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Microbial Agents and Antimicrobial Resistance Patterns in Blood Cultures of Intensive Care Unit Patients: Pre- and Post-COVID-19 Pandemic Analysis

Yoğun Bakım Ünitesi Hastalarının Kan Kültürlerinde Mikrobiyal Etkenler ve Antimikrobiyal Direnç Paternleri: COVID-19 Pandemisi Öncesi ve Sonrası Analiz

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ABSTRACT

Objective: This study aimed to identify the microbial agents isolated from blood cultures of intensive care unit (ICU) patients and their antibiotic resistance rates before and after the Coronavirus Disease 2019 (COVID-19) pandemic.

Methods: Blood culture samples from general ICU-1 and ICU-2, collected between 2018-2022, were retrospectively analyzed.

Results: Of the samples analyzed, 44.4% showed positive culture growth, 46.1% showed no growth, and 9.5% were determined to be skin contaminants. In both ICUs, coagulase-negative staphylococci were the most frequently isolated microorganisms, followed by Enterococcus species. Methicillin resistance *in Staphylococcus aureus* significantly decreased in ICU-1 after the pandemic but increased significantly in ICU-2. Resistance rates to vancomycin and teicoplanin in *Enterococcus* species significantly increased during the pandemic in both ICUs. No colistin resistance was detected in *Escherichia coli*, but colistin resistance rates significantly increased in other Gram-negative isolates during the pandemic, except for *Pseudomonas aeruginosa* in ICU-1. After the pandemic, *Klebsiella pneumoniae* in ICU-1 and *Acinetobacter baumannii* in ICU-2 showed the highest colistin resistance rates.

ÖZ

Amaç: Bu çalışmada Koronavirüs Hastalığı 2019 (COVID-19) pandemisi öncesi ve sonrası yoğun bakım ünitesinde (YBÜ) yatan hastalara ait kan kültürlerinde üreyen etkenleri ve antibiyotik direnç oranlarını tespit etmeyi amaçladık.

Yöntemler: 2018-2022 tarihleri arasında genel (YBÜ-1) ve 2 (YBÜ-2)'de yatan hastalara ait kan kültür örnekleri retrospektif olarak değerlendirildi.

Bulgular: Kan kültürü örneklerinin %44,4'ünde üreme saptanırken %46,1'inde üreme olmadığı, %9,5'inin cilt flora bakterileriyle kontamine olduğu saptanmıştır. Her iki yoğun bakımda da en sık Koagülaz negatif stafilokokların izole edildiği, bunu Enterokok türlerinin takip ettiği görülmüştür. Metisilin direnci *Staphylococcus aureus'da* genel YBÜ-1'de pandemi öncesine göre anlamlı şekilde azalırken genel YBÜ-2'de anlamlı şekilde artmıştır. *Enterococcus* spp. izolatlarında vankomisin ve teikoplanin direnç oranları her iki yoğun bakımda da COVID-19 pandemisiyle birlikte istatistiksel olarak anlamlı artış göstermiştir. *Escherichia coli* izolatlarında kolistin direnci saptanmamış, pandemiyle beraber kolistin direnç oranlarının genel YBÜ-1'den izole edilen *Pseudomonas aeruginosa* türleri hariç diğer gram negatif izolatlarda anlamlı oranda arttığı; pandemi sonrası genel YBÜ-1'den izole edilen *Klebsiella pneumoniae*, genel YBÜ-2'de ise *Acinetobacter baumannii* izolatlarının en yüksek kolistin direnç oranlarına sahip olduğu görülmüştür.

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Conclusion: This study revealed that, during the pandemic, there was a shift in the distribution of isolated pathogens, accompanied by increased resistance rates even to last-resort antibiotics such as vancomycin and colistin.

Keywords: Blood culture, COVID-19, pathogen microorganisms, antimicrobial resistance, pandemic, intensive care unit

Sonuç: Çalışmamızda pandemiyle birlikte kan kültüründe üreyen etkenlerin dağılımının değiştiği, direnç oranlarının ise vankomisin ve kolistin gibi son çare antibiyotiklerde bile arttığı saptanmıştır.

Anahtar Sözcükler: Kan kültürü, COVID-19, patojen mikroorganizmalar, antimikrobiyal direnç, pandemi, yoğun bakım ünitesi

INTRODUCTION

Bloodstream infections (BSIs) are among the most significant causes of morbidity and mortality worldwide. The primary diagnostic method, regarded as the gold standard, is blood culture testing, which is frequently conducted using automated blood culture systems. Blood culture allows for the identification of causative microorganisms and the determination of their antibiotic susceptibility patterns. This facilitates appropriate treatment for patients, thereby reducing morbidity and mortality rates (1-3).

