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Investigation of the ST131 Clone by Real-time PCR in *Escherichia coli* Strains Isolated in Patients from Intensive Care Units and Other Services

Yoğun Bakım ve Diğer Servislerde Yatan Hastalardan İzole Edilen *Escherichia coli* İzolatlarında ST131 Klonunun Gerçek-zamanlı PZR ile Araştırılması

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Abstract

Introduction: The prevalence and spread rate of the *Escherichia coli* ST131 clone continue to be an important issue requiring investigation. This study investigated the presence of the ST131 clone in *E. coli* isolates isolated from intensive care units (ICUs) and other services using real-time polymerase chain reaction (PCR).
Materials and Methods: A total of 200 *E. coli* isolates were included in the study. The double-disk synergy method was used to show the presence of extended-spectrum beta-lactamases (ESBL). The ST131 clones were investigated using the real-time PCR method in all isolates.
Results: *E. coli* ST131 clones were identified in 17 of 100 strains isolated from the specimens from ICUs. Of these strains, 15 (29.4%) of the 51 ESBL positive isolates and two (4.1%) of the 49 ESBL negative isolates were identified as *E. coli* ST131 clones. *E. coli* ST131 clones were identified in 13 of 100 strains isolated from non-ICUs. Among these strains, 12 (34.3%) of 35 ESBL positive isolates and one (1.5%) of the 65 ESBL negative isolates were identified as *E. coli* ST131 clones.
Conclusion: While the *E. coli* ST131 clone was common in ESBL-producing isolates, its presence did not differ in intensive or non-ICUs.
Keywords: *E. coli*, ST131, intensive care unit

Öz

Giriş: *Escherichia coli* ST131 klonunun görülme sıklığı ve yayılım hızı, araştırılması gereken önemli bir konu olmaya devam etmektedir. Bu çalışmanın amacı, yoğun bakım ve diğer servislerinde yatan hastalardan izole edilen *E. coli* izolatları içinde, ST131 klonu varlığının gerçek-zamanlı polimeraz zincir reaksiyonu (PZR) yöntemi ile araştırılmasıdır.
Gereç ve Yöntem: Çalışmaya toplam 200 adet *E. coli* izolatı dahil edilmiştir. Genişlemiş spektrumlu beta-laktamaz (GSBL) varlığını göstermek için çift disk sinerji yöntemi kullanılmıştır. Gerçek-zamanlı PZR yöntemi ile tüm *E. coli* izolatlarında, ST131 klonu varlığı araştırılmıştır.
Bulgular: Yoğun bakımda yatan hastalardan izole edilen 100 suştan 17 tanesi *E. coli* ST131 klonu olarak tespit edilmiştir. Bu suşlardan GSBL pozitif olan 51 izolatın 15 (%29,4) tanesi, GSBL negatif olan 49 izolatın iki (%4,1) tanesi *E. coli* ST131 klonu olarak tespit edilmiştir. Yoğun bakım dışı servislerde yatan hastalardan izole edilen 100 suşun 13 tanesi *E. coli* ST131 klonu olarak tespit edilmiştir. Bu suşlardan GSBL pozitif olan 35 izolatın 12 (%34,3) tanesi, GSBL negatif olan 65 izolatın bir (%1,5) tanesi *E. coli* ST131 klonu olarak tespit edilmiştir.
Sonuç: *E. coli* ST131 klonu, GSBL üreten izolatlarda daha yaygın iken, yoğun bakım ünitelerinde veya yoğun bakım dışı ünitelerde bulunma açısından bir fark görülmemiştir.
Anahtar Kelimeler: *E. coli*, ST131, yoğun bakım ünitesi

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Introduction

Escherichia coli strains in the intestinal flora of every healthy individual can cause intestinal and extraintestinal infections in healthy and immune-suppressed individuals. Urinary tract infections, bacteremia, meningitis, and diarrhea are among the most observed clinical syndromes caused by the limited number of *E. coli* pathogenic clones[1].

Recently, an unknown clone of *E. coli* ST131 was detected in different countries. Today, ST131 is considered an important clone among extraintestinal pathogenic *E. coli* isolates and is widely known to produce extended-spectrum beta-lactamases (ESBL) such as CTX-M-15, are resistant to a variety of antibiotics, particularly fluoroquinolones. The spread of *E. coli* ST131 clone led to a worldwide increase in the ESBL-producing *E. coli* isolates in community and hospital settings[2].

