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RESEARCH

Prevalence and resistance trends of Gram positive cocci *Staphylococcus aereus* and *Enterococcus* spp. in a tertiary care hospital

Üçüncü basamak bir hastanede Gram pozitif koklar *Staphylococcus aereus* ve *Enterococcus spp.* prevalansı ve direnç eğilimleri

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Abstract

Purpose: Antimicrobial resistance is a silent pandemic. In this study, it was aimed to determine the distribution and resistance trends of Staphylococcus aureus (S. aureus), Enterococcus faecium (E. faecium) and Enterococcus faecalis (E. faecalis) isolates, which are among the priority pathogens of the World Health Organization in bloodstream infections.

Materials and Methods: This was a retrospective study conducted between January 1, 2021 and March 31, 2023. The blood cultures (n=1261), of which microbiologically tested by using an automated system, were screened in the relevant period via laboratory information system.

Results: Of the total 941 isolates, 51.9% were S. aureus (n=488), 22.9% were E. faecalis (n=216), and 25.2% were E. faecium (n=237). Of the patients, 89.1% were inpatients (n=838), and 10.9% were outpatients (n=103). The most of patients were hospitalized in internal medicine departments (53.3%) and in intensive care units (35%). Among S. aureus isolates, the resistance rate to benzylpenicillin was 93.8%, to methicillin 44.3%, to teicoplanin 3.7%, and to linezolid 0.4%. Vancomycin and tigecycline resistances were not detected. Of the E. faecalis isolates, 5.5% were resistant to ampicillin, 3.3% to vancomycin, 3.2% to linezolid, and 0.7% to tigecycline. The resistance rates for E. faecium strains were 90.9% for ampicillin, 28.7% for vancomycin, 17.7% for tigecycline, 4.3% for linezolid, and 0.8% for teicoplanin. When the susceptibilities of all three bacteria were compared, a statistically significant difference was found between teicoplanin, vancomycin, tigecycline and linezolid susceptibilities. The methicillin resistance was higher in internal medicine and intensive care units. The levofloxacin and vancomycin resistance were more prevalent in intensive care units.

Öz

Amaç: Antimikrobiyal direnç sessiz bir salgındır. Bu çalışmada Dünya Sağlık Örgütü'nün kan dolaşımı enfeksiyonlarında öncelikli patojenler arasında yer verdiği Staphylococcus aureus (S. aureus), Enterococcus faecium (E. faecium) ve Enterococcus faecalis (E. faecalis) izolatlarının dağılımı ve direnç eğilimlerinin belirlenmesi amaçlandı.

Gereç ve Yöntem: Retrospektif olan bu çalışma, 1 Ocak 2021 ile 31 Mart 2023 tarihleri arasında gerçekleştirildi. Otomatik sistem kullanılarak mikrobiyolojik incelemesi yapılan kan kültürleri (n=1261) ilgili dönemde laboratuvarı bilgi sistemi ile tarandı.

Bulgular: Çalışmaya dahil edilen toplam 941 izolatın %51.9'u S. aureus (n=488), %22.9'u E. faecalis (n=216) ve %25.2'si E. faecium (n=237) idi. Hastaların %89.1'i yatan (n=838), %10.9'u ayaktan (n=103) hastalardı. Hastaların büyük çoğunluğu dahili servislerde (%53.3) ve yoğun bakım ünitelerinde (%35) yatmaktaydı. S. aureus izolatlarında benzilpenisiline direnç oranı %93.8, metisilin'e %44.3, teikoplanine %3.7 ve linezolide %0.4 direnç gösterdi. Vankomisin ve tigesiklin direnci saptanmadı. E. faecalis izolatlarının %5.5'inin ampisiline, %3.3'ünün vankomisine, %3.2'sinin linezolide ve %0.7'sinin tigesikline dirençli olduğu belirlendi. E. faecium suşlarında direnç oranları ampisilin için %90.9, vankomisin için %28.7, tigesiklin için %17.7, linezolid için %4.3 ve teikoplanin için %0.8 olarak belirlendi. Her üç bakterinin duyarlılıkları karşılaştırıldığında, teikoplanin, vankomisin, tigesiklin ve linezolid duyarlılıkları arasında istatistiksel olarak anlamlı farklılık bulunduğu saptandı. Metisilin direnci dahiliye ve yoğun bakım ünitelerinde daha yüksekti. Levofloksasin ve vankomisin direnci yoğun bakım ünitelerinde daha yaygındı.