Antibiotic resistance is a global public health problem worldwide. The widespread use of antibiotics is one of the most important reasons that triggers antibiotic resistance (4). Patients in intensive care units (ICUs) are particularly susceptible to resistant infections due to factors such as broad-spectrum antibiotic use, compromised immune systems, prolonged hospital stays, and invasive procedures. In particular, the Coronavirus Disease 2019 (COVID-19) pandemic has intensified the need for ICU care among COVID-19-positive patients and has led to an increase in broad-spectrum antibiotic use (5-7). While antibiotic resistance has risen over the years, the effect of the COVID-19 pandemic on this resistance has varied across different healthcare settings. Therefore, this study aimed to determine the pathogens that grew in the blood cultures of ICU patients over a four-year period, assess antibiotic resistance rates, and evaluate the effect of the COVID-19 pandemic on microbial resistance within our hospital.

MATERIALS AND METHODS

Study Design

This retrospective study involved the analysis of blood culture samples submitted to the microbiology laboratory from patients admitted to general ICU-1 and ICU-2 at Tekirdağ Namık Kemal University Hospital. The study period extended from March 11, 2018, to March 10, 2022. Our hospital is a tertiary care institution with a capacity of 430 beds (11 beds each for ICU-1 and ICU-2). It also provided uninterrupted care to all patients during the COVID-19 pandemic. ICU-1 was designated for intubated patients with confirmed COVID-19 infection [SARS-CoV-2 polymerase chain reaction (PCR)-positive], while ICU-2 catered to intubated patients with non-COVID-19 conditions (SARS-CoV-2 PCR-negative). Patients hospitalized in both ICU-1 and ICU-2, had similar clinical presentations except for their COVID-19 status (positive or negative). The study period was divided into two phases: the two years preceding the date when the first case was reported in Türkiye (March 11, 2018-March 10, 2020) and the two years following the pandemic onset (March 11, 2020-March 10, 2022).

Demographic data (gender and age) and clinical data (pathogen, antimicrobial susceptibility tests, inpatient service, etc.) were retrieved from the hospital information management system. Ethics Committee approval Tekirdag Namik Kemal University, Non-Interventional Clinical Research Ethics Committee, (decision number: 2023.133.06.19, date: 23.06.2023).

Microbiological Evaluation

For patients suspected of BSI, blood culture samples were collected in two sets, comprising a total of four bottles (two aerobic and two anaerobic bottles). These samples were monitored using the BD BACTEC automated blood culture system (Becton Dickinson, USA). Upon detecting a positive growth signal, the samples underwent Gram staining, and preliminary Gram results were promptly reported to the relevant clinical units. In our hospital, empirical treatment is initiated with the notification of blood culture gram-stain results; vancomycin is frequently preferred when gram-positive bacteria (GPB) are seen, and carbapenems are frequently preferred when gram-negative bacteria (GNB) are seen. Afterwards, de-escalation is performed according to the culture-antibiogram results of the microbiology laboratory.

Blood culture bottles with positive signals were inoculated onto blood agar (Bes-Lab, Türkiye), eosin methylene blue agar (Bes-Lab, Türkiye), and chocolate agar (Bes-Lab, Türkiye). All plates were incubated at 37 °C for 24-48 hours. Isolates were identified using conventional methods (colony morphology, gram staining, catalase, coagulase, and oxidase tests) and automated identification systems (VITEK®2 Compact, Biomerieux, France, and BD Phoenix System, Becton Dickinson, USA). The presence of coagulase-negative staphylococci (CoNS) in culture was evaluated based on guideline recommendations. If growth was observed in a single bottle, it was considered a potential skin flora contaminant, and species identification and methicillin resistance testing were performed, with results reported as contamination. If growth occurred in both bottles, species identification and antibiogram testing were conducted. When the same microorganism was isolated in both bottles, it was deemed the causative agent, whereas the isolation of different CoNS species was considered contamination. Mixed skin flora growth in culture was directly reported as contamination (8).

Antibiotic susceptibility tests were performed using manual Kirby-Bauer disc diffusion (Bioanalyse, Türkiye, and Oxoid, UK) and automated antibiogram systems (VITEK®2 Compact, Biomerux, France, and BD Phoenix System, Beckton Dickinson, USA) in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (9):

• Methicillin resistance in staphylococcal isolates was determined using the disk diffusion method with cefoxitin disk (Oxoid, UK).

• Vancomycin and teicoplanin resistance detected in enterococcal isolates was confirmed using gradient tests (Bioanalyse, Türkiye).

• Carbapenem resistance in *Klebsiella pneumoniae* isolates was evaluated via the combined disc diffusion method (Bioanalyse,

Türkiye).

• Colistin resistance was assessed using the broth microdilution method (Micronaut-S, Merlin, Germany).

