



Investigation of Extended Spectrum Beta Lactamase Frequency in *Escherichia coli* and *Klebsiella pneumoniae* Strains Isolated From Individuals Affected By a Major Earthquake

Büyük Depremden Etkilenen Bireylerden İzole Edilen Escherichia coli ve Klebsiella pneumoniae Suşlarında Genişletilmiş Spekturum Beta Laktamaz Sıklığının Araştırılması

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ABSTRACT

Aim: Extended-Spectrum β -Lactamase (ESBL) - producing *Escherichia coli* (E.coli) and *Klebsiella pneumoniae* (K.pneumoniae) are threatening pathogens which are more resistant to antibiotics in healthcare settings all around the world. The spread of these pathogens may be triggered by natural disasters such as earthquake and flood.

Material and Method: A total of 3129 subjects were included in this study and evaluated retrospectively between January 2023 and July 2024 just before and after a major earthquake. VITEK[®] 2 compact automatized system was used to identify the strains. Extended-spectrum β -lactamase production was determined by double disc synergy test and combine disc synergy test. VITEK[®] 2 compact automatized system was used to determine the antibiotic resistance of strains. All statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) program. Chi-square test was applied in comparing the variables between the groups. Statistical significance threshold was found to be P value of <0.05.

Results: Nine hundred and fifty two E.coli and 355 K.pneumoniae strains were isolated from 3129 clinical samples. The 478 E. coli (50.21%) and 102 K.pneumoniae strains (28.73%) were found to be ESBL-positive. ESBL-producing E.coli strains were mostly resistant to cefuroxime (97.48%), ceftriaxone (84.51%) and 97.05% resistance rate was determined in ESBL-positive K.pneumoniae strains against ceftriaxone.

Conclusion: Although ESBL positivity was high in E.coli strains and not significantly different when compared with literature, it was found to be relatively moderate in K.pneumoniae strains. It can be said that despite the major earthquake disaster, these findings are promising.

Key words: ESBL; E.coli; K.pneumoniae; nosocomials; antibiotic resistance

ÖZET

Amaç: Genişletilmiş spekturum beta laktamaz (GSBL) üreten *Escherichia coli* (E.coli) ve *Klebsiella pneumoniae* (K.pneumoniae) türleri dünya genelinde sağlık hizmetleri açısından yüksek oranlarda antibiyotik dirençleri sebebiyle önemli patojenler arasında yer almaktadır. Bu patojenlerin yayılımı deprem ve sel gibi doğal afetler sebebiyle tetiklenebilmektedir.

Materyal ve Metod: Bu çalışmaya, büyük depremin hemen öncesi ve sonrasında Ocak 2023 ile Temmuz 2024 tarihleri arasında sağlık kuruluşuna başvuran hastalardan alınan 3129 örnek retrospektif olarak dâhil edilmiştir. Suşların identifikasyonu için VITEK[®] 2 kompakt otomatize sistem kullanılmıştır. Genişletilmiş spekturum beta laktamaz üretimi çift disk sinerji testi ve kombine disk sinerji testi ile belirlenmiştir. Suşların antibiyotik direnci VITEK[®] 2 kompakt otomatize sistemle belirlenmiştir. Tüm istatistiksel veriler IBM Sosyal Bilimlerde İstatistik Paket Programı (SPSS) kullanılarak analiz edildi. Gruplar arasındaki değişkenlerin karşılaştırılmasında Ki-kare testi uygulandı. İstatistiksel anlamlılık eşiği P <0,05 olarak belirlendi.

Bulgular: Klinik örneklerden izole edilen 3129 mikroorganizmadan 952'sinin E.coli ve 355'inin K.pneumoniae suşu olduğu tespit edilmiştir. E.coli suşlarından 478'i (%50,21) ve K.pneumoniae suşlarından 102'sinin (%28,73) GSBL-pozitif suşlar olduğu belirlenmiştir. GSBL-pozitif E.coli suşlarının çoğunlukla sefuroksim (%97,48), seftriakson (%84,51) dirençli olduğu belirlenirken, GSBL-pozitif K.pneumoniae suşlarının %97,05 oranda seftriakson direnci olduğu bulunmuştur.

