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Gram-Negative Bacteria and Antimicrobial Resistance Profiles Isolated from Blood Cultures of Patients Hospitalized in Adult Intensive Care Units of Atatürk University Faculty of Medicine Research Hospital

Objective: This study aimed to evaluate the distribution of Gram-negative bacterial agents isolated from blood cultures and their antibiotic resistance profiles in our hospital between 2017 and 2021.

Materials and Methods: This retrospective study included positive blood culture samples obtained from intensive care unit patients between 2017 and 2021. Bacterial identification and isolation were performed using an automated system, and antibiotic susceptibility was assessed according to EUCAST criteria.

Results: The most frequently isolated pathogen was *A. baumannii* (41.8%), followed by *K. pneumoniae* (23.1%), *E. coli* (14.9%), and *P. aeruginosa* (7.2%). ESBL positivity was found in 44.2% of *E. coli* and 42.8% of *K. pneumoniae* isolates. The highest antibiotic susceptibility was observed for colistin in both *P. aeruginosa* and *K. pneumoniae*.

Conclusion: The identification of pathogens and their resistance profiles in intensive care patients can guide empirical treatment choices. Increasing resistance to many antibiotics other than colistin highlights the importance of rational antibiotic use and strict infection control measures.

Key Words: *Healthcare-associated infections, blood culture, gram-negative bacteria*

Atatürk Üniversitesi Tıp Fakültesi Araştırma Hastanesi'nin Yetişkin Yoğun Bakım Ünitelerinde Yatan Hastaların Kan Kültürlerinden İzole Edilen Gram-Negatif Bakteriler ve Antimikrobiyal Direnç Profilleri

Amaç: Bu çalışmanın amacı, hastanemizde 2017–2021 yılları arasında kan kültürlerinden izole edilen gram negatif bakteriyel etkenlerin dağılımını ve bu etkenlerin antibiyotiklere karşı direnç profillerini değerlendirmektir.

Gereç ve Yöntem: Retrospektif olarak planlanan bu çalışmada, 2017–2021 yılları arasında yoğun bakım ünitesi hastalarından alınan pozitif kan kültürü örnekleri incelenmiştir. İzolasyon ve tanımlama işlemleri otomatize sistemle gerçekleştirilmiş, antibiyotik duyarlılıkları EUCAST kriterlerine göre değerlendirilmiştir.

Bulgular: Toplamda en sık izole edilen etkenin *Acinetobacter baumannii* (%41.8) olduğu belirlenmiştir. Bunu sırasıyla *Klebsiella pneumoniae* (%23.1), *Escherichia coli* (%14.9) ve *Pseudomonas aeruginosa* (%7.2) izlemiştir. *E. coli* izolatlarında ESBL pozitifliği %44.2, *K. pneumoniae* izolatlarında ise %42.8 olarak saptanmıştır. En yüksek antibiyotik duyarlılığı *P. aeruginosa* ve *K. pneumoniae* için kolistine karşı gözlenmiştir.

Sonuç: Yoğun bakım hastalarında izole edilen etkenler ve bunların antibiyotik direnç profilleri, ampirik tedavi seçiminde yol gösterici olabilir. Kolistin dışındaki pek çok antibiyotiğe karşı artan direnç oranları, uygun antibiyotik kullanımının ve enfeksiyon kontrol önlemlerinin önemini bir kez daha ortaya koymaktadır.

Anahtar Kelimeler: *Sağlık hizmeti ilişkili enfeksiyonlar, kan kültürü, gram negatif bakteri*

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Introduction

Healthcare-associated infections (HAIs) are major global health problems that also cause significant morbidity and mortality in our country (1). They occur more frequently in intensive care units (ICUs) (2), where hospital-acquired bloodstream infections and pneumonia can increase mortality two- to threefold (3). Among HAIs, bloodstream infections (BSIs) hold particular importance. According to a recent systematic review and meta-analysis, BSIs account for 13–26% of HAIs in low- and middle-income countries, including Turkey, and 10–23% in high-income Western countries (4).