Sonuç: Metisiline dirençli S. aureus (%44.3), vankomisin (%28.7) ve tigesikline (%17.7) dirençli E. faecium oranları

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Conclusion: MRSA (44.3%), vancomycin (28.7%) and tigecycline (17.7%) resistant E. faecium rates are well above the national data. The epidemiology on resistance trends, which will guide clinicians in the management of bloodstream infections, should be carried out periodically.
Keywords:. Enterococcus faecium, Enterococcus faecalis, antimicrobial resistance, methicillin resistance, vancomycin resistance, linezolid resistance, tigecycline resistance, MRSA, VRSA, VRE.

ulusal verilerin oldukça üzerindedir. Kan dolaşımı enfeksiyonlarının tedavisinde klinisyenlere yol gösterecek direnç eğilimlerine ilişkin epidemiyolojik çalışmaların periyodik olarak yapılması gerekmektedir.

Anahtar kelimeler: Enterococcus faecium, Enterococcus faecalis, antimikrobiyal direnç, metisilin direnci, vankomisin direnci, linezolid direnci, tigesiklin direnci, MRSA, VRSA, VRE.

INTRODUCTION

Antimicrobial resistance (AMR), which is a global public health problem, is a silent pandemic. It was estimated that there was a total of 6.22 million deaths in 2019, directly and indirectly due to the bacterial AMR[1]. According to the World Health Organization (WHO), AMR caused more deaths than tuberculosis and HIV/AIDS in the Western Pacific Region in 2020, and is expected to cause 5.2 million more deaths by 2030. Moreover, deaths due to AMR are close to those from diabetes, cirrhosis, and breast cancer when compared to the non-communicable diseases[2].

The pathogens with high priority AMR include methicillin-resistant or vancomycin-resistant *Staphylococcus aureus* (*S. aureus*) and vancomycin-resistant *Enterococcus faecium* (*E. faecium*) of Gram positive cocci[3]. According to the clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), cefoxitin (30µg) screening test zone diameter for methicillin-resistant *S. aureus* (MRSA) is >22 mm, minimum inhibitory concentration (MIC) is >2 mg/L for vancomycin-resistant *S. aureus* (VRSA), and MIC for vancomycin resistance in enterococci (VRE) is >4 mg/L[4].

It has been reported that MRSA isolates alone caused more than 100.000 deaths in 2019. The pathogens and rates vary from region to region according to socioeconomic income level. For instance, *S. aureus* is responsible for 26.1% of direct AMR-related deaths and 25.4% of AMR-associated deaths in high-income regions[1]. Bloodstream infections (BSI), which progress with high morbidity and mortality (14-37%), especially in intensive care units, can be controlled with early diagnosis, rational and appropriate antimicrobial therapy[5]. The most common Gram positive bacteria causing BSIs are *S. aureus* and enterococci[6]. The distribution of high priority pathogens with AMR varies geographically depending on income level, antibiotic use habits, health policies of countries and health status of the individuals.

In this study, it was aimed to determine the distribution and resistance trends of *S. aureus, E. faecium* and *E. faecalis* isolates, which are among the priority pathogens of the WHO, in BSIs. Thus, it was also aimed to support the practical applications of clinicians with the local data of a tertiary regional hospital, and to contribute to the surveillance studies.