• Antifungal susceptibility testing of yeast isolates was performed using the microdilution method (Mikronaut-AM, Bruker, Germany).

For patients with multiple samples, only the first isolate was included in the study. When two sets of blood cultures obtained simultaneously yielded the same pathogen, the isolates were counted as one. SARS-CoV-2 polymerase chain reaction testing was performed using the Bio Speedy SARS-CoV-2 RT-qPCR kit (Bioeksen, Türkiye).

Statistical Analysis

The data obtained from the study were entered into SPSS version 22.0 (SPSS Inc, Chicago, IL, USA) for statistical analyses. Categorical data were given as percentages. The chi-square test was used to compare independent groups with categorical variables. Cases where the p-value was below 0.05 were considered statistically significant.

RESULTS

Over the course of four years, 1,702 blood culture sets (3,942 bottles) were submitted from 728 patients. The demographic data indicated that 59.2% (n=431) of the patients were male and 40.8% (n=297) were female. The distribution by ICU was similar: ICU-1 had 59.4% male and 40.6% female patients, while ICU-2 had 59% male and 41% female patients. The mean patient age was 66.5±16.6 years (range: 17-100 years), with no significant differences in age or gender between the patients in ICU-1 (mean age: 67.5±16.3 years) and those in ICU-2 (mean age: 65.2±16.7 years) (p>0.05).

Before the pandemic, 198 patients were followed up in ICU-1, with 466 blood culture sets requested. During the pandemic, the number of patients in ICU-1 decreased to 191, while the number of sets increased to 524. In contrast, in ICU-2, the number of patients rose from 160 to 179, while the number of sets decreased from 385 to 327.

Of the samples analyzed, 44.4% showed positive culture growth, 46.1% showed no growth, and 9.5% were determined to be skin contaminants. After the COVID-19 pandemic, both ICUs showed decreases in culture positivity and contamination rates, with an increase in no-growth samples. (Before pandemic ICU-1; culture positive 48.4%, culture negative 41.8%, contamination 9.7%, ICU-2; culture positive 42.2%, culture negative 46.3%, contamination 11.5%. After pandemic ICU-1; culture positive 44.3%, culture negative 48.6%, contamination 7.1%, ICU-2; culture positive 41.9%, culture negative 47.6%, contamination 10.5%). However, these changes were not statistically significant (p>0.05). When blood culture contamination rates were evaluated separately over the years, contamination rates decreased in 2019 and 2020 in both units. However, these changes were not statistically significant (ICU-1: 2018-14.2%, 2019-6.8%, 2020-6.0%, 2021-9.2%, ICU-2: 2018-14.4%, 2019-9.2%, 2020-10.1%, 2021-11.6%) (p>0.05).

Among isolates deemed clinically significant and subjected to susceptibility testing, 55.7% were GPB, 38.1% were GNB, and 6.2% were yeast species (*Candida* spp.). Over four years, ICU-1 isolates consisted of 54.3% GPB, 38.7% GNB, and 7.0% yeast, while ICU-2 isolates comprised 57.7% GPB, 37.3% GNB, and 5.0% yeast. The comparison of the pre- and post-pandemic data revealed a decline in GNB isolation in both ICUs. While ICU-1 showed an increase in yeast isolation, ICU-2 showed an increase in GPB isolation. In both units, CoNS were the most frequently isolated pathogens, followed by *Enterococcus* spp. (*E. faecium/E. faecalis*). Among GNB, *Acinetobacter baumannii* was most commonly isolated in ICU-1, while *Pseudomonas aeruginosa* was predominantly observed in ICU-2 (Table 1).

The rate of methicillin-resistant *Staphylococcus aureus* (MRSA) was 43.8% in ICU-1 (pre-pandemic: 50.0%, post-pandemic: 33.3%) and 58.8% in ICU-2 (pre-pandemic: 33.3%, post-pandemic: 62.5%). The MRSA rate significantly decreased in ICU-1 after the pandemic p=0.015 but significantly increased in ICU-2 (p≤0.001). The rate of methicillin resistance in CoNS was 77.9% in ICU-1 (pre-pandemic: 78.0%, post-pandemic: 77.6%) and 72.0% in ICU-2 (pre-pandemic: 72.6%, post-pandemic: 71.4%), with no significant changes (p>0.05). No resistance to vancomycin, teicoplanin, or linezolid was observed in either *S. aureus* or CoNS isolates (Table 2).

Among *Enterococcus* spp. isolates, vancomycin and teicoplanin resistance rates were 10.5% in ICU-1 and 9.5% in ICU-2. Resistance significantly increased in both units during the pandemic (p=0.038 for ICU-1, p=0.018 for ICU-2) (Table 2).