The most frequent ICU pathogens are *Acinetobacter spp*., *Staphylococcus aureus*, *Candida spp*., *P. aeruginosa*, and coagulase-negative staphylococci (CNS) (5). Resistance rates in ICU infections are high, mainly due to extended-spectrum β-lactamase (ESBL) production in enteric Gram-negative bacteria, especially *E. coli* and *K. pneumoniae*. *Pseudomonas* and *Acinetobacter* species also show extremely high resistance, limiting treatment options (6).

Uncontrolled antibiotic use contributes directly to resistance. Rational use and effective infection control measures reduce resistant organisms, mortality, and costs (7). Preventing transmission via healthcare personnel, ensuring rapid diagnosis, selecting appropriate antibiotics, and early recognition of outbreaks are essential to minimize infection rates (1, 8).

This study aimed to evaluate the distribution and resistance profiles of Gram-negative bacteria isolated from blood cultures of ICU patients at Atatürk University Faculty of Medicine Hospital between 2017 and 2021.

Materials and Methods

Research and Publication Ethics: Ethical approval was obtained from the Atatürk University Faculty of Medicine Clinical Research Ethics Committee (Approval No: B.30.2.ATA.0.01.00/667; Date: October 27, 2022).

This retrospective study analyzed blood culture results from patients aged ≥18 years hospitalized in ICUs between January 2017 and December 2021. Blood samples were collected aseptically and incubated in aerobic and anaerobic bottles using the BacT/Alert automated system (bioMérieux, Marcy l'Etoile, France).

Bacterial identification was performed by conventional methods (colony morphology, Gram staining, motility, biochemical tests) and automated systems—Phoenix (Becton Dickinson, USA) and Vitek 2 (bioMérieux, France)—used in different years.

Antimicrobial susceptibility testing was performed by disk diffusion, and results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. The same antibiotic disks (Bioanalyse, Türkiye) were used throughout the study, and quality control followed EUCAST recommendations.

A priori power analysis (G*Power 3.1.9.7) was performed using one-way ANOVA (effect size f=0.40, α=0.05, 1–β=0.80). The required sample size (n=80; 16/group) was adequate to detect large effects (actual power=0.803).

Statistical analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics summarized demographic and microbiological data. Independent Samples t-test and Chi-square test were used for group comparisons. The Kruskal–Wallis H test analyzed annual carbapenem resistance variations. A *p*-value <0.05 was considered significant.

Results

A total of 709 patients with Gram-negative growth in blood cultures, hospitalized in adult intensive care units of Atatürk University Health Research and Application Center Research Hospital between January 1, 2017, and December 31, 2021, were included in the study. Repeated samples with identical growth and antibiotic susceptibility patterns from the same patient were excluded to avoid duplication.

Of the patients, 51.3% (n=364) were male and 48.7% (n=345) were female. The mean age of male patients was 62.74±15.84 years, while the mean age of female patients was 67.37±16.43 years. No statistically significant difference was observed between the sexes in terms of Gram-negative growth in blood cultures (*p*=0.5394).

Explanation (for Table 1): In total, 808 Gram-negative bacterial isolates were identified from 709 patients included in the study. The combined table below presents a comprehensive overview of the distribution of isolates by bacterial species, intensive care units, and years.

- The species distribution section summarizes the relative frequencies of the most commonly isolated Gram-negative bacteria.
- The ICU-based distribution highlights the departments with the highest incidence of Gram-negative bacterial growth.
- The yearly distribution illustrates the annual variation in the number of isolates throughout the five-year study period.

