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Evaluation of antibiotic susceptibility in enterococci isolated from blood culture samples

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ABSTRACT

Aims: Increased vancomycin resistance in enterococci is an important cause of life-threatening bloodstream infections in hospitalized patients. The aim of this study is to determine the antibiotic susceptibility rates of *Enterococcus* strains isolated from blood cultures in hospitalized patients.

Methods: The antibiotic resistance rates of *Enterococcus* strains isolated from blood cultures of patients hospitalized in the service and intensive care units (ICU) between 1 January 2018 and 30 December 2022 were examined retrospectively. Blood samples were studied with the BacT/ALERT 3D culture system (Biomerieux, France). Bacterial identification was performed using conventional methods, Matrix Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) and VITEK 2 (Biomerieux, France) systems. Antimicrobial susceptibility tests were performed with VITEK 2 (Biomerieux, France) systems. Ampicillin, vancomycin, teicoplanin, high-level gentamicin resistance (HLGR) and linezolid susceptibility of isolated strains were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. Vancomycin minimal inhibitory concentration (MIC) values of vancomycin resistant strains were studied by microdilution gradient strip test (Bioanalyse).

Results: A total of 623 strains of enterococci were isolated from blood culture samples. Of the enterococci, 305 (48.9%) were identified as *Enterococcus faecalis*, 281 (45.6%) *Enterococcus faecium*, 12 (1.9%) *Enterococcus avium*, 11 (1.8%) *Enterococcus gallinarum*, 7 (1.2%) *Enterococcus casseliflavus*, 2 (0.4%) *Enterococcus durans* and 1 *Enterococcus hirae* (0.2%). Ampicillin and HLGR resistance rates of isolated *E. faecalis* strains were 11 (3.6%) and 72 (23.6%), respectively, and all strains were found to be susceptible to vancomycin, teicoplanin and linezolid. The ampicillin, vancomycin, teicoplanin and HLGR resistance rates of *E. faecium* strains were determined as 229 (81.5%), 36 (12.8%), 30 (10.7%) and 142 (50.5%), respectively, and all strains were found to be susceptible to linezolid.

Conclusion: In infections caused by enterococci, identification and determination of antibiotic susceptibility rates according to culture antibiogram results would be the right approach. Knowing the current susceptibility rates of enterococci isolated from blood culture samples in our hospital will contribute for clinicians' planning of empirical treatment.

Keywords: Enterococci, blood culture, antibiotic susceptibilities

INTRODUCTION

Enterococci are found as a flora element in human intestines. *Enterococcus faecalis* is the most common human fecal *Enterococcus* species. It can colonize the oropharynx, vagina, and skin. Because of a member of the normal intestinal flora, it causes hospital and community-acquired infections. They cause colonization, bacteremia, peritonitis, endocarditis, wound and urinary tract infections, especially in hospitalized patients.

Protein and carbohydrate virulence factors play major role in the pathogenesis of enterococcal infections. Aggregation substance released by enterococci is responsible for its attachment to heart valves and renal cells. Enterococci colonize the urinary tract, heart valves, and catheters by producing biofilms.[1,2] Although such virulence factors are more common in *Enterococcus faecalis* (*E. faecalis*) strains in the hospital setting, *Enterococcus faecium* (*E. faecium*) strains are more resistant to antibiotics.[3] Depending on the geographical location, most of the nosocomial infections due to VREs are caused by *E. faecium* and only 2-20% of them are caused by *E. faecalis* strains.[4]

In enterococcal infections, penicillin G, ampicillin, vancomycin and teicoplanin are used as cell wall-active bacteriostatic antibiotics. Gentamicin is included in the treatment as a bactericidal agent. Enterococci are intrinsically resistant to trimethoprim-sulfomethoxazole (TMP-SXT), penicillin, cephalosporin, lincosamide, and aminoglycosides.[5]

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Resistance rates in bloodstream infections caused by *E. faecalis* and *E. faecium* are increasing in hospitals and can progress with high morbidity and mortality rates. Therefore, considering the regional resistance data, appropriate and rational use of antibiotics will affect the patient's prognosis.[6]

The aim of this study is to determine the antibiotic susceptibility rates of *E. faecalis* and *E. faecium* strains isolated from the blood cultures of patients hospitalized in our hospital between 2018-2022.

METHODS

The study was carried out with the permission of Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date 08.06.2023, Decision No: 8/27) All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Antibiotic resistance rates of *Enterococcus* strains isolated from blood culture samples of patients hospitalized in the service and intensive care units (ICU) of our hospital between January 1, 2018 and December 30, 2022 were examined retrospectively. Blood samples were incubated with the BacT/ALERT 3D culture system (Biomerieux, France) for 5-7 days. Gram stain was done for each sample during routine culture procedures. In addition, the samples were inoculated on 5% sheep blood agar (BA), chocolate agar (CA) and Eosin Methylene Blue agar (EMB) (RTA, Türkiye) and incubated at 37°C for 18-24 hours. Bacterial identification was performed using conventional methods, Matrix Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometer (MALDI-TOF MS) and VITEK 2 (Biomerieux, France) systems. Antimicrobial susceptibility tests were performed with VITEK 2 (Biomerieux, France) systems in line with the manufacturer's recommendations. Ampicillin, vancomycin, teicoplanin, high-level gentamicin resistance (HLGR) and linezolid susceptibility of isolated strains were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. Vancomycin minimal inhibitory concentration (MIC) values of strains found to be resistant to vancomycin were studied by microdilution gradient strip test (Bioanalysis) method.