For *A. baumannii* isolates, resistance rates to all tested antibiotics except colistin decreased during the pandemic in both ICUs. In ICU-1, decreases in carbapenem and ciprofloxacin resistance were statistically significant (p=0.001 and p=0.002, respectively). In ICU-2, significant decreases were observed for ciprofloxacin and trimethoprim/sulfamethoxazole resistance (p=0.001 and p≤0.001, respectively). However, colistin resistance rates increased in both ICUs, revealing a statistically significant (p=0.050) (Table 3).

For *P. aeruginosa*, resistance to piperacillin/tazobactam (TZP), cephalosporins, and carbapenems increased in both ICUs during the pandemic, while amikacin resistance decreased. TZP and cephalosporin resistance significantly increased in both units, while meropenem resistance significantly increased only in ICU-1 (p<0.05). Colistin resistance decreased in ICU-1 but increased significantly in ICU-2 (p=0.013) (Table 3).

In ICU-1, the resistance of *K. pneumoniae* isolates to all tested antibiotics, including colistin, increased during the pandemic. This increase was statistically significant for all antibiotics except gentamicin (p<0.05). In ICU-2, resistance to cephalosporins and ciprofloxacin decreased, while resistance to TZP, carbapenems, gentamicin, amikacin, trimethoprim/sulfamethoxazole, and colistin increased significantly (p<0.05) (Table 4).

No colistin resistance was observed for *E. coli* in either ICU. However, resistance rates to all other antibiotics increased after the pandemic, except for gentamicin in ICU-2. Significant increases were observed for all antibiotics in ICU-1 except ertapenem, which approached the statistical significance level of 0.05. In ICU-2, significant increases were observed for ceftazidime, cefepime, and ciprofloxacin resistance (p<0.05) (Table 4).

Table 1: Distribution of microorganisms isolated from blood cultures (n/%)

Microorganisms	Before COVID-19								After COVID-19								Four-year period						
	2018				2019				2020				2021				TOTAL						
	ICU-1		ICU-2		ICU-1		ICU-2		ICU-1		ICU-2		ICU-1		ICU-2		ICU-1		ICU-2		Total		
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
GPB																							
	S. aureus	6	5.1	3	2.8	8	5.2	6	5.9	8	5.5	6	5.4	1	1.0	2	3.3	23	4.5	17	4.5	40	4.5
	CoNS	**36**	**30.8**	**37**	**34.9**	**46**	**29.7**	**25**	**24.8**	**42**	**29.0**	**46**	**41.4**	**25**	**26.3**	**24**	**39.3**	**149**	**29.1**	**132**	**34.8**	**281**	**31.5**
	Enterococcus spp.	**23**	**19.7**	**21**	**19.8**	**26**	**16.8**	**16**	**15.8**	**19**	**13.1**	**16**	**14.4**	**27**	**28.4**	**10**	**16.4**	**95**	**18.6**	**63**	**16.6**	**158**	**17.7**
	Other GPB	1	0.9	1	1.0	3	1.9	3	3.0	4	2.8	1	0.9	3	3.2	2	3.3	11	2.1	7	1.8	18	2.0
GNB																							
NFGN	*A. baumannii*	**10**	**8.5**	8	7.5	6	3.8	6	5.9	**23**	**15.9**	**11**	**9.9**	**12**	**12.6**	5	8.2	**51**	**10.0**	30	7.9	**81**	**9.1**
	P. aeruginosa	**10**	**8.5**	4	3.8	**16**	**10.3**	**12**	**11.9**	16	11.0	10	9.0	5	5.3	7	**11.5**	47	9.2	**33**	**8.7**	80	9.0
	Other NFGN	3	2.7	2	1.9	14	9.1	10	9.9	2	1.4	2	1.8	3	3.2	-	-	22	4.3	14	3.7	36	4.0
Enterobacterales	*K. pneumoniae*	**10**	**8.5**	7	6.6	4	2.6	5	5.0	13	9.0	6	5.4	7	7.4	2	3.3	34	6.6	20	5.4	54	6.1
	E. coli	**10**	**8.5**	7	6.6	12	7.7	9	8.9	3	2.1	4	3.6	2	2.1	3	4.9	27	5.3	23	6.1	50	5.6
	Others	8	6.8	6	5.7	7	4.5	7	6.9	1	0.6	5	4.6	1	1.0	3	4.9	17	3.3	21	5.5	38	4.3
Fungi																							
	Candida spp.	-	-	**10**	**9.4**	13	8.4	2	2.0	14	9.6	4	3.6	9	9.5	3	4.9	36	7.0	19	5.0	55	6.2
Total			117	100	106	100	155	100	101	100	145	100	111	100	95	100	61	100	512	379	100	891	100