Table 1. Combined Distribution of Gram-negative bacteria isolated from blood cultures by species, ıntensive care units, and years

Bacteria (n, %)	ICU (n, %)	Year (n, %)
Acinetobacter spp. (338, 41.8)	Anesthesiology (279, 34.5)	2017 (104, 12.9)
K.pneumoniae (187, 23.1)	Internal Medicine (217, 26.9)	2018 (177, 21.9)
E. coli (120, 14.9)	Neurology (106, 13.1)	2019 (147, 18.2)
P.aeruginosa (58, 7.2)	Neurosurgery (52, 6.5)	2020 (161, 19.9)
S.maltophilia (34, 4.2)	General Surgery (39, 4.8)	2021 (219, 27.1)
Enterobacter spp. (22, 2.7)	Pulmonology (33, 4.1)	Total (808, 100.0)
Proteus spp. (19, 2.4)	Other (82, 10.1)	
Other (30, 3.7)	Total (808, 100.0)	
Total (808, 100.0)		

Table 2. Comparison of Antibiotic Susceptibility (S/R %) among the most common Gram-negative bacteria

Antibiotic	*A. baumannii* (S/R %)	*P. aeruginosa* (S/R %)	*E. coli* (S/R %)	*K. pneumoniae* (S/R %)
Colistin	88.7 / 11.3	83.3 / 16.7	100 / –	75.0 / 25.0
Amikacin	9.9 / 89.5	85.9 / 12.7	88.3 / 5.5	53.2 / 34.2
Meropenem	2.4 / 97.0	69.5 / 26.0	88.2 / 6.3	26.2 / 61.2
Imipenem	3.3 / 92.8	45.6 / 29.4	83.3 / 6.7	30.7 / 37.0
Ciprofloxacin	1.7 / 96.8	46.4 / 26.8	29.4 / 66.7	14.2 / 84.4
Levofloxacin	4.3 / 86.6	19.4 / 36.2	31.7 / 56.1	11.7 / 78.0
Ceftazidime	-	36.1 / 38.9	38.7 / 55.9	9.3 / 86.0
Cefepime	-	45.7 / 31.5	40.4 / 45.7	9.4 / 87.3
Ertapenem	-	-	79.5 / 14.9	19.9 / 76.5
Ceftriaxone	-	-	37.6 / 60.4	9.1 / 81.3
Cefuroxime	-	-	27.1 / 67.3	7.3 / 88.0
Trimethoprim–sulfamethoxazole	33.0 / 54.6	-	-	26.1 / 72.0
Gentamicin	10.3 / 87.6	-	-	54.7 / 42.3
Tigecycline	-	-	84.1 / 5.9	-
Piperacillin	-	41.4 / 32.9	-	8.9 / 87.4
Aztreonam	-	6.2 / 31.3	-	-

S: Susceptible, R: Resistant

Table 3. Distribution of ESBL-producing *E. coli* and *K. pneumoniae* isolates over the study period

Years	*E. coli*				*K. pneumoniae*				Total			
	ESBL +		ESBL-		ESBL +		ESBL-		ESBL +		ESBL-	
	n	%	n	%	n	%	n	%	n	%	n	%
2017	12	44.4	15	55.6	8	42.1	11	57.9	20	43.4	26	56.5
2018	11	40.7	16	59.3	10	40.0	15	60.0	21	40.4	31	59.6
2019	12	48.0	13	52.0	16	45.7	19	54.3	28	41.6	32	58.3
2020	8	44.4	10	55.6	20	42.5	27	57.4	28	43.4	37	59.6
2021	10	43.4	13	56.6	26	42.6	35	57.3	36	42.8	48	57.2
	ESBL+: %44.2				ESBL+: %42.8							
	*p**: 0.990				*p**: 0.995							

* Result obtained with chi-square test

Table 4. Distribution of carbapenem resistance among frequently isolated bacteria by year

Years		*A. baumannii*		*K. pneumonia*		*E. coli*		*P. aeruginosa*	
		IMP	MEM	IMP	MEM	IMP	MEM	IMP	MEM
2017	n	35	38	18	19	20	27	7	5
	%	80.0	92.1	16.7	42.1	0.0	3.7	42.9	60.0
2018	n	88	93	35	34	30	30	19	20
	%	97.7	98.9	31.4	47.1	6.7	6.7	15.8	30.0
2019	n	70	70	40	39	27	27	8	8
	%	97.1	98.6	20.0	43.5	7.4	11.1	37.5	25.0
2020	n	65	65	50	50	19	19	14	14
	%	96.9	96.7	44.0	68.0	5.3	0.0	21.4	21.4
2021	n	48	72	52	64	21	24	20	22
	%	81.2	95.8	53.8	79.7	14.3	8.3	40.0	18.1
p		**0.1058**	**0.2566**	**0.0030**	**0.0003**	**0.4649**	**0.5884**	**0.3967**	**0.4038**