Statistical Analysis

Statistical Packages for the Social Sciences software version 22.0 (SPSS Inc., Chicago, USA) was used in the statistical analysis of the study. Antibiotic susceptibility results by years were analyzed by the Chi-square test. The degree of statistical significance (p-value) was determined as 0.05 in all analyses.

RESULTS

A total of 623 strains of enterococci were isolated from blood culture samples. Of the enterococci, 305 (48.9%) were identified as *Enterococcus faecalis*, 281 (45.6%) *Enterococcus faecium*, 12 (1.9%) *Enterococcus avium*, 11 (1.8%) *Enterococcus gallinarum*, 7 (1.2%) *Enterococcus casseliflavus*, 2 (0.4%) *Enterococcus durans* and 1 *Enterococcus hirae* (0.2%). Ampicillin and HLGR resistance rates of isolated *E. faecalis* strains were 11 (3.6%) and 72 (23.6%), respectively, and all strains were found to be susceptible to vancomycin, teicoplanin and linezolid. The ampicillin, vancomycin, teicoplanin, and HLGR resistance rates of *E. faecium* strains were determined as 229 (81.5%), 36 (12.8%), 30 (10.7%) and 142 (50.5%), respectively, and all strains were found to be susceptible to linezolid (Table 1). The MIC values of the strains found to be resistant to vancomycin with the automated system VITEK 2 were determined as >256 µg/ml with the microdilution gradient strip test. The distribution of the isolated *E. faecalis* and *E. faecium* strains according to the clinics they were isolated from is given in Table 2 and the distribution of resistance rates by years is given in Table 3.

Table 1. Resistance rates of *E. faecalis* and *E. faecium* strains

Antibiotics	*E. faecalis* (n: 305) (%)	*E. faecium* (n: 281) (%)	Toplam (n: 586) (%)
Ampicillin	11 (3.6)	229 (81.5)	240 (40.9)
Vancomycin	0	36 (12.8)	30 (6.1)
Teicoplanin	0	30 (10.7)	30 (5.1)
HLGR	72 (23.6)	142 (50.5)	214 (36.5)
Linezolid	0	0	0
Total	305	281	586

Table 2. Distribution rates of *E. faecalis* and *E. faecium* strains according to clinics from which they are isolated

Clinics	*E. faecalis* (n: 305) (%)	*E. faecium* (n: 281) (%)	Toplam (n: 586) (%)
Intensive care unit	149 (48.8)	155 (55.1)	304 (51.8)
Internal diseases	38 (12.4)	42 (14.9)	80 (13.6)
Neurology	21 (6.8)	7 (2.4)	28 (4.7)
Infectious diseases	13 (4.2)	2 (0.7)	15 (2.5)
Pediatry	10 (3.2)	4 (1.4)	14 (2.3)
Urology	3 (0.9)	5 (1.7)	8 (1.3)
Brain surgery	3 (0.9)	3 (1.0)	6 (1.0)
Cardiology	6 (1.9)	7 (2.4)	13 (2.2)
Gynecology	2 (0.6)	1 (0.3)	3 (0.5)
Cardiovascular surgery	10 (3.2)	4 (1.4)	14 (2.3)
General surgery	35 (11.4)	40 (14.2)	75 (12.7)
Thoracic surgery	10 (3.2)	2 (0.7)	12 (2.0)
Other clinics	5 (1.6)	9 (3.2)	4 (0.6)
Total	305	281	586

In our study, an increase was observed in ampicillin, HLGR, vancomycin and teicoplanin resistance over the years, and the difference between the resistance rate for teicoplanin in 2022, which was 23%, from other years was statistically significant (p:0.001).