Sonuç: Genişletilmiş spekturum beta laktamaz pozitifliği E.coli suşlarında yüksek ancak literatürle karşılaştırıldığında anlamlı farklılık göstermemesine rağmen K.pneumoniae suşlarında nispeten orta düzeyde bulundu. Bölgemizde yaşadığımız büyük deprem felaketine rağmen elde edilen bu bulgular, umut vaat edici denilebilir.

Anahtar kelimeler: GSBL; E.coli; K.pneumoniae; nozokomiyaller; antibiyotik direnci

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Introduction

The production of beta-lactamase enzymes in gram-negative bacteria is a major factor contributing to antibiotic resistance. In particular, extended-spectrum beta-lactamases (ESBLs) are enzymes that confer resistance to a wide range of beta-lactam antibiotics and are closely associated with multidrug resistance¹. ESBL-producing strains of *Escherichia coli* and *Klebsiella pneumoniae* are increasingly implicated in both community-acquired and hospital-acquired infections, significantly diminishing the effectiveness of beta-lactam antibiotics². These pathogens can cause various infections which associated with higher morbidity, increased treatment costs, and prolonged hospital stays³. Antimicrobial resistance is a pressing global issue, contributing to an estimated 1.27 million deaths worldwide in 2019⁴.

ESBLs were first reported in Germany in 1983⁵. Since then, *E.coli* and *K.pneumoniae* have emerged as prominent ESBL-producing pathogens, especially in health-care settings^{6–8}. While *K.pneumoniae* strains are commonly found in soil, water, and environmental sources, ⁹ *E.coli* strains are well-known foodborne pathogens¹⁰. Both are major contributors to community and hospital infections, colonizing mucosal surfaces such as the upper respiratory, gastrointestinal, and urinary tracts^{11,12}.

These ESBL-producing strains are responsible for a range of infections, including gastroenteritis, urinary tract infections, septicemia, and neonatal meningitis^{13,14}. Importantly, the resistance traits can be rapidly transferred to other bacteria via clonal spread and plasmid-mediated conjugation, facilitating outbreaks of resistant strains¹⁵. Continuous exposure to beta-lactam antibiotics can lead to beta-lactamase overproduction and mutations, resulting in enzymes capable of hydrolyzing penicillins, cephalosporins, and monobactams (e.g., aztreonam), hence the designation “extended-spectrum beta-lactamases”^{15,16}. Resistance to oxyimino-cephalosporins and aztreonam in *E.coli* and *K.pneumoniae* due to ESBL production is well documented¹⁷.

Given the increasing resistance rates and the associated clinical challenges, it is essential to monitor the prevalence of ESBL-producing Gram-negative bacteria¹⁸. This study aims to assess the frequency of ESBL production and resistance profiles of *E.coli* and *K.pneumoniae* strains isolated from various departments of a tertiary

care hospital following a major earthquake in our region in February 2023. It was hypothesized that the spread of resistant strains may have increased under the adverse post-disaster conditions.

Materials and Methods

A total of 3129 clinical samples (blood, urine, wound swab, vaginal swab, sputum, pleural liquid, tracheal aspirate) were collected between the dates Jan 2023 and July 2024 from various departments of a tertiary care hospital and incubated on 5% sheep blood agar and Eosin Methylene Blue (EMB) agar at 35–37°C for 24–48 hours. Conventional methods such as colony morphology, Gram staining, carbohydrate and citrate use, urease test were performed to isolate Enterobacteriaceae. *E.coli* and *K.pneumoniae* strains were identified by PhoenixTM 100 automatized identification system (BD Phoenix System, Beckton Dickinson, USA). Extended-spectrum β -lactamase production was detected by double disc synergy test and combine disc synergy test. Antibiotic resistance profiles of the isolates was tested with VITEK² compact automatized system and the results were evaluated according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria EUCAST 2019¹⁹.

Double Disk Synergy Test

This test was performed on Mueller-Hinton agar (Oxoid) in accordance with the standards of the disk diffusion method. Amoxicillin-clavulanic acid (10+20 μ g) disk was placed in the center, and aztreonam (30 μ g), ceftazidime (30 μ g) and cefotaxime (30 μ g) disks were placed on the periphery at a distance of 25 mm from center to center. The results were evaluated after the strains were incubated at 35°C for 18–20 hours. The expansion of the inhibition zones of the antibiotics towards the clavulanic acid disk or the observation of a region where no growth occurred in the area where bacteria grew between the two inhibition zones was interpreted as ESBL-positive.