Table 2 presents a comparative overview of the antibiotic susceptibility (S/R %) patterns of the most frequently isolated Gram-negative bacteria: *A. baumannii, P. aeruginosa, E. coli,* and *K. pneumoniae.* Overall, *A. baumannii* exhibited high resistance to multiple antibiotic classes, particularly carbapenems and cephalosporins, while *P. aeruginosa* demonstrated notable resistance to β-lactam and carbapenem antibiotics. *E. coli* isolates generally showed higher susceptibility rates compared to other species, although resistance to fluoroquinolones and third-generation cephalosporins was evident. *K. pneumoniae* isolates displayed markedly high resistance rates across several antibiotic groups, especially carbapenems, β-lactams, and fluoroquinolones, underscoring their clinical significance.

Although fluctuations were observed in the proportion of ESBL-producing *E. coli* and *K. pneumoniae* strains over the years, these changes were not statistically significant. The annual distribution of ESBL positivity in these species is summarized in Table 3.

Table 4 demonstrates the annual changes in carbapenem resistance among the major Gram-negative pathogens, highlighting the overall trend of increasing non-susceptibility over the study period.

Discussion

Healthcare-associated infections (HAIs), which pose a serious global public health problem, negatively affect treatment outcomes (9). In our study, *A. baumannii* was the most frequently isolated microorganism (41.8%, n=338), followed by *K. pneumoniae* (23.1%), *E. coli* (14.9%), and *P. aeruginosa* (7.2%). Unlike our findings, studies from other countries reported *E. coli* as the most common isolate (10, 11). Similar bacterial distributions have been documented in other national and international studies (12, 13).

According to the 2020 WHO CAESAR report, *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were the predominant species in blood cultures from Turkey (14).

The majority of isolates were obtained from the Anesthesiology and Reanimation ICU (34.5%) and Internal Medicine ICU (26.9%), together accounting for 61.4% of all isolations. Comparable distributions have been reported both in a study from Elazığ (15) and in international reports, particularly from medical and surgical ICUs with high patient density, frequent invasive procedures, and prolonged hospitalization (16, 17). This indicates that the distribution of bacterial isolates is more closely related to clinical and procedural factors than to regional variations.

The number of bacterial isolations varied across the study years. The highest number of isolates was detected in 2021, whereas the lowest was observed in 2017. The increase seen in 2021 may be associated with the COVID-19 pandemic, during which hospital and ICU admissions markedly increased. Similar findings were reported in both national and international studies, indicating that the pandemic period led to a rise in healthcare-associated infections and antimicrobial resistance due to prolonged hospitalization and increased antibiotic use. This pattern suggests that changes in bacterial isolation rates over time may be influenced by extraordinary conditions such as pandemics, in addition to variations in patient density. (18, 19) In our study, *A. baumannii* isolates demonstrated high resistance rates. Compared to the 2011–2013 data from the same hospital, a notable decrease in susceptibility was observed, particularly for imipenem and ciprofloxacin. The emergence of colistin-resistant strains further indicates a growing therapeutic challenge (20). Similar findings have been reported in recent international studies, showing that *A. baumannii* isolates exhibit extremely high carbapenem resistance and a gradual decline in colistin susceptibility, emphasizing that this trend is not limited to a single region but represents a global concern (21).

These results highlight the importance of continuous resistance monitoring and implementation of effective infection control measures to limit the spread of multidrug-resistant *A. baumannii*.