MATERIALS AND METHODS

Study design and data collection

This retrospective observational microbiological study was carried out using the records of laboratory information system, regarding the time period between January 1, 2021 and March 31, 2023, corresponding to the blood cultures positive for *S. aureus, E. faecium* and *E. faecalis* isolates, at Cukurova University Faculty of Medicine Balcalı Hospital, which was a tertiary care hospital with 1150 beds at that period, before the 6th February Kahramanmaras Earthquake. The ethical approval for this study was obtained from Cukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (2023/53), and the study was conducted in accordance with the Declaration of Helsinki. The identification and antimicrobial susceptibility tests of the isolates, which have been carried out by microbiology specialist physicians and certified, experienced laboratory personnel, were retrospectively screened by comparing it with the Hospital Data Processing Unit records.

The inclusion criteria of the study: All *S. aureus, E. faecium* and *E. faecalis* isolated from at least two bottles of the blood culture set sent from various clinics in the relevant period (n=1261) were included. Only the first isolate per patient was included in the study. Isolates with different antibiotic susceptibility patterns from the same patient were considered as different individual isolates.

The exclusion criteria: The blood cultures, in which the same bacteria was isolated and had the same antibiotic susceptibility test (AST) result and duplicate isolates of the same patient up to 14 days (n=175) were excluded from the study. If *Bacillus* spp., *Corynebacterium* spp., micrococci, *Propniobacterium acnes* and coagulase negative Staphylococci, which belong to the skin microbiota, grew in only one of the blood samples taken from the same patient at the same time, this was considered as contamination and excluded (n=145). If the positive blood culture obtained on day ≥3 after hospital admission (nosocomial), it was considered as inpatient, and all others were accepted as outpatient.Since the study was retrospectively performed in vitro, and the personal data confidentiality was protected, there was no need to get signed informed consent.

Identification and susceptibility of relevant isolates

The blood cultures were inoculated and incubated in fully automated blood culture system BACTEC-FX (Becton Dickinson, USA) for five days. Specimens that had a signal of growth were examined by Gram staining and methylene blue staining under Biosafety Level-2 conditions. Then, they were passaged into the 5% sheep blood agar, MacConkey agar and chocolate agar media by single colony planting method, and incubated at 37°C for 24-48 hours. If Gram positive cocci were observed in Gram staining, but there was no growth in the culture, a vancomycin disc (5 µg) was placed in the blood agar for vacomycin-dependent enterococci.

The identification and ASTs of the isolates were performed using conventional methods, and the VITEK 2 Compact ID/AST (bioMeriéux, Marcy-l'Étoile, France) automated system. Cefoxitin disc (30 µg) was used to detect MRSA by Kirby-Bauer disc diffusion test, and the isolates with a zone diameter of <22 mm were considered as MRSA, and they were reported to be resistant to all beta-lactam antibiotics. The isolates with vancomycin resistance were confirmed by E-test (Lioflchem, Abruzzo, Italy). *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 strains were used as the quality control strains. All results were evaluated according to the EUCAST (2023 version) guidelines[4].

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics Version 20.0 (IBM Corp. Armonk, USA) statistical software package[7]. The categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and min-max where appropriate. Antimicrobial resistance profiles were compared according to gender, age groups, application date, clinics and status of patient by using Pearson Chi-Square Test or Fisher's Exact Test was used depending on whether the expected value problem arises or not. The statistical level of significance for all tests was considered to be 0.05.

RESULTS

The mean age of patients with BSIs due to the relevant Gram positive cocci (*S. aureus, E. faecalis, E. faecium*) (n=941) during the study period was 47.6±26.871 years, and the median age was 55.0 (range of 0-90 years). Of the patients, 44.2% were ≥60 years (n=416), 19.8% were 18-59 years old (n=331), and 20.6% were younger than 18 years (n=194). The female patients were 40.5% (n=381) and males were 59.5% (n=560). Of the total 941 isolates, 51.9% were *S. aureus* (n=488), 22.9% were *E. faecalis* (n=216), and 25.2% were *E. faecium* (n=237). Of the patients, 89.1% were inpatients (n=838), and 10.9% were outpatients (n=103). The patients were 53.3% (n=502) in internal medicine, 35% (n=329) in intensive care units and 11.7% (n=110) in surgery departments. 44.9% (n=422) of the strains were isolated in 2022, 43.0% (n=405) in 2021 and 12.1% (n=114) in 2023 (Table 1).