It is important to monitor this clone to prevent its spread, track the condition of its resistance genes, determine effective empirical antibiotic treatment options, and develop new strategies to control antibiotic resistance. Determining the reservoirs of *E. coli* ST131 clones, which have become an important public health threat, and understanding the risk factors and resistance transfer pathways are among the critical issues leading to multidrug resistance and its global spread[3].

Detecting multidrug-resistant bacteria and their colonization in intensive care units (ICUs) will positively contribute to the fight against nosocomial infections[4].

This study investigated the presence and rate of ST131 clones in *E. coli* isolates isolated from inpatients in ICUs and non-ICUs using real-time polymerase chain reaction (PCR) methods.

Materials and Methods

Isolates: This study was performed with the permission of 2018/293 granted by the Ondokuz Mayıs University Clinical Research Ethics Committee on 25.06.2018.

The isolates included in the study were obtained from different clinical specimens routinely sent to the microbiology laboratory between January 2017 and January 2018 from ICUs (n=100) and non-ICUs (n=100). One isolate per patient was included in the study. Of these isolates, 86 ESBL-producing and 114 ESBL-non-producing isolates were included in the study. The strains were stored at -20 °C until the study was conducted.

A routine identification procedure was performed for isolates included in the study. Samples were inoculated onto the 5% sheep blood agar (bioMérieux, France) and eosin methylene blue agar (bioMérieux, France). The inoculated plates were incubated at 35 °C for 18-24 hours, and the bacteria that grew after incubation were evaluated using the routine identification procedure. The Vitek-MS (bioMérieux, France) system was used to identify the isolates.

Extended-spectrum beta-lactamases detection: The double-disk synergy method was used to determine the presence of ESBL. The bacterial suspension was prepared from tested microorganisms, adjusted to a 0.5 McFarland standard and streaked onto the Mueller-Hinton agar (Oxoid, England). Amoxicillin-clavulanic acid (Oxoid) was placed in the center of the plate. Ceftazidime (Oxoid), ceftriaxone (Oxoid), and cefotaxime (Oxoid) were placed 20 mm away from the amoxicillin-clavulanic acid. The plates were incubated at 37 °C for 18-24 h. After the incubation, enlargement of the inhibition zones around the ceftazidime, ceftriaxone, and cefotaxime toward the amoxicillin-clavulanic acid is interpreted as the presence of ESBL[5,6].

DNA extraction in *E. coli* isolates: DNA extraction of *E. coli* strains was performed by modifying the boiling method of Tchesnokova et al.[7]. Then, the supernatants were collected into clean tubes and checked using a NanoDrop (Thermo, USA). DNA samples were stored at -20 °C until real-time PCR.

Real-time PCR process: Real-time PCR (BIO-RAD, USA) was used to detect the *E. coli* ST131 clone and the primary sets of Dhanji et al.[8] were used in the PCR process. The base sequences of the primer sets are presented in Table 1.

The mixture content for the real-time PCR process was prepared as 5 µl Cyber Green Supermix (BIO-RAD), 0.5 µl F primer, 0.5 µl R primer, 2 µl DNA, and 2 µl pcr-grade water. Amplification was performed with the following thermal cycling profile: 98°C three min pre-denaturation, 95 °C 15 sec, and 60 °C 20 sec in 40 cycles. In the SNP qPCR tests performed on ST131 *E. coli* clones with these primers, Tm values were 79.50 °C adenine (A) and 80.50 °C thymine (T) in the melting curve analyses, respectively. After the PCR process, melting curve analysis was performed based on the manufacturer's recommendations, and the results were interpreted.

Statistical Analysis

The frequency of *E. coli* ST131 clone detection, the relationship between hospitalization in ICUs and non-ICUs, and whether to produce ESBL was investigated using the chi-square test. Data were analyzed by the Statistical Package for the Social Sciences

Table 1. Base sequences of the primer sets

Primary	Primary sequences
ST131TF	(5'-GGTGCTCCAGCAGGTG-3')
ST131TR	(5'-TGGGCGAATGTCTGC-3')
ST131AF	(5'-GGCAATCCAATATGACCC-3')
ST131AR	(5'-ACCTGGCGAAATTTTTCG-3')

software package program, version 21. The level of significance was accepted as $p<0.05$

Results

A total of 200 *E. coli* isolates were included in the study. Non-intensive care services comprised 43% internal medicine, 24% emergency, 7% urology; 6% general surgery, 5% pediatric diseases, 4% infection; 4% cardiology, and 7% other services (neurology, chest diseases, neurosurgery, gynecology).