According to the report prepared by the Turkish Public Health Agency in 2022, aminoglycoside resistance in bloodstream infections caused by *A. baumannii* was reported as 82.5%, colistin resistance as 6.9%, and quinolone resistance as 96.5% (17, 22). In our study, aminoglycoside resistance was 88.2%, colistin resistance 11.3%, and quinolone resistance 93.4%. While aminoglycoside and colistin resistance rates in our isolates were higher than the Turkish national average, quinolone resistance remained at a similar level. Comparable findings have been reported in international surveillance data, such as the WHO CAESAR report, which shows that *A. baumannii* strains in neighboring countries exhibit similarly high quinolone resistance (90–95%) and a gradually increasing colistin resistance trend, indicating a common regional challenge (23).

In our study, *P. aeruginosa* was identified as a significant healthcare-associated infection (HAI) pathogen, consistent with previous national reports (24). Colistin showed the highest susceptibility rate among the tested antibiotics, as all isolates were found to be sensitive to it, which is similar to the findings of Türk Dağı et al. (25). The resistance rate to amikacin was 12.7%, which aligns with earlier studies reporting rates between 2% and 34% (26, 27).

Resistance to ceftazidime and cefepime was found to be 38.9% and 31.5%, respectively, which is comparable to the rates reported by Kılınç et al. (28) (17.1%), and Çetin et al. (29) (24–37%). Ciprofloxacin resistance was 26.8%, consistent with reports by Çetin et al. (29) (26–37%), Falagas et al. (30) (33.3%). Compared with findings by Gültekin et al. (20) from 6–8 years earlier, susceptibility to most antibiotics—except amikacin and cefepime—has decreased, suggesting a gradual worsening of resistance trends over time.

E. coli is a frequently detected bacterium with high rates among the agents isolated from blood cultures. In our study, the antibiotic to which *E. coli* was most sensitive was colistin with 100%. The sensitivity percentages of aminoglycosides and carbapenems were determined to be high compared to other antibiotics.

K. pneumoniae is a significant and frequently encountered pathogen in hospital-acquired infections. In our study, the highest resistance rates were observed against cephalosporins and piperacillin/tazobactam. Similarly, Doğanay et al. (31) also reported high cephalosporin resistance in their study. Findings from various national and international studies are consistent with our results, showing increasing resistance rates to third-generation cephalosporins and β-lactam/β-lactamase inhibitor combinations, which suggests that cephalosporins may no longer be reliable options for empirical therapy in *K. pneumoniae* infections (28, 29, 32) In our study, colistin showed the highest activity against *K. pneumoniae*, with a resistance rate of 25%.

Infections caused by extended-spectrum β-lactamase (ESBL)-producing bacteria represent a major therapeutic challenge, as many commonly used antibiotics become ineffective. Failure to detect ESBL may lead to prolonged hospitalization, increased morbidity and mortality, and a significant economic burden (33). In our study, ESBL positivity was found to be 44.2% for *E. coli* and 42.8% for *K. pneumoniae* over the five-year period, with no significant variation observed between years. This finding is particularly noteworthy, as both the number of isolates and resistance rates to multiple antibiotic groups increased during the same period. Several factors may explain this observation. While the proportion of ESBL-producing strains remained relatively stable, other resistance mechanisms-such as AmpC β-lactamases and carbapenemases-may have become more prevalent, contributing to the overall rise in resistance. Additionally, the circulation of specific resistant clones and selective antibiotic pressure in ICU environments might have reinforced this trend (23, 34, 35).

In contrast, Bayraktar et al. (36) reported lower ESBL positivity rates (35% for *E. coli* and 31% for *K. pneumoniae*), with significant year-to-year variation. Similarly, studies from Germany and other European countries have documented ESBL positivity rates below 20%, often showing periodic fluctuations (37). These findings indicate that ESBL production remains a substantial and persistent problem in our region, despite potential improvements in infection control measures. Therefore, continued surveillance is essential to monitor evolving resistance mechanisms, including emerging β-lactamases, and to guide empirical antibiotic use effectively.