In the evaluation of AST results of *S. aureus* isolates, the resistance rate to benzylpenicillin was 93.8%, to methicillin (MRSA) 44.3%, to clindamycin 7.2%, and to erythromycin 0.4% among the primary reported antibiotics. Among the second line drugs, 3.7% resistance to teicoplanin, 2.1% to tetracycline, 13.6% to ciprofloxacin, 10.9% to levofloxacin and 15% to fusidic acid were detected. While no resistances to vancomycin and tigecycline were detected in *S. aureus* strains, 0.4% (2/488) resistance was observed to linezolid, which is the last-resort drugs (Table 2).

Of the *E. faecalis* isolates, 5.5% were resistant to ampicillin, 3.3% to vancomycin (7/216), 3.2% to linezolid (7/216) and 0.7% to tigecycline (1/216). There was no resistance to teicoplanin.

The AMR rates for *E. faecium* strains were 90.9% for ampicillin (189/237), 28.7% for vancomycin

(68/237), 17.7% for tigecycline (35/237), 4.3% for linezolid (10/237), 0.8% for teicoplanin (2/237).

When the susceptibilities of all three bacteria were compared, a statistically significant difference was found between teicoplanin, vancomycin, tigecycline and linezolid susceptibilities ($p<0.005$).In the comparison of demographic and clinical features with antibiotic resistance, it was observed that methicillin resistance was higher in internal medicine and intensive care units ($p<0.005$), levofloxacin and vancomycin resistance were more prevalent in intensive care units ($p<0.005$). In addition, it was found to be statistically significant that AMR was more frequent in the patients who were hospitalized at the time of BSI diagnosis (nosocomial BSI) compared to the patients who were diagnosed as outpatients ($p<0.005$) (Table 2).

Table 1. Species distribution and characteristics of *Staphylococcus aureus, Enterococcus faecalis* and *Enterococcus faecium* isolates.

Characteristics	Number	%
Number of isolates		
Staphylococcus aureus	488	51.9
Enterococcus faecalis	216	22.9
Enterococcus faecium	237	25.2
Total	941	100
Gender		
Female	381	40.5
Male	560	59.5
Age groups (years)		
0-2	78	8.3
3-17	116	12.3
18-44	161	17.1
55-59	170	18.1
60-69	175	18.6
≥70	241	25.6
Date		
2021	405	43.0
2022	422	44.9
2023	114	12.1
Clinical departments		
Intensive care unit	329	35.0
Internal medicine[a]	502	53.3
Surgical medicine[b]	110	11.7
Situation		
Inpatient	838	89.1
Outpatient	103	10.9

[a]Including departments of hematology, infectious diseases medicine, cardiology, gastroenterology, endocrinology, rheumatology, oncology, pediatrics, and dermatology.
[b]Including departments of urinary surgery, basic surgery, neurosurgery, orthopedics, and chest surgery.

Table 2. Data comparison between antimicrobial resistance profiles and characteristics in BSIs.