ICU: Intensive care unit, GPB: Gram-positive bacteria, CoNS: Coagulase-negative *Staphylococci*, Other GPB: Other Gram-positive bacteria, GNB: Gram-negative bacteria, NFGN: Non-fermentative gram-negative, COVID-19: Coronavirus Disease 2019

Table 2. Antibiotic resistance rates of CoNS and Enterococcus spp. isolates before and after the pandemic (%)

Antibiotic	**CoNS**						***Enterococcus* spp.**					
	Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)		p-value		Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)		p-value	
	ICU-1 (n=82)	ICU-2 n=62	ICU-1 n=67	ICU-2 n=70	ICU-1	ICU-2	ICU-1 n=49	ICU-2 n = 37	ICU-1 n = 46	ICU-2 **(n=46)**	ICU-1	ICU-2
MET	78.0	72.6	77.6	71.4	0.996	0.753	-	-	-	-	-	-
PEN	79.3	77.4	97.0	77.1	**≤0.001**	0.997	-	-	-	-	-	-
CIP	64.6	66.1	68.7	67.1	0.547	0.881	-	-	-	-	-	-
LEV	-	-	-	-	-	-	40.8	21.6	23.9	11.5	**0.010**	0.060
VAN	0.0	0.0	0.0	0.0	-	-	6.1	5.4	15.2	15.4	**0.038**	**0.018**
TEC	0.0	0.0	0.0	0.0	-	-	6.1	5.4	15.2	15.4	**0.038**	**0.018**
GEN	45.1	45.2	46.3	45.7	0.887	0.886	-	-	-	-	-	-
E	78.0	77.4	83.6	64.3	0.279	**0.044**	-	-	-	-	-	-
DA	54.9	54.8	65.7	55.7	0.112	0.887	-	-	-	-	-	-
TE	56.1	46.8	38.8	35.7	**0.016**	0.114	-	-	-	-	-	-
LIN	0.0	0.0	0.0	0.0	-	-	0.0	0.0	0.0	0.0	-	-

CoNS: Coagulase-negative *Staphylococci*, COVID-19: Coronavirus Disease 2019, ICU: Intensive care unit, MET: Methicillin, PEN: Penicillin, CIP: Ciprofloxacin, LEV: Levofloxacin, VAN: Vancomycin, TEC: Teicoplanin, GEN: Gentamicin, E: Erythromycin, DA: Dlindamycin, TE: Tetracycline, LIN: Linezolid

Table 3. Antibiotic resistance rates of Acinetobacter baumannii and Pseudomonas aeruginosa isolates before and after the pandemic (%)

Antibiotic	Antibiotic											
	A. baumannii						***P. aeruginosa***					
	Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)		p-value		Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)		p-value	
	ICU-1 (n=16)	ICU-2 (n=14)	ICU-1 (n=35)	ICU-2 (n=16)	ICU-1	ICU-2	ICU-1 (n=26)	ICU-2 (n=16)	ICU-1 (n=21)	ICU-2 (n=17)	ICU-1	ICU-2
TZP		-	-	-	-	-	-	46.2	37.5	61.9	52.9	**0.023**	**0.033**
CAZ		-	-	-	-	-	-	34.6	31.3	61.9	58.8	**≤0.001**	**≤0.001**
FEP		-	-	-	-	-	-	34.6	31.3	57.1	52.9	**0.002**	**0.002**
IMP		100	92.9	88.6	87.5	**0.001**	0.228	46.2	18.8	57.1	29.4	0.120	0.071
MER		100	92.9	88.6	87.5	**0.001**	0.228	38.5	18.8	57.1	23.5	**0.011**	0.389
GEN		93.8	71.4	91.4	68.8	0.579	0.758	-	-	-	-	-	-
AK		100	85.7	97.1	81.3	0.081	0.341	34.6	18.8	4.8	11.8	**≤0.001**	0.171
CIP		100	92.9	91.4	75.0	**0.002**	**0.001**	53.8	31.3	42.9	41.2	0.120	0.141
TMP-SXT		81.3	85.7	74.3	62.5	0.236	**≤0.001**	-	-	-	-	-	-
COL		6.3	14.3	11.4	25.0	0.205	**0.050**	11.5	0.0	4.8	5.9	0.076	**0.013**

ICU: Intensive care unit, COVID-19: Coronavirus Disease 2019, TZP: Piperacillin-tazobactam, CAZ: Ceftazidime, FEP: Cefepime, IMP: Imipenem, MER: Meropenem, GEN: Gentamicin, AK: Amikacin, CIP: ciprofloxacin, TMP-SXT: trimethoprim-sulfamethoxazole, COL: colistin

Table 4. Antibiotic resistance rates of ***Klebsiella pneumoniae*** and ***Escherichia coli*** isolates before and after the pandemic (%)