Combine Disc Synergy Test

Ceftazidime (30 μ g) and cefotaxime (30 μ g) disks with and without clavulanic acid (10 μ g) are placed on Mueller-Hinton medium on which a bacterial suspension of McFarland 0.5 standard density is spread. After incubation at 35°C overnight, the zones of inhibition around the disks with and without clavulanic acid are measured and compared. Strains in which the

zone of inhibition around the combination disks is ≥ 5 mm wider than the zone of inhibition around the disk without clavulanic acid are considered positive for ESBL production.

This study was approved by Non-invasive Clinical Researches Ethics Committee meeting date 30/10/2024 with the decision number of 11/26. In the study, the relevant provisions of the 1964 Declaration of Helsinki and its later updates and the regulations issued by the Ministry of Health of the Republic of Türkiye were complied with.

Statistical Analysis

All statistical data were analyzed using IBM Statistical Package for Social Sciences (SPSS) program version 23 (IBM Inc., Chicago, IL, USA). Chi-square test was applied in comparing the variables between the groups. Statistical significance threshold was found to be P value of <0.05 .

Results

When examined, it was seen that the majority of the isolated pathogens were gram negative bacteria (1954/3129; 62.44%) which followed by gram positive bacteria (923/3129; 29.49%) and *Candida* spp. yeasts (252/3129; 8.05%).

Department of urology was the highest source of isolated microorganisms in total with a number of 753 (24.06%) followed by internal diseases intensive care unit (ICU) (387; 12.36%) and internal diseases various services (381; 12.17%). The distribution of pathogens obtained from various services and ICUs was given in Table 1.

Among the 1954 gram negative bacteria 952 (48.72%) *E.coli* and 355 (18.16%) *K.pneumoniae* strains have been determined. These strains were mostly isolated from urine cultures with a rate of 79.93% for *E.coli* and 58.31% for *K.pneumoniae*. Of the 952 *E.coli* strains, 638 (67.01%) were isolated from female patients and remained 314 (32.99%) from male patients. The number of female patients whom *K.pneumoniae* strains were isolated was 150 (42.25%) and 205 (56.34%) *K.pneumoniae* strains were isolated from male patients. Four hundred and seventy eight (50.21%) *E.coli* isolates were ESBL-positive and 102 (28.73%) ESBL-producing *K.pneumoniae* strains were determined (Table 2).

Table 2. The distribution of *E.coli* and *K.pneumoniae* strains by clinical specimens

Clinical sample	<i>E.coli</i> (n) (%)	<i>K.pneumoniae</i> (n) (%)
Urine	761 (79.93)	207 (58.31)
Wound swab	121 (12.71)	48 (13.52)
Blood	51 (5.35)	35 (9.85)
Tracheal aspirate	7 (0.73)	42 (11.83)
Sputum	5 (0.52)	16 (4.51)
Other	7 (0.73)	7 (1.97)
TOTAL	952 (100)	355 (100)

Table 3 presents the distribution of isolated ESBL-positive and ESBL-negative *E.coli* and *K.pneumoniae* strains according to the hospital services and intensive care units from which they were isolated.

A panel of antibiotics has been selected to determine the antibiotic resistance profiles of ESBL (+) and ESBL (-) *E.coli* and *K.pneumoniae* strains by automated system. Accordingly, ESBL-negative *E.coli* strains

Table 1. The distribution of pathogens isolated from various departments and ICUs

Departments/ICUs	n (%)	Departments/ICUs	n (%)
Department of Urology	753 (24.06)	Department of General Surgery	68 (2.17)
Internal Diseases ICU	387 (12.36)	Department of Pediatric Surgery	68 (2.17)
Internal Diseases Various Departments	381 (12.17)	Department of Plastic, Aesthetic and Reconstructive Surgery	60 (1.91)
Department of Obstetrics and Gynecology	335 (10.70)	Department of Neurology	56 (1.78)
Department of Orthopedics and Traumatology	234 (7.47)	Brain and Nerve Surgery ICU	52 (1.66)
Anesthesiology and Reanimation ICU	209 (6.67)	Coronary ICU	46 (1.47)
Department of Infection Diseases	192 (6.13)	Other (miscellaneous)	20 (0.63)
Surgery ICU	139 (4.44)		
Department of Dermatology	129 (4.12)		

ICU: intensive care unit.