	Benzylpenicillin n[a] (%)	Ampicillin n (%)	Methicillin n (%)	Clindamycin n (%)	Erythromycin n (%)	Fusidic acid n (%)
Gender						
Female	160 (92.5)	83 (51.9)	74 (43.8)	33 (22.8)	2 (1.1)	21 (18.1)
Male	292 (94.5)	115 (54.2)	136 (44.6)	42 (17.6)	3 (0.9)	26 (13.2)
p value	0.433	0.675	0.923	0.233	0.877	0.255
Age groups						
0-2	30 (100.0)	19 (55.9)	23 (79.3)	7 (30.4)	0 (0.0)	4 (21.1)
3-17	87 (93.5)	12 (63.1)	41 (44.1)	17 (23.3)	0 (0.0)	16 (26.2)
18-44	102 (92.7)	20 (46.5)	47 (43.9)	12 (14.3)	0 (0.0)	15 (21.7)
45-59	82 (95.3)	37 (52.9)	26 (31.3)	7 (9.9)	2 (2.3)	2 (3.3)
60-69	70 (98.6)	46 (56.1)	40 (57.1)	20 (34.5)	0 (0.0)	4 (9.3)
>70	81 (88.0)	64 (51.6)	33 (35.9)	12 (16.0)	3 (3.1)	6 (9.8)
p value	0.055	0.574	-[d]	-	0.159	0.000
Date (year)						
2021	189 (92.2)	69 (58.0)	93 (45.8)	22 (10.6)	0 (0.0)	9 (11.3)
2022	199 (93.9)	106 (52.0)	91 (44.2)	11 (5.2)	5 (2.2)	32 (16.9)
2023	64 (98.5)	23 (46.9)	26 (40.0)	35 (7.2)	0 (0.0)	47 (15.0)
p value	0.180	0.372	0.719	0.046	0.077	0.515
Clinics						
ICU[b]	108 (96.4)	97 (53.9)	62 (55.9)	16 (14.2)	3 (2.4)	8 (10.5)
IM[c]	283 (91.6)	74 (48.7)	114 (54.3)	16 (5.2)	2 (0.6)	34 (17.3)
Surgery	61 (100.0)	27 (67.5)	34 (16.2)	3 (4.9)	0 (0.0)	5 (12.2)
p value	0.014	0.102	0.001	0.009	0.212	0.365
Situation						
Inpatients	375 (94.2)	194 (54.3)	167 (42.8)	59 (18.7)	5 (1.2)	33 (12.9)
Outpatient	77 (91.7)	4 (26.7)	43 (51.2)	16 (23.2)	0 (0.0)	14 (24.6)
p value	0.453	0.061	0.183	0.404	0.595	0.038
Isolates						
S. aureus	452 (93.8)	-	210 (44.3)	35 (7.2)	2 (0.4)	47 (15.0)
E. faecalis	-	9 (5.5)	-	-	-	-
E. faecium	-	189 (90.9)	-	-	-	-

Table 2 (continued). Data comparison between antimicrobial resistance profiles and characteristics in BSIs.