*Antibiotic	*K. pneumoniae*						*E. coli*					
	Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)		p-value		Before COVID-19 (2018-2019)		After COVID-19 (2020-2021)		p-value	
	ICU-1 (n=14)	ICU-2 (n=12)	ICU-1 (n=20)	ICU-2 (n=8)	ICU-1	ICU-2	COVID-19	ICU-2 (n=16)	ICU-1 (n=5)	ICU-2 (n=7)	ICU-1	ICU-2
AMC	50.0	75.0	90.0	75.0	**≤0.001**	-	(2018-2019)	68.8	100	71.4	**≤0.001**	0.758
TZP	50.0	50.0	80.0	75.0	**≤0.001**	**≤0.001**	27.3	31.3	60.0	42.9	**≤0.001**	0.079
CRO	50.0	83.3	85.0	62.5	**≤0.001**	**0.001**	72.7	68.8	100	71.4	**≤0.001**	0.758
CAZ	42.9	83.3	80.0	75.0	**≤0.001**	0.165	68.2	75.0	100	85.7	**≤0.001**	**0.050**
FEP	42.9	66.7	70.0	50.0	**≤0.001**	0.015	63.6	50.0	80.0	71.4	**0.012**	**0.002**
ERT	28.6	33.3	50.0	75.0	**0.002**	**≤0.001**	27.3	25.0	40.0	28.6	0.051	0.524
IMP	28.6	25.0	45.0	62.5	**0.019**	**≤0.001**	18.2	18.8	40.0	28.6	**0.001**	0.098
MER	21.4	25.0	45.0	62.5	**≤0.001**	**≤0.001**	18.2	18.8	40.0	28.6	**0.001**	0.098
GEN	57.1	25.0	60.0	62.5	0.667	**≤0.001**	31.8	50.0	60.0	42.9	**≤0.001**	0.321
AK	7.1	16.7	55.0	50.0	**≤0.001**	**≤0.001**	-	-	-	-	-	-
CIP	50.0	66.7	85.0	62.5	**≤0.001**	**0.553**	54.5	37.5	80.0	57.1	**≤0.001**	**0.007**
TMP-SXT	28.6	58.3	60.0	87.5	**≤0.001**	**≤0.001**	50.0	68.8	100	71.4	**≤0.001**	0.758
COL	7.1	0.0	30.0	12.5	**≤0.001**	**≤0.001**	0.0	0.0	0.0	0.0	-	-

ICU: Intensive care unit, COVID-19: Coronavirus Disease 2019, AMC: Amoxicillin-clavulanate, TZP: Piperacillin-tazobactam, CRO: Ceftriaxone, CAZ: Ceftazidime, FEP: Cefepime, ERT: Ertapenem, IMP: Imipenem, MER: Meropenem, GEN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, TMP-SXT: Trimethoprim-sulfamethoxazole, COL: Colistin

DISCUSSION

BSIs are a significant health concern for hospitalized patients. COVID-19 infections have necessitated the admission of patients to ICUs and have exposed them to numerous secondary infections, including BSIs. Blood culture holds a pivotal role in diagnosis, and the proper collection of samples is critically important for accuracy (10,11). In our study, while the number of patients monitored in ICU-1 slightly decreased during the pandemic, an increase in the number of requested blood culture sets was observed. In contrast, in ICU-2, where patients intubated for reasons other than COVID-19 were monitored, the patient count increased, but the number of sets decreased. This discrepancy can be attributed to the frequent presentation of fever in COVID-19 infections. When analyzing blood culture results, the rate of culture positivity decreased in both clinics during the pandemic, likely due to the increased number of blood culture sets requested.

In addition to proper sampling, adequate skin antisepsis and the prevention of contamination are crucial steps in diagnosis (12,13). Aygar et al. (10) evaluated blood culture contamination rates in ICU patients before and during the pandemic and found an increase in contamination rates. In the current study, contamination rates decreased in both ICUs during the pandemic compared to pre-pandemic levels. An annual analysis showed a reduction in contamination rates in ICU-1, in 2019 compared to 2018, reaching the lowest levels during 2020 (the first pandemic year), followed by an increase thereafter. During the COVID-19 pandemic, infection control measures in our hospital were increased, and antisepsis rules were followed more strictly. This decrease in contamination rates may be related to the enhanced cleaning and antisepsis measures implemented in our hospital during the pandemic. However, since the detected contamination rate still exceeds the national target value of 3% (8), additional measures are deemed necessary in both clinics to further reduce these rates.

