBL-producing bacteria are known to be isolated more of-
ten from hospital-acquired bacteremia. Ndir et al.[19] re-
ed that 11.6% of ESBL-positive Enterobacteriaceae isolates in blood culture were isolated from community-acquired bacteremia and 88.4% from nosocomial bacteremia. In a study conducted in Turkey, it was determined that 61.4% of ESBL-producing E. coli strains were isolated from nosocomial infections.[16] In particular, antibiotic resistance rates were observed to be higher in ICUs. Yilmaz et al.[23] found an ESBL rate of 56% E. coli and 63% K. pneumoniae strains isolated from blood cultures of patients with nosocomial infections hospitalized in ICUs. Sağlam et al.[14] reported that 37.8% of ESBL-positive E. coli strains in blood cultures were isolated from patients hospitalized in ICUs.

In our study, although ESBL-positive isolates were not de-
ined separately as nosocomial or community-acquired in-
fected, it was determined that 28.1% of these strains were isolated from patients hospitalized in emergency services, 26.1% of them from ICUs, and 45.8% from inpatients in other clinics. The high rate of ESBL-positivity in admissions to the emergency department suggest that antibiotics should be carefully selected for empirical treatment of community-acquired infections. Previous use of third-generation cephalosporins and fluoroquinolones has been reported to increase the risk of infection with community-acquired ESBL-producing strains.[10]

ESBL-positive strains are also resistant to beta-lactam antibiotics as well as other antibiotic groups, compared with ESBL-negative strains, and treatment of infections caused by these strains continues to be problematic.[24, 25]

Although carbapenems are among the most effective agents in the treatment of infections caused by ESBL-producing bacteria, frequent and inappropriate use may lead to the development of resistance to these antibiotics.[6] In a study conducted in our country encompassing the years 2005 to 2009, the rate of imipenem, meropenem, and ertapenem resistance in the ESBL-positive E. coli and Klebsiella isolates obtained from BSIs was found to be 5.7%, 1.9%, and 2.4%, respectively.[26]

In another study conducted in this country, ESBL-positive E. coli strains isolated from various clinical samples did not demonstrate imipenem or meropenem resistance. However, resistance to ertapenem was found in 0.8% of ESBL-positive E. coli strains and the rate of resistance in K. pneumoniae isolates was 3.6% for all 3 antibiotics.[27]

In several studies conducted in Europe, the resistance rate of E. coli strains isolated from BSIs was found to range between 3.2% and 6.7% for meropenem and 1.6% and 6.5% for imipenem. In one of these studies, resistance to meropenem and imipenem was not detected in K. pneumoniae isolates, in another study, the resistance rate was 65.1% for meropenem and 67.5% for imipenem.[3, 9]

In a 10-year study of 77,618 blood cultures in India, where the resistance to carbapenem and piperacillin tazobactam was monitored in E. coli and K. pneumoniae strains, the increase in the resistance rate to these antibiotics over the years was not statistically significant for E. coli, but it was significant for K. pneumoniae. The increase in the resistance rate in that study was interpreted as a result of ESBL prevalence in third-generation cephalosporins, and these antibiotics were replaced by carbapenems and piperacillin tazobactam in the treatment of serious infections.[28]

In our study, the rate of resistance to imipenem, meropenem, ertapenem, and piperacillin tazobactam caused by ESBL-positive E. coli and K. pneumoniae strains isolated from blood cultures was 0.7%, 0%, 3.3%, 23.7% and 11.2%, 12%, and 45.5%, respectively. The increase in the resistance rate to all of these antibiotics over the years studied was statistically significant in for K. pneumoniae (p<0.001), whereas it was not significant for E. coli. It has been reported that insufficiency in empirical treatment increases mortality rates in invasive infections caused by ESBL-producing strains.[10]

Considering the ESBL rates in our hospital, the use of carbapenem or amikacin in empirical treatment and de-escalation according to AST results may be good practice in patients with suspected Gram-negative bacteremia.

There are some limitations of this study. Due to the retrospective nature, we could not evaluate the duration of hospital stay or patient transfers between ICU and other services, and hospital and community-acquired infections were not evaluated separately. In addition, the resistance state of multiple isolated microorganisms was not identified using molecular methods. However, this study is one
of the rare examples in our country of research conducted with a large number of blood culture isolates. 

*E. coli* and *K. pneumoniae* strains isolated from blood cultures in our hospital showed ESBL-positivity and carbapenem resistance rates had increased over the years studied. *E. coli* and *K. pneumoniae* are important factors in the empirical treatment of BSIs. Considering the increased carbapenem resistance in *Klebsiella spp.*, it would be appropriate to review the treatment when AST results are obtained.

**Disclosures**

**Ethics Committee Approval:** The study was approved by the Local Ethics Committee.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

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