	Ciprofloxacin n (%)	Levofloxacin n (%)	Tetracycline n (%)	Teicoplanin n (%)	Vancomycin n (%)	Tigecycline n (%)	Linezolid n (%)
Gender							
Female	59 (46.5)	97 (33.3)	6 (3.4)	5 (1.3)	35 (9.2)	17 (5.6)	9 (2.4)
Male	54 (26.5)	126 (30.1)	4 (1.3)	15 (2.7)	40 (7.2)	19 (4.2)	10 (1.8)
p value	0.000	0.367	0.181	0.133	0.271	0.487	0.638
Age groups							
0-2	6 (21.4)	16 (27.6)	3 (8.8)	0 (0.0)	10 (12.8)	1 (1.8)	0 (0.0)
3-17	7 (15.9)	9 (10.1)	0 (0.0)	0 (0.0)	11 (9.5)	0 (0.0)	3 (2.6)
18-44	12 (15.4)	24 (21.4)	0 (0.0)	5 (3.1)	7 (4.3)	2 (1.5)	5 (3.1)
45-59	15 (35.7)	45 (34.6)	3 (3.5)	4 (2.4)	13 (7.7)	10 (7.6)	1 (0.6)
60-69	34 (48.6)	40 (31.0)	1 (1.4)	1 (0.6)	18 (10.4)	16 (10.9)	4 (2.4)
>70	39 (56.5)	89 (46.3)	3 (3.3)	8 (3.3)	16 (6.7)	7 (3.5)	6 (2.8)
p value	-[a]	-	0.009	0.292	-	-	0.453
Date (year)							
2021	105 (32.9)	58 (25.1)	4 (2.0)	5 (1.2)	32 (8.0)	18 (6.3)	9 (2.2)
2022	8 (72.7)	133 (36.3)	6 (2.8)	10 (2.4)	41 (9.7)	15 (4.2)	9 (2.2)
2023	0 (0.0)	32 (28.3)	0 (0.0)	5 (4.4)	2 (1.8)	36 (4.8)	1 (0.9)
p value	0.130	0.012	0.453	0.126	0.010	0.293	0.767
Clinics							
ICU[b]	48 (42.5)	108 (42.2)	6 (5.3)	8 (2.4)	43 (13.2)	20 (7.3)	10 (3.1)
IM[c]	53 (30.6)	94 (24.7)	4 (1.3)	10 (2.0)	27 (5.4)	14 (3.6)	8 (1.6)
Surgery	12 (26.7)	21 (28.4)	0 (0.0)	2 (1.8)	5 (4.5)	2 (2.4)	1 (0.9)
p value	0.067	0.000	0.028	0.305	0.000	0.061	0.316
Situation							
Inpatients	109 (36.6)	211 (33.5)	9 (2.2)	19 (2.3)	75 (100.0)	36 (5.4)	19 (2.3)
Outpatient	4 (12.1)	12 (15.0)	1 (1.2)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
p value	0.004	0.001	1.000	0.746	0.000	0.026	0.254
Isolates							
S. aureus	26 (13.6)	44 (10.9)	10 (2.1)	18 (3.7)	0 (0.0)	0 (0.0)	2 (0.4)
E. faecalis	-	-	-	0 (0.0)	7 (3.3)	1 (0.7)	7 (3.2)
E. faecium	-	-	-	2 (0.8)	68 (28.7)	35 (17.7)	10 (4.3)

[a,b,c] n; Number; ICU; Intensive care unit. IM; Internal medicine. BSI: Blood stream infection.
[d] No statistics was computed because the variable was a constant or susceptibility testing is not recommended.

DISCUSSION

This was a study that determined the incidence and resistance trends of *S. aureus, E. faecalis* and *E. faecium* strains isolated from blood cultures in a tertiary hospital serving as a regional hospital. BSIs, which progress with high morbidity and mortality (14-37%), especially in intensive care units, can be controlled with early diagnosis and appropriate treatment[5].

It was previously reported that *S. aureus* was both the most frequently isolated cause of BSI in many countries, and that MRSA isolates are the significant factor associated with the higher mortality among all of the pathogens[6-8]. As a member of the human microbiota, *S. aureus* can easily cause BSIs by colonizing 25-32% of patients, especially in intensive care units, under certain conditions[9,10]. Therefore, it was not surprising that the causative agents of BSIs in our study were *S. aureus* (51.9%), *E. faecium* (25.2%) and *E. faecalis* (22.9%), in order of frequency. In the studies conducted in Türkiye, it was reported that

Gram positive cocci are at a rate of 28.1-71.1% among BSI agents, of which 2.7-25% was *S. aureus*, 0.7-4.0% *E. faecalis*, 1.7-4.5% *E. faecium*[11-15]. Since the epidemiological data of the pathogens can be affected by the time, geographical location, treatment approaches, hospital flora and personal factors of the patients, the rates of their incidences may differ.

It was previously reported that the incidence of *S. aureus* associated BSIs was high in Australia, especially in men over 60 years of age, and in another study, being over 70 years of age with MRSA was a significant risk factor for 30-day mortality[16,17]. Similarly, in our study, 59.5% of the patients with BSI were male. And 34.8% of the patients with MRSA were over 60 years of age, and 44.6% of them were also male. In addition, the majority of these patients (55.9%) were under treatment in the intensive care unit. It has been reported in previous studies that being in the intensive care unit will adversely affect both the risk of conversion to BSI of colonization, morbidity and mortality, since it indicates the presence of intravascular medical equipment applications, intensive drug therapy applications, and possibly underlying comorbidities that may be immunosuppressive[5,17-20].