Table 3. The distribution of ESBL (+/-) strains isolated from various departments and ICUs

Departments / ICUs	ESBL (-) <i>E.coli</i> strains. n; %	ESBL (+) <i>E.coli</i> strains. n; %	ESBL (-) <i>K.pneumoniae</i> strains. n; %	ESBL (+) <i>K.pneumoniae</i> strains. n; %
Department of Urology	166; 17.43	203; 21.32	50; 14.08	37; 10.42
Department of Obstetrics and Gynecology	94; 9.87	47; 4.93	16; 4.50	8; 2.25
Internal Diseases Various Departments	42; 4.41	47; 4.93	27; 7.60	23; 6.47
Department of Infection Diseases	32; 3.36	40; 4.20	15; 4.22	8; 2.25
Internal Diseases ICU	31; 3.25	16; 1.68	34; 9.57	1; 0.28
Department of Pediatric Surgery	6; 0.63	37; 3.88	—	—
Surgery ICU	10; 1.05	13; 1.36	20; 5.63	3; 0.84
Department of General Surgery	11; 1.15	9; 0.94	12; 3.38	1; 0.28
Department of Orthopedics and Traumatology	12; 1.26	6; 0.63	8; 2.25	—
Anesthesiology and Reanimation ICU	8; 0.84	2; 0.21	39; 10.98	—
Other	62; 6.51	58; 6.09	32; 9.01	21; 5.91
p	<0.001		<0.001	

ICU: intensive care unit.

Table 4. Antibiotic resistance in ESBL (+/-) *E.coli* and *K.pneumoniae* strains

ANTIBIOTICS	ESBL (-) <i>E.coli</i> (n=474) (%)	ESBL (+) <i>E.coli</i> (n=478) (%)	ESBL (-) <i>K.pneumoniae</i> (n=253) (%)	ESBL (+) <i>K.pneumoniae</i> (n=102) (%)
Ampicillin	248 (52.32)	476 (99.58)	253 (100)	102 (100)
AMC	178 (37.55)	366 (76.57)	184 (72.72)	68 (66.66)
Amikacin	11 (2.3)	23 (4.81)	89 (35.17)	5 (4.9)
Ceftazidime	54 (11.39)	394 (82.42)	158 (62.45)	98 (96.07)
Ceftriaxone	58 (12.23)	404 (84.51)	151 (59.68)	99 (97.05)
Cefuroxime	95 (20.04)	466 (97.48)	169 (66.79)	102 (100)
Nitorfurantoin	25(5.27)	11 (2.30)	46 (18.18)	29 (11.46)
SXT	155 (32.70)	262 (54.81)	96 (37.94)	72 (70.59)
TZP	86 (18.14)	121 (25.31)	171 (67.58)	49 (48.04)
Gentamicin	36 (7.59)	71 (14.85)	20 (7.90)	16 (15.68)
Ciprofloxacin	147 (31.01)	340 (71.12)	157 (62.03)	78 (76.47)
Imipenem	0 (0)	0 (0)	0 (0)	0 (0)
Meropenem	0 (0)	0 (0)	0 (0)	0 (0)

AMC: amoxicillin/clavulanic acid, SXT: trimethoprim/sulfamethoxazol, TZP: piperacillin/tazobactam.

showed the highest resistance to ampicillin, with a rate of 52.32%. These strains were highly susceptible to amikacin (97.2%). The Amoxicillin/clavulanic acid (AMC), Trimethoprim/sulfamethoxazol (SXT), and ciprofloxacin resistance rates of ESBL (-) *E.coli* strains were found to be 37.55%, 32.70%, 31.01%, respectively. On the other hand, the rate of the resistant ESBL (+) *E.coli* strains were higher, as expected, against these antibiotics by 76.57%, 54.81%, and 71.12%, respectively. But higher resistance rates were detected

against to ampicillin (99.58%), cefuroxime (97.48%), ceftriaxone (84.51%) and ceftazidime (82.42%). These strains exhibited the highest susceptibility to imipenem and meropenem (100% each), followed by amikacin (95.19%).