The double-disk synergy method confirmed that among isolates isolated from ICU samples included in the study, 51 produced ESBL, while 49 did not. In addition, out of 100 isolates isolated from non-ICUs samples, 35 produced ESBL, while 65 did not.

Seventeen of 100 strains isolated from ICU patients were identified as *E. coli* ST131 clones. Of these strains, 15 were ESBL-positive, and two were negative.

Thirteen of the 100 strains isolated from non-ICU patients were identified as *E. coli* ST131 clones. Of these strains, 12 were ESBL-positive isolates, and one was negative.

The number and rates of ST131 clones detected in all strains isolated from ICUs and non-ICUs are presented in Table 2.

As a result of the statistical analysis, no statistically significant difference was found in the ST131 clone isolated from patients hospitalized in ICUs or non-ICUs. Whether the isolates produced, ESBL was significant regarding ST131 incidence ($p=0.001$).

Discussion

E. coli is one of the most common gram-negative pathogenic microorganisms capable of producing ESBL and may cause infections that are costly and difficult to treat with high morbidity and mortality, especially in the hospital environment[9].

Oğuz Mızrakçı et al.[10] investigated the risk factors for gastrointestinal colonization of ESBL-producing *K. pneumoniae* and *E. coli* isolates. They found that the risk of getting infected with the ESBL-positive isolates of these two species was significantly higher in patients who stayed in ICU for a long time.

The *E. coli* ST131 clone is found worldwide to cause antimicrobial-resistant infection. Diseases caused by this clone vary from cystitis to life-threatening sepsis and are similar to other *E. coli* clones. Urinary tract infections are among the most commonly observed diseases caused by the *E. coli* ST131 clone, and the infection can be seen in all age groups[11].

Health institutions' awareness of their patient profiles, the microorganisms that make up the hospital flora, and their resistance patterns will enable them to develop the right strategies[12].

In 2008, for the first time in Turkey, a study by Yumuk et al.[13] reported that one of the clones from the 17 ESBL-producing *E. coli* isolates was ST131.

In 2015, Can et al.[14] investigated the ST131 clone in their study, which included 294 *E. coli* isolates, the causative agent of urinary tract infection. Of all the isolates included in the study, 35 (12%) were ST131 and 259 non-ST131. While 21 (60%) of the *E. coli* isolates identified as ST131 were ESBL-positive, 49 (19%) of the non-ST131 isolates produced ESBL. The researchers concluded that the *E. coli* ST131 clone is directly associated with global resistance in urinary tract infections and predicts treatment failure. They also emphasized that early and rapid detection of *E. coli* ST131 would be useful in treating urinary tract infections.

Can et al.[15] found that of the 297 *E. coli* strains isolated from blood culture, 49 (16%) isolates were ST131 clones. In that study, ESBL positivity in all isolates was 45%, and the ST131 clone rate in the ESBL-producing isolates was 31%. In addition, the researchers reported that the prevalence of ST131 increased from 13% to 23% in four years. As a result, the researchers reported that the *E. coli* ST131 clone rapidly increased in blood culture isolates worldwide.

Ismail et al.[16] identified 65 (29.4%) *E. coli* isolates isolated from 221 young women diagnosed with urinary tract infections as ST131 clones. They also found that ST131 clone isolates were more resistant to quinolones than other ST clones.

Different methods were developed to detect the *E. coli* ST131 clones. Lafolie et al.[17] worked on a mass spectrometric method and reported that 39 of 40 ST131 clones and 49 of 49 non-

Table 2. The number and rates of ST131 clones detected in all strains

	Intensive care units n=100		Non-intensive care units n=100	
	ESBL+ n=51 (%)	ESBL- n=49 (%)	ESBL+ n=35 (%)	ESBL- n=65 (%)
ST131	15 (29.4)	2 (4.1)	12 (34.3)	1 (1.5)
Non-ST131	36 (70.6)	47 (95.9)	23 (65.7)	64 (98.5)

ESBL: Extended-spectrum beta-lactamases

ST131 clones were correctly identified. They also concluded that using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS), the ST131 clones could be detected easily and quickly in the laboratory and can contribute to infection control and prevention measures in multidrug-resistant *E. coli* isolates.