In this study, the rate of methicillin resistance (MRSA) in *S. auerus* isolates was 44.3%. While MRSA was below 5% in European countries such as Finland, Netherlands, Norway, ≥25% MRSA was reported in Belarus, Croatia, Greece, Italy, North Macedonia, Portugal, Romania and Serbia, including Türkiye. In Türkiye, MRSA rates showed an increasing trend between 2016 and 2020, and were reported as 22.7%, 25.8%, 29.6%, 31.3% and 33.4%, respectively[21]. Recently, the WHO published that the rate of MRSA in invasive samples in Türkiye was 30.7%[22]. The incidence of MRSA in our hospital was higher than these rates. International reports may lag behind real-life data. When local and multicenter epidemiological data are documanted at regular intervals, we may be able to determine the real-life rates. As MRSA rates increase, there is a parallel increase in the use of vancomycin, one of the last-choice drugs, and there may be a risk of developing VRSA, which has not yet been reported in Türkiye, including this study[23]. It was previously reported that the prevalence of VRSA between 2000 and 2019 in the world was 6%, and the rate of VRSA was 1.2% before 2010, while it was 2.4% with a 2-fold increase after 2010[24,25]. The prevalence of VRSA was reported to be higher in Asian countries such as Iran and India, where 67% of the isolates belong, and this may be due to the high number of developing countries in Asia, the high population density, inadequate hygiene habits and the difference in antimicrobial consumption habits. The high human mobility between countries in recent years may be a risk factor for the spread of resistance worldwide.

In the recent COVID-19 Pandemic, it was reported that when the patients infected with SARS-CoV-2 were co-infected with *S. aureus*, especially when exposed to mechanical ventilation and/or receiving steroid therapy, the risk of mortality increased, and even the 30-day mortality was 67%[26,27]. Local epidemiological studies are needed in this regard.

Enterococci are the second most common bacterial species isolated from BSIs among Gram positive cocci. The most important issue in enterococcal infections is AMR[21,28-29]. Enterococci are intrinsically resistant to many antibiotics (fusidic acid, cephalosporins, low-level aminoglycosides, macrolides, sulfonamides) which are commonly used in the treatment of other Gram positive cocci (such as staphylococci and streptococci). The management of enterococcal infections becomes difficult, especially when there is a high resistance to ampicillin and vancomycin in *E. faecium* isolates[29-31]. The cornerstone of treatment for enterococcal infections is ampicillin, but most strains of *E. faecium* exhibit high levels of penicillin resistance due to expression of low-affinity penicillin-binding protein (PBP) 5. In this study, *E. faecium* and *E. faecalis* isolates had an ampicillin resistance rate of 90.9% and 5.5%, respectively. Although ampicillin resistance was found to be lower in *E. faecalis* strains, active β-lactams have low affinity for *E. faecalis* PBPs resulting with low bactericidal activity, and thus, this leads to the treatment failure in bacteremia with high bacterial load[29]. In this study, AMR rates were found to be higher in *E. faecium* isolates compared to *E. faecalis* strains (Table 2).

The main mechanism of VRE is a change in the target site of the compound and decreased affinity of the drug[32]. VRE is more common in *E. faecium* strains, as in this study. It was previously reported that vancomycin resistance in *E. faecium* isolates increased from 11.6% in 2016 to 16.8% in 2020 in Europe, and increased from 14.6% to 15.8% in Türkiye, in the relevant years[21.22]. The rate in our hospital (28.7%) was higher than these reports, which can be explained by the diversity of the hospital flora and the majority of intensive care unit patients. It may also be due to

the fact that local real-life data may differ from the international epidemiological data.