Studies report that the growth rate of GPB in blood culture samples ranges from 59.3% to 70.3%, GNB from 22.1% to 40.2%, and *Candida* species from 7.1% to 14.8%. The frequency of *Candida* isolation has been reported to increase during the pandemic (5-7,10). In our study, the distribution of microorganisms that grew in blood cultures was consistent with the literature. The isolation frequency of *Candida* species increased in ICU-1 but decreased in ICU-2 during the pandemic. Risk factors such as the use of broad-spectrum antimicrobial agents, prolonged ICU stays, and mechanical ventilation requirements in COVID-19 patients may have predisposed them to *Candida* infections.

At the species level, CoNS were the most frequently isolated pathogens, consistent with previous studies (5-7,14). *Enterococcus* spp. were also commonly isolated GPB (15). Among GNB, *Klebsiella* spp. and *Acinetobacter* spp. were frequently isolated, although their order of prevalence varies across studies (1,10). Arslan et al. (5) reported that *Acinetobacter* spp. were predominant among Gram-negative isolates before the pandemic, whereas *Klebsiella* spp. became more prominent during the pandemic. Aytaç et al. (7) demonstrated that while *Klebsiella* spp. were more frequently

identified before the pandemic, the prevalence of *Acinetobacter* spp. increased during the pandemic. In our study, CoNS and *Enterococcus* spp. were the most frequently isolated agents before and after the pandemic in both clinics. GNB were generally the third most frequently isolated agents, with *P. aeruginosa* being more common before the pandemic and *A. baumannii* increasing in prevalence after the pandemic. Rapid diagnosis of BSIs and initiation of appropriate empirical treatment are crucial for reducing mortality. Although the distribution of causative agents was similar in both ICUs, changes in isolation frequencies were observed during the pandemic. Therefore, regular analysis of the distribution of pathogens isolated from blood cultures in clinical settings is essential to guide empirical treatment strategies effectively. In addition, effective infection control measures should be taken to prevent the spread of infectious agents within the hospital and to prevent the transfer of pathogenic microorganisms between patients or clinics (16). Despite the stricter attention paid to isolation measures implemented in our hospital during the pandemic, the fact that the same physicians provided consultation services to patients in both ICUs may have contributed to the similar distribution of agents.

Methicillin resistance is a critical factor in the treatment of staphylococcal infections. Recent studies report methicillin resistance rates in staphylococcal species isolated from blood cultures ranging from 34.0% to 90.4% for CoNS and 28.2% to 61.5% for *S. aureus* (6,15,17). Our findings are consistent with these rates and similarly indicate higher methicillin resistance in CoNS compared to *S. aureus* (14). Methicillin resistance in CoNS remained consistent during the pandemic, while it decreased in *S. aureus* isolates from ICU-1 and increased in ICU-2, with the differences being statistically significant.

Vancomycin, teicoplanin, and linezolid are critical antibiotics for treating resistant GPB infections (18). In our study, all staphylococcal species isolated over the four-year period were sensitive to these agents. However, vancomycin resistance in *Enterococcus* species increased significantly from pre-pandemic levels of approximately 5-6% to 15% during the pandemic. According to the 2019 data from the Central Asian and Eastern European Surveillance on Antimicrobial Resistance (CAESAR) network, vancomycin resistance rates in *Enterococcus* species isolated from blood cultures were 1% in *E. faecalis* and 14% in *E. faecium* (19). In our study, the isolated *Enterococcus* species consisted of *E. faecium* and *E. faecalis*, with vancomycin resistance rates reported as aggregate data. The resistance rates we observed appear consistent with CAESAR data, considering that *E. faecium* isolates are more frequently implicated in hospitalized patients, particularly in ICUs (20). Furthermore, when clinicians suspect a BSI, they often initiate empirical antibiotic treatment, and frequently choose vancomycin to target GPB. Given the established link between antibiotic use and the development of resistance, the observed increase in resistance during the pandemic likely is attributed to the increased use of vancomycin.

Carbapenems are critical antimicrobials used in the empirical treatment of GNB-related BSIs in ICUs. Unfortunately, the emergence of carbapenem-resistant *Pseudomonas* and *Acinetobacter* species has become a significant public health issue globally (21). Bayraktar (20) reported meropenem resistance rates in ICU isolates collected between 2022 and 2023 as follows: 96.2% for *A. baumannii*, 45.5% for *K. pneumoniae*, 25.0% for *P. aeruginosa*, and 8.7% for *E. coli*. Similarly, Albayrak et al. (22) observed imipenem and meropenem resistance in *A. baumannii* isolates from blood cultures at a rate of 95% in 2017, 81% in 2018, and 90% in 2019. Another study by Çınar et al. (23) assessed febrile neutropenia episodes in patients with hematologic malignancies from 2019 to 2021, finding imipenem and meropenem resistance rates of 79.1% for *A. baumannii*, 48.1% for *K. pneumoniae*, 45.9% for *P. aeruginosa*, and 29.5% for *E. coli*. Arslan et al. (5) examined carbapenem resistance before and after the COVID-19 pandemic and reported increases across several species, including *Klebsiella* spp. (75% to 79.4%), *Acinetobacter* spp. (imipenem: 88.2% to 92.9%, meropenem: 88.2% to 85.7%), *Pseudomonas* spp. (0% to 60%), and *E. coli* (imipenem: 9.1% to 40%, meropenem: 27.3% to 53.3%).