Table 3. Distribution of *E. faecalis* and *E. faecium* strains isolated from blood cultures and antibiotic resistance by years

	E. faecalis						*E. faecium*						p value
	2018 n:64	2019 n:31	2020 n:75	2021 n:66	2022 n:69	Total n:305	2018 n:43	2019 n:45	2020 n:61	2021 n:58	2022 n:74	Total n:281	
Ampicillin	2 (3.1)	4 (12.9)	0	0	5 (7.2)	11 (3.6)	38 (88.3)	36 (80.0)	50 (81.9)	42 (72.4)	69 (93.2)	235 (83.6)	0.236
Vancomycin	0	0	0	0	0	0	3 (6.9)	3 (6.6)	8 (13.1)	3 (5.1)	13 (17.5)	30 (10.6)	0.088
Teicoplanin	0	0	0	0	0	0	3 (6.9)	1 (2.2)	3 (4.9)	6 (10.2)	17 (22.9)	30 (10.6)	0.001
HLGR*	14 (21.8)	10 (32.2)	17 (22.6)	14 (21.2)	17 (24.6)	72 (23.6)	21 (48.8)	21 (46.6)	28 (45.9)	27 (46.5)	45 (60.8)	142 (50.5)	0.362
linezolid	0	0	0	0	0	0	0	0	0	0	0	0	

*HLGR: High-level gentamicin resistance.

DISCUSSION

Enterococci cause nosocomial infections due to their ability to spread easily through patients and healthcare personnel. The increased incidence and antimicrobial resistance in enterococcal infections are important problems.[7] Many antibiotics used in the treatment of gram-positive bacterial infections are not effective in enterococcal infections. Owing to these enterococci can develop antibiotic resistance to many antibiotics intrinsically and/or acquired mechanisms. In addition, enterococci have the ability to transfer this resistance to new generations.[8-10] The increase of vancomycin-resistant enterococci (VRE) strains is particularly important.[11] For this reason, local determination of antibiotic resistance of enterococcal strains at regular intervals will guide treatment planning.[12]

Çelik et al.[6] found ampicillin resistance in enterococci isolated from blood cultures 84.8% for *E. faecium* strains and 5.2% for *E. faecalis* between 2015-2017. They found 90.7% for *E. faecium* strains and 1.6% for *E. faecalis* between 2018-2020. In a meta-analysis study examining 291 studies from different parts of the world, ampicillin resistance was found to be 78% for *E. faecium* strains and 4% for *E. faecalis* in enterococci isolated from blood cultures.[13] In our study, the ampicillin resistance rate was 81.5% for *E. faecium* strains and 3.6% for *E. faecalis*, which is consistent with the literature.

In the 1980s, enterococci developed a resistance to beta-lactam antibiotics and aminoglycosides, and vancomycin was used instead.[5] Vancomycin and teicoplanin are antibiotics that show effective activity against both *E. faecalis* and *E. faecium*.[8] Vancomycin-resistant enterococci strains were reported for the first time in 1986 from France and England.[14] In our country, vancomycin resistance was first reported from rectal swab samples by Vural et al.[15] in 1998. Later, in studies conducted in different regions, increased vancosimine resistance was reported in *Enterococcus* strains over the years. Gök et al.[3] reported vancomycin resistance to *E. faecium* at a rate of 8.2% from blood and various samples of hospitalized patients from Konya in 2020. Çelik et al.[6] did not detect vancomycin resistance in *E. faecalis* strains isolated from the blood samples of hospitalized patients in 2021, but they reported 3.3% vancomycin resistance in *E. faecium* strains. Şanlı et al.[12] reported the rates of resistance to vancomycin as 1.5% and 32.1%, respectively, in *E. faecalis* and *E. faecium* isolated from the blood culture of ICU patients in the Istanbul region in 2022.

In our study, vancomycin and teicoplanin resistance was not detected in *E. faecalis* strains isolated from blood culture. Vancomycin and teicoplanin resistance rate was 10.7% in *E. faecium* isolates. MIC values of vancomycin-resistant *E. faecium* isolates were found to be >256 µg/ml. In addition to the variability in regional data, the steady increase in resistances over the years should be noted and monitored. While evaluating the results of our study, the data in our region revealed that it is necessary to be careful about the increase in resistance over the years. In the present study, especially vancomycin and teicoplanin resistance has increased due to their widespread use in hospital infections in recent years, and the resistance rate for teicoplanin in 2022, which was found to be 23%, was found to be statistically significant compared to other years (p:0.001).

High-level aminoglycoside resistance in enterococci is mediated by aminoglycoside-modifying enzymes.[16] In a study conducted in our country, the HLGR rates in *E. faecalis* and *E. faecium* isolated from blood cultures hospitalized in the ICU were found to be 39.4% and 74.3%, respectively.[12] In another study, HLGR rates of 37% in *E. faecalis* and 62% in *E. faecium* were found in blood and various body fluid samples from hospitalized patients.17 In the presented study, the resistance rates of HLGR in *E. faecalis* and *E. faecium* were found to be 23.6% and 50.5%, respectively.

Linezolid has a broad gram positive spectrum covering methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and penicillin-resistant *Streptococcus pneumoniae* strains. Linezolid

is a watch and reserve drug approved by the Food and Drug Administration (FDA) to treat serious infections (infective endocarditis, bacteremia, and central nervous system infections) caused by VRE with high levels of aminoglycoside resistance.[14] In our study, similar to some studies in our country, all enterococcal strains isolated from the blood cultures of hospitalized and ICU patients were found to be susceptible to linezolid