Extended-spectrum β -lactamase (-) *K.pneumoniae* strains showed the highest resistance to ampicillin (100%) and amoxicillin-clavulanate (72.72%), followed by piperacillin-tazobactam (67.58%), cefuroxime (66.79%), and ceftazidime (62.45%). Ampicillin

and cefuroxime resistance was 100% in ESBL (+) *K.pneumoniae* isolates. Ceftriaxone and ceftazidime resistance of these isolates were also quite high (97.05% and 96.07, respectively). Extended-spectrum β -lactamase (+) *K.pneumoniae* strains were fully susceptible to carbapenems (100%) and the susceptibility rate was 95.1% against amikacin (Table 4, and Table 5).

Table 5. Antibiotic resistance in ESBL (+/-) *E.coli* and *K.pneumoniae* strains isolated from various ICUs

ANTIBIOTICS	ESBL (+) <i>E.coli</i> strains isolated from ICUs (n=34) (%)	ESBL (+) <i>K.pneumoniae</i> strains isolated from ICUs (n=12) (%)
Ampicillin	34 (100)	12 (100)
AMC	26 (76.47)	4 (33.33)
Amikacin	2 (5.88)	0 (0)
Ceftazidime	32 (93.75)	11 (91.66)
Ceftriaxone	29 (85.29)	12 (100)
Cefuroxime	34 (100)	12 (100)
Nitrofurantoin	1 (2.94)	3 (0.25)
SXT	18 (52.94)	11 (91.66)
TZP	15 (44.11)	4 (33.3)
Gentamicin	4 (11.76)	1 (8.33)
Ciprofloxacin	28 (82.35)	9 (75)
Imipenem	0 (0)	0 (0)
Meropenem	0 (0)	0 (0)

AMC: amoxicillin/clavulanic acid, SXT: trimethoprim/sulfamethoxazol,
TZP: piperacillin/tazobactam.

Discussion

The treatment of infections caused by Gram-negative enteric bacteria has become increasingly difficult in recent years²⁰. ESBL-producing *Escherichia coli* and *K.pneumoniae* are frequently isolated from various infections, particularly in settings with poor healthcare conditions^{17,21} and also these strains are more resistant against antibiotics²².

Gram negative bacteria are leading causes of serious infections with high morbidity and mortality in worldwide^{23,24}. These strains can often be isolated from various clinical samples. In a study which conducted by Forouzani and co-workers, it was demonstrated a 64.2% isolation rate for gram negative bacteria²⁵. Parmar and co-workers presented a 87.13% isolation rate for gram negative bacteria from clinical specimens in their study. Among these isolates, *E.coli* was the prominent bacterial strain with a 48.4% rate followed by *K.pneumoniae* (13.6%)²⁶. In another study

conducted in Türkiye, Gürbüz and colleagues analyzed 1.460 clinical samples, of which 69.17% were Gram-negative bacteria. Among these isolates *E.coli* was again the prominent pathogen with a rate of 22.32% followed by *K.pneumoniae* (17.80%)²⁷. In our study 3129 subjects were evaluated and the majority of these isolates (1954; 62.44%) were found to be gram negative bacteria in process. The isolation rate was 48.72% for *E.coli* and 18.16% for *K.pneumoniae* strains. *E.coli* strains were most frequently isolated from the urology department, with an isolation rate of 38.76%. Urology service was also the major origin of *K.pneumoniae* strains with an isolation rate of 24.5%. Eighty (8.4%) *E.coli* and 97 (27.32%) *K.pneumoniae* strains were detected in clinical samples obtained from ICUs.

Gram negative bacteria are isolated at different rates from many different clinical samples such as urine, wound, blood, tracheal aspirate and sputum, and etc. However, these pathogens are most commonly isolated from urine specimens, wound swabs and surgical site infections in healthcare settings²⁸⁻³³. Ali and co-workers examined the urine specimens and according to their results *E.coli* strains found to be most frequently seen in urine cultures with a rate of 52.6%, they reported. *K.pneumoniae* strains showed an isolation rate of 9.0% in this study³⁴. When the results of a study were examined, which conducted in our country, it was reported that 57.61% of the *E.coli* strains were isolated in urine samples taken from ICUs. In the same study, it was stated that 47.15% of *E.coli* and 43.38% of *K.pneumoniae* strains were grown in the urine cultures of patients receiving treatment in inpatient services³⁵. In a study in Türkiye performed by Avcıküçük and Altın, it was investigated that the gram positive and gram negative bacterial strains isolated from urine cultures and the isolation rates of *E.coli* and *K.pneumoniae* strains were given as 70.4% and 12.4% respectively³⁶. In our study it was determined the majority of urinary isolates were *E.coli*, compatible with literature, but with a higher prevalence (79.93%). *K.pneumoniae* was second most common bacterial pathogen isolated from urinary samples with a rate of 58.31%.