Aktaş et al.[18] included 203 ESBL-positive *E. coli* samples isolated from urinary tract infections in their study. This study investigated *E. coli* ST131 clones using MALDI-TOF MS and Real-Time PCR. At the end of the study, 81 (39.9%) of the 203 samples were identified as ST131 clones using PCR and 75 (36.9%) using MALDI-TOF MS.

Çizmeci et al.[3] investigated the presence of the ST131 clone in ESBL-positive *E. coli* isolates. They included 301 *E. coli* strains; of them, 251 were urine, and 50 were non-urine and used double-disk synergy to demonstrate ESBL production in the strains studied. Of 301 *E. coli* isolates, 110 (36.6%) PCR and 92 (30.6%) MALDI-TOF MS ST131 clones produced ESBL. They did not find a significant difference in the presence of ST131 between urine samples and non-urine samples. In conclusion, they suggested that further investigation of this high-risk clone would be beneficial in developing new strategies to control antibiotic resistance.

Al-Agamy et al.[19] investigated ESBL positivity, the ST131 clone, and epidemiological typification using pulsed-field gel electrophoresis among 152 *E. coli* isolates. Of the isolates, 31 were found to be ESBL-positive using phenotypic methods. Among these 31 ESBL-producing *E. coli* isolates, 29 were different pulse types, and 20 (64.5%) were ST131 clones.

In the study by Liu et al.[20], phylogenetic group, virulence gene profiles, ST types, and fluoroquinolone susceptibility were investigated in 174 *E. coli* isolates isolated from dogs and cats in the Shaanxi province of China. The study showed that ST69 (18.4%) was the most common ST. The frequencies of other ST types were ST648 (13.2%), ST73 (10.9%), ST405 (10.3%), ST12 (10.3%), and ST131 (9.8%).

Demirci et al.[21] investigated the ST131 clone in 101 *E. coli* isolates isolated from the urine specimens of hospitalized patients and outpatients. They reported that the ST131 clone was detected in 14 of 42 (33.33%) hospitalized patients, nine of 59 (15.25%) outpatients, and a total of 23 of 101 (22.27%) patients. Also, the ST131 clone was detected in 16 of 51 (31.37%) ESBL-positive isolates and seven of 50 (14%) ESBL-negative isolates, and the presence of the ST131 clone was significantly higher in ESBL-positive isolates.

Of the 200 isolates in the present study, 30 (15%) were identified as *E. coli* ST131 isolates.

In the statistical analysis based on the ESBL production of the isolates included in the present study, 27 (31.4%) of 86 ESBL-positive isolates and three (2.6%) of 114 ESBL-negative isolates were identified as *E. coli* ST131 clones. In the statistical study, isolates' ESBL production ability was significant regarding ST131 incidence (p=0.001).

As a result of this study, of the *E. coli* strains isolated from the ICU patients, 15 (29.4%) of the 51 ESBL-positive isolates and two (4.1%) of the 49 ESBL-negative isolates were identified as *E. coli* ST131 clones. Of the strains isolated from non-ICU, 12 (34.3%) of the 35 ESBL-positive isolates and one (1.5%) of 65 ESBL-negative isolates were identified as *E. coli* ST131 clones. There was no significant difference between the *E. coli* strains isolated from ICU and non-ICU patients regarding the ST131 clone (p=0.55).

Study Limitations

The limitations of the study are that molecular epidemiological analysis of the isolates could not be performed, and the prognosis of the patients from whom the specimens were isolated was not evaluated.

Conclusion

Knowing the rate and epidemiological spread of the *E. coli* ST131 isolate, considered high-risk for treatment, plays a key role while fighting against these isolates. The presence and distribution of these isolates may contribute to developing treatment protocols. Further molecular epidemiologic studies should be conducted to acquire new and more comprehensive data.

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Ethics

Ethics Committee Approval: This study was carried out with the permission of 2018/293 granted by the Ondokuz Mayıs University Clinical Research Ethics Committee on 25.06.2018.

Informed Consent: Informed consent was not obtained because study-only isolates were tested, and patients' electronic data were used without ID information.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: F.H., K.B., Design: F.H., K.B., A.B., Data Collection or Processing: F.H., K.B., Y.T.Ç., Analysis or Interpretation: F.H., K.B., H.K., Literature Search: F.H., K.B., Writing: F.H.

Conflict of Interest: The authors declare that they have no conflicts of interest.

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