In recent years, the number of multidrug-resistant (MDR) strains resistant to multiple antibiotic classes has increased, and carbapenems have become widely used as last-resort agents (38). Our findings are consistent with previous studies conducted in Turkey, which have also reported extremely high carbapenem resistance rates in *A. baumannii*. Between 2010 and 2012, Cesur et al. (39) reported 94% resistance to imipenem and 90% to meropenem; in 2015, Uğur and Genç (40) found 98% and 99%, respectively. According to the Turkish Public Health Agency (22), the imipenem and meropenem resistance rates in bloodstream infections caused by *A. baumannii* were 93.7% and 93.1%, respectively. The ECDC 2022–2023 report, based on 2020–2021 data, revealed carbapenem resistance rates of 93.1–93.3% in Turkey, 94.6–96.9% in Greece, 80.8–86.9% in Italy, 77.9–82.9% in Bulgaria, 98.0–98.6% in Serbia, and 96.4–99.5% in Croatia. In countries geographically close to Turkey, resistance levels were similarly high, while Western and Northern European countries demonstrated much lower rates: 3.1–3.3% in France, 3.1–4.3% in Germany, 0.7–7.1% in Sweden, and 4.7–5.9% in Denmark (23, 32). These findings highlight that carbapenem resistance in *A. baumannii* remains alarmingly high in Turkey and neighboring regions. The markedly lower rates in Western and Northern Europe likely reflect stricter antibiotic stewardship, controlled antimicrobial use, and effective infection control programs, whereas uncontrolled antibiotic use in our country may have contributed to the current situation.

Imipenem resistance of *K. pneumoniae* strains in our study varied between 16.6% and 53.8% over the years. The change in imipenem resistance over the years was found to be significant, with the least resistance being 16.6% in 2017 and the most resistance being 53.8% in 2021. The lowest meropenem resistance was found to be 42.1% in 2017 and the highest was found to be 79.6% in 2021. The change in resistance to meropenem over the years was found to be significant ($p<0.05$). According to the report prepared by the Turkish Public Health Agency in 2022, the resistance rate for imipenem was 60.1% and for meropenem 63.2% in healthcare-associated bloodstream infections caused by *K. pneuomoniae*. The data in our study are similar to the data in the published report (22). In the report published by ECDC in 2022 and 2023, carbapenem resistance rates according to 2020 and 2021 data were given as 48.2-49.1 in Turkey, 28.1-46.3 in Bulgaria, 66.3-73.7 in Greece, 48.3-54.5 in Romania, 29.5-26.7 in Italy, 0.5-0.8 in Germany, 0.5-0.8 in France, 0.1-0.3 in Norway, and 0.3-0.2 in Sweden, respectively (23, 32). According to the data, similar resistance rates were detected in similar geographical regions.

In our study, carbapenem resistance in *E. coli* isolates was evaluated. The differences in imipenem and meropenem resistance rates between the years were not found to be statistically significant ($p>0.05$).

In our study, imipenem resistance in *P. aeruginosa* was between 15.7% and 42.8%. The change in imipenem and meropenem resistance between years was not found to be significant ($p>0.05$). In the report published by the Turkish Public Health Agency in 2022, imipenem resistance was determined as 81.6% and meropenem resistance as 50.3% in bloodstream infections caused by *P. aeruginosa* (22). The resistance rates detected in the study are higher than the data we found in our study.

In conclusion, intensive care units remain the primary settings where healthcare-associated infections are most frequently observed. Through effective infection control and antibiotic stewardship programs, resistant pathogens can be identified early, and unnecessary antibiotic use can be minimized. Consequently, infection rates, patient morbidity, and mortality can be substantially reduced.

However, as this was a retrospective study, several limitations must be acknowledged. The data were derived from existing laboratory and hospital records, which may not fully reflect clinical outcomes or treatment responses. Additionally, changes in sampling practices or diagnostic methods over the years may have influenced the observed resistance rates. The study was conducted in a single tertiary-care center, which may limit the generalizability of the findings to other institutions or regions. Furthermore, molecular typing was not performed, and therefore, potential clonal relationships or specific resistance genes could not be evaluated.

Despite these limitations, this study provides valuable insight into the long-term antimicrobial resistance trends in bloodstream infections in intensive care units. Continuous national surveillance and multicenter molecular studies are recommended to better understand evolving resistance mechanisms and to inform evidence-based infection control strategies.

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