Wound and surgical site infections (SSIs) are the important source of gram negative bacterial growth in healthcare settings. SSIs are the second most prevalent nosocomial infections, representing around 25% of all hospital-acquired infections³⁷. Wounds provide a suitable environment for pathogens³⁸. Gram negative bacteria can easily grow in the wound in suitable

conditions and effect deeper tissues³⁹. Various studies have reported that gram-negative bacteria are frequently isolated from wound infections. Watanabe and co-workers have demonstrated 105 gram negative bacteria isolated from 227 blood cultures (46.25%) in their study⁴⁰. In a study it was shown a 62% rate of gram negative bacteria isolated from wound swabs. Among these strains the rate of *K.pneumoniae* isolates was 13.9% and 12.6% for *E.coli* have been determined⁴¹. The prevalences of *K.pneumoniae* and *E.coli* strains isolated from superficial incisional surgical site infections were 39.58% and 29.17%, respectively, have been reported in a study which performed by Ali and Al-Jaff⁴². Tanriverdi Çaycı and co-workers determined 65.1% isolation rate for gram negative bacteria isolated from wound swabs between 2015 and 2017⁴³. *E.coli* was found to be the dominant microorganism and 20.5% isolation rate for *E.coli* has been given. The rate of isolated *K.pneumoniae* strains was 9.8% and the frequency of *K.pneumoniae* and *E.coli* isolated from wound swabs was found to be 13.52% and 12.71, respectively, in this study⁴³.

In recent years, both the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) have closely monitored antibiotic resistance in Enterobacterales species producing ESBLs. According to the WHO's Global Antimicrobial Resistance and Use Surveillance System (GLASS), as of 2022, 27.4% of bloodstream infections caused by *E.coli* were reported to be resistant against third-generation cephalosporins. This incidence is even higher in low- and middle-income countries. Another comprehensive surveillance initiative established by the WHO, known as the "Tricycle Protocol," adopts a multi-sectoral approach to monitor ESBL-producing organisms in humans, animals, and the environment. Data obtained through this protocol revealed that the carriage rate of ESBL-producing *E.coli* in human fecal samples exceeded 40% in regions such as Southeast Asia and Africa⁴⁴.

According to the CDC's *Antibiotic Resistance Threats Report* published in 2019, an estimated 197.400 infections caused by ESBL-producing *E.coli* occur annually in the United States, with approximately 9.100 of these resulting in death. Furthermore, the CDC highlighted the increasing trend of healthcare-associated infections due to ESBL-producing organisms and recommended enhanced active surveillance and stricter infection control measures targeting these pathogens. These findings

emphasize the urgent need for continuously updated, strengthened, and globally coordinated strategies for the surveillance and control of antibiotic-resistant bacteria⁴⁵.