The main mechanism of resistance to linezolid, which is one of the last treatment options in enterococcal infections, is 23S rDNA mutation in the genome and other point mutations with genetic recombination[33]. Exposure to the drug itself, prolonged use of antibiotics, hospitalization in hematology or intensive care units, and immunodeficiency are some risk factors for the development of resistance to linezolid[31]. In studies conducted since the early 2000s, the incidence of linezolid resistance has been reported to be between 0.16-5.2%, especially for *E. faecium* strains[31,34]. It was reported that the first case in Türkiye developed in 2012, in the third week of linezolid administration with the diagnosis of VRE coinfection, in a 66-year-old MRSA patient treated in the intensive care unit[35]. In a study conducted in Izmir, it was reported that linezolid resistances in *E. faecalis* and *E. faecium* isolated from various clinical specimens were 1% and 6%, respectively[36]. In this study, linezolid resistances were found to be 3.2% and 4.3% for *E. faecalis* and *E. faecium*, respectively, which is consistent with these literature data. Linezolid resistance has not yet been included in international surveillance studies as it appears sporadically[37,38].

Although tigecycline resistance, which occurs with overexpression of efflux pumps, especially in multidrug-resistant bacteria, is rare, making patient management difficult[39,40]. Tigecycline resistance in the world was reported as 0.2-0.16% for *E. faecalis* and 0.15-0.5% for *E. faecium* isolates[31,41]. In this study, tigecycline resistance was detected in one *E. faecalis* strain and 17.7% of *E. faecium* isolates, which is quite high for *E. faecium*. This may be due to the fact that international and local epidemiological data are not followed up to date, or it may be due to the fact that our data are the results of an automated system and not verified by an advanced diagnostic method.

In this study, the identification and AST of the isolates were based on the results of an automated system, and due to the retrospective nature of the study, confirmation could not be made with a molecular method such as sequencing analysis. The limitations of the study were that this was a single-center study, the lack of the clinical data of the patients and clinical outcomes, the lack of data on daptomycin, and the lack of antibiotic resistance of enterococcal strains other than *E. faecalis* and *E. faecium*. Both direct and indirect effects of empirical, prophylactic and therapeutic antimicrobial drug use should be revealed and followed up with genotypic epidemiological studies.

In conclusion, The rates of *S. aureus* (51.9%), *E. faecium* (25.2%) and *E. faecalis* (22.9%) as BSI agents were found to be higher in this study than the rates previously reported in Türkiye. Since the epidemiological data of the pathogens can be affected by the time, geographical location, treatment approaches, hospital flora and individual factors, the rates of incidences may differ.

The incidence of MRSA (44.3%) in this study was higher than the recent WHO report on MRSA in Türkiye (30.7%), which may lag behind real-life data. The incidence of VRE was also found to be higher in this study (28.7%) compared to the recent WHO report (15.8%). These rate differences point to the importance of evaluating and documenting local and national epidemiological studies at regular intervals. The presence of VRSA has not yet been reported in Türkiye, including this study. However, 2.4% VRSA has been reported worldwide, and given the significant increase in human mobility between countries in recent years, it may be good to consider that AMR may spread to countries with negative resistance.

In this study, linezolid resistances were found to be 3.2% and 4.3% for *E. faecalis* and *E. faecium*, respectively. Tigecycline resistance was quite high (17.7%) than the international rates for *E. faecium* isolates. This may be due to the fact that international and local epidemiological data are not followed up to date, or it may be due to the fact that our data are the results of an automated system and not verified by an advanced molecular diagnostic method. Nevertheless, teicoplanin, vancomycin, tigecycline and linezolid are still the most potent antibiotics for resistant *S. aureus, E. faecalis* and *E. faecium* isolates. These and similar epidemiological studies, which will guide clinicians in the management of bloodstream infections, should be carried out periodically, resistance trends and changes should be documented, and laboratory infrastructure should be at a level to allow advanced diagnostic molecular methods.

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