In our study, *A. baumannii* showed the highest resistance to carbapenems. Resistance rates dropped from 100% and 92.9% before the pandemic to 88.6% and 87.5% after the pandemic in ICU-1 and ICU-2, respectively, and this reduction was statistically significant. For *P. aeruginosa*, imipenem resistance in ICU-1 increased from 46.2% to 57.1%, and meropenem resistance increased significantly from 38.5% to 57.1%. In ICU-2, although resistance rates were lower, a non-significant increase was observed. For *K. pneumoniae*, resistance rates increased significantly, from 21-29% before the pandemic to 45-50% after the pandemic in ICU-1 and from 25-34% to 63-75% in ICU-2. For *E. coli*, carbapenem resistance in ICU-1 increased significantly from a pre-pandemic range of 18-27% to 40% during the pandemic. In ICU-2, resistance rose from 19-25% to 29%, but this increase was not statistically significant. We attribute the observed rise in resistance to the increased use of carbapenems during the pandemic. This highlights the necessity of avoiding unnecessary and prolonged antibiotic use in hospitalized patients to mitigate the development of resistance.

The increasing prevalence of carbapenemase-producing GNB infections and the associated treatment challenges have brought colistin back into focus, despite its historical decline in use due to side effects. Although reserved as a last-line therapy, resistance to colistin is rising globally and in Turkey. According to EUCAST recommendations, colistin susceptibility testing should be performed using the broth microdilution method for reliable results (24,25). Recent reports from centers in Turkey using this method indicate colistin resistance rates of 16.7-41.7% for *K. pneumoniae*, 0-8.2% for *A. baumannii*, and 0-12.5% for *P. aeruginosa* (26-29). Süzük Yıldız et al. (30) conducted a study involving 28 hospitals from level-II statistical regions in Türkiye, reporting colistin resistance rates of 8.7% for *E. coli* and 28.4% for *K. pneumoniae* in 2019. Global studies have documented colistin resistance rates of 10.0-19.9% in *Enterobacterales,* 2.5-4.0% in *A. baumannii*, and 1.0-5.0% in *P. aeruginosa* (31-35). In our study, colistin resistance was not detected in *E. coli* isolates, while rates for *K. pneumoniae* and *P. aeruginosa* were consistent with the literature. However, post-pandemic resistance rates for *A. baumannii* increased to 14.3% in ICU-1 and 25.0% in ICU-2. Resistance rates for colistin increased significantly with the pandemic in all isolates except for *P. aeruginosa* in ICU-1. The highest colistin resistance after the pandemic was observed in *K. pneumoniae* isolates from ICU-1 and *A. baumannii* isolates from ICU-2. These findings underscore the critical need to address colistin resistance in our hospital and suggest that resistance may become a severe issue if preventive measures are not implemented.

Furthermore, the pandemic has likely contributed to the increase in resistance rates, complicating the treatment of carbapenem-resistant GNB infections (24-25).

Study Limitations

The retrospective design of our study, the small number of microorganism species included, and the lack of access to clinical information about the patients are the limitations of the study.

CONCLUSION

In conclusion, BSIs are a significant cause of mortality among hospitalized patients, necessitating the rapid initiation of pathogen-specific empirical treatment. The most important reason for antibiotic resistance is the long-term use of antibiotics, which creates a vicious circle and makes treatment difficult. During the COVID-19 pandemic, the widespread use of broad-spectrum antibiotics and prolonged ICU stays affected blood culture and antibiogram results. In our study, pathogen distribution shifted during the pandemic, with resistance rates increasing even for last-resort antibiotics such as vancomycin and colistin. To reduce antibiotic resistance rates in our hospital, adherence to restricted antibiotic policies is essential. Additionally, enhancing infection control measures is critical to prevent the spread of resistant microorganisms.

Ethics

Ethics Committee Approval: Ethics Committee approval Tekirdag Namik Kemal University, Non-Interventional Clinical Research Ethics Committee, (decision number: 2023.133.06.19, date: 23.06.2023).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Concept: B.E., H.D., Design: B.E., B.B., H.D., N.K., Y.U., Data Collection or Processing: B.E., B.B., Analysis or Interpretation: B.E., H.D., Literature Search: B.E., H.D., Writing: B.E., H.D., N.K., Y.U.

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