It is well known that the ESBLs existence in gram negative bacteria lead multi drug resistance against various antibiotics. Dramatical increase in bacterial resistance can easily be detected by time in worldwide and also in Türkiye. Bedzichowska and co-workers investigate the prevalence of ESBL positivity in *K.pneumoniae* and *E.coli* strains in a five year period. Among the pediatric patients, ESBL (+) *E.coli* and *K.pneumoniae* strains were prominent pathogens with 43.5% and 36.9%, respectively in their study⁴⁶. It was reported the ESBL frequency in *E.coli* strains by 8.09% between 1996–2001, 10.61% between 2002–2007, and 28.17% between 2007–2012 in studies performed in Türkiye⁴⁷. One more study performed by Şenol and co-workers, presented the ESBL existence in gram negative bacteria with a rate of 60% in 2021²⁹. Bayraktar and co-workers demonstrated a 35% ESBL frequency in *E.coli* and 31% in *K.pneumoniae* strains which isolated from blood cultures in a three year period in their study⁴⁸. Extended-spectrum β -lactamase positivity was found to be 48% in *E.coli* and 67% in *K.pneumoniae* strains in another study which performed by Parlak and co-workers⁴⁹. Onuk and co-workers have reported a 66.7% ESBL positivity in *E.coli* strains and 30.0% in *K.pneumoniae* strains isolated from various ICUs in their study⁵⁰. Due to lack of sanitation and hygiene after major earthquake in our region, we aimed to assess the prevalence of ESBL-producing *E.coli* and *K.pneumoniae* strains during the study, even it was monocentric. So, it was determined a 50.21% ESBL positivity in *E.coli* and 28.73% in *K.pneumoniae* isolates. This result is promising despite adverse conditions, although a few studies from our country have reported higher ESBL frequencies in the post-earthquake period. For example, in a recent study which performed by Bursal and co-workers the frequency of ESBL positivity in *E.coli* and *K.pneumoniae* strains isolated from urinary tract infections were presented as 41.4% and 53.2% respectively, by a reflection of a single centric research on post-earthquake period in our country⁵¹. Akineden and co-workers showed a 47.63% ESBL positivity in *E.coli* strains. They indicated 61.16% rate for ESBL existence in *K.pneumoniae* strains isolated from various clinical specimens in a tertiary care hospital recently⁵². The distribution of

ESBL-positive *E.coli* and *K.pneumoniae* strains by clinics is presented in the Table 3 ($p = <0.001$).

Since ESBL-producing Enterobacteriaceae present multi-drug resistance, it is crucial to well documented the antibiotic resistance profiles of isolated strains. In several studies it was reported various resistance rates for ESBL (+) *E.coli* and *K.pneumoniae*. In this context, Garba and co-workers have demonstrated the resistance patterns of ESBL (+) *E.coli* and *K.pneumoniae* strains isolated from clinical specimens. Accordingly, the higher resistance in these strains was against ciprofloxacin (94.1% and 94.0%, respectively), SXT (83.4% and 89.7%, respectively) and TZP (68.0% and 76.9%, respectively)⁵³. Although it is a one of the first line antibiotic choice in empirical treatment option, nitrofurantoin resistance in especially ESBL-producing *K.pneumoniae* strains was alarming with 74.4%⁵⁴. As known the gram negative bacteria isolated from ICUs are more resistant when compared with non-ICU strains, so it would be better to investigate the resistance rates of gram negative bacteria isolated from ICUs and non-ICUs separately. Wani and co-workers showed a high prevalence of nitrofurantoin resistance (71.6%) in gram negative bacteria isolated from ICU patients⁵⁵. Nitrofurantoin resistance in inpatient services was 2.09% and 25.49% for ESBL (+) *E.coli* and *K.pneumoniae* strains, respectively. The similar findings were obtained in terms of nitrofurantoin resistance in ESBL (+) *E.coli* and *K.pneumoniae* strains isolated from ICUs (2.09% and 25, respectively) in our study. The highest resistant rates for ICUs strains were against ceftazidime (93.75% in ESBL (+) *E.coli* and 91.66% in ESBL (+) *K.pneumoniae*), ceftriaxone (85.29% in ESBL (+) *E.coli* and 100% in ESBL (+) *K.pneumoniae*), and SXT (52.94% in ESBL (+) *E.coli* and 91.66% in ESBL (+) *K.pneumoniae*) we found. In ESBL (+) non-ICUs *E.coli* strains and *K.pneumoniae* strains, the resistance against ceftriaxone was 77.82% and 85.29%, respectively, in our study. Imipenem was the most effective antibiotic agent against both ICUs and non-ICUs ESBL (+/-) *E.coli* and *K.pneumoniae* strains (100% for both) followed by amikacin (between 0% – 5.88% for all ESBL (+) *E.coli* and *K.pneumoniae* strains). The data obtained from various clinical studies performed in Türkiye was compatible with our results. Coşkun and co-workers have demonstrated that ESBL (+) *E.coli* and *K.pneumoniae* isolates were mostly resistant against ampicillin-sulbactam (80.3% and 85.2% respectively). These strains were completely susceptible against amikacin and imipenem⁵⁴. Aydoğmuş

and co-workers indicated no resistance against imipenem and meropenem among isolated ESBL (+) *E.coli* and *K.pneumoniae* strains in their study. They have determined that these isolates were completely resistant against ceftriaxone (100%)⁵⁶.

The antimicrobial resistance patterns of gram negative bacteria isolated from blood cultures were given in a recent study performed by Öner and co-workers. According to their findings the ceftazidime resistance was 60.3% and 84.2%, ciprofloxacin resistance was 55.7% and 77.1%, SXT resistance was 48.7% and 56.1% for *E.coli* and *K.pneumoniae*, respectively. The most effective antibiotic was found to be amikacin in this study (susceptibility rates were 85.6% for *E.coli* and 68.7% for *K.pneumoniae*)⁵⁷.

Keskin and co-workers demonstrated the antibiotic resistance in *E.coli* and *K.pneumoniae* strains isolated from urinary tract infections and the resistance of *E.coli* isolates against imipenem, amikacin, gentamicin, nitrofurantoin, piperacillin/tazobactam (TZP), ciprofloxacin, amoxicillin/clavulanic acid (AMC), cefuroxime, trimethoprim/sulfamethoxazol (SXT), ampicillin was reported as; 0.1% (0.1–1.3), 2.2% (1.9–6.3), 13.1% (12.4–25), 2.3% (2.2–3.8), 13.6% (13.3–18.8), 26.3% (25.3–42.5), 29.4% (28.8–40), 37.2% (35.7–62.5) 34.8% (33.9–49.4), 61.7% (60.9–74.4), respectively. These rates were given as; 10.3% (5.8–43.2) against imipenem, 10.6% against amikacin, 27.9% against gentamicin, 25.3% against TZP, 22.8% against ciprofloxacin, 44.4% against AMC, 46.6% against cefuroxime, and 41.6% against SXT in *K.pneumoniae* strains⁵⁸. In compatibility with the antibiotic panel of this study, to compare our findings were as follows; in ESBL-producing *E.coli* isolates the resistance rate against ampicillin was 99.58%, AMC was 76.57%, amikacin was 4.81%, ceftazidime was 82.42%, ceftriaxone was 84.51%, cefuroxime was 97.48%, nitrofurantoin was 2.30%, SXT was 54.81%, TZP was 25.31%, gentamicin was 14.85%, ciprofloxacin was 71.12%, and imipenem was 0%. The resistance rates in ESBL-producing *K.pneumoniae* isolates were as follows; ampicillin (100%), AMC (66.66%), amikacin (4.9%), ceftazidime (96.07%), ceftriaxone (97.05%), cefuroxime (100%), nitrofurantoin (11.46%), SXT (70.59%), TZP (48.04%), gentamicin (15.68%), ciprofloxacin (76.47%), and imipenem (0%).

Conclusion

Treatment of infections caused by gram negative bacteria should be based on rational use of antibiotics in healthcare settings. In our study, it was determined high resistance against third generation cephalosporins, ciprofloxacin, and SXT as reported in various studies in Türkiye. Nitrofurantoin serves as an effective antimicrobial choice against ESBL (+) *E.coli* and *K.pneumoniae* strains isolated from both inpatient services and ICUs. Despite the high prevalence of these strains and the lack of sanitation and hygiene following the major earthquake in our region in February 2023, the observed ESBL frequency was consistent with the literature and considered promising. It should be considered that poor living conditions, malnutrition, psychogenic stress factors and change in demographic structure may also trigger these infections in process. Extended-spectrum beta-lactamase production significantly contributes to antimicrobial resistance in *E.coli* and *K.pneumoniae*, leading to limited treatment options and increased morbidity. The frequency of ESBL-producing strains directly influences resistance patterns, making it a critical concern in healthcare settings. To mitigate this issue, several strategies can be implemented in clinical practice. These include strict adherence to infection control measures, routine surveillance of resistance patterns, antimicrobial stewardship programs to reduce inappropriate antibiotic use, and the use of rapid diagnostic tools for early detection. Additionally, clinicians should consider local resistance data when selecting empirical therapies to ensure effective treatment. Ultimately, a multifaceted approach is essential to control the spread of ESBL-producing pathogens and to optimize patient outcomes.

Ethical Approval

This study was approved by Hatay Mustafa Kemal University Non-invasive Clinical Researches Ethical Committee with a meeting date on 30.10.2024 and decision number of 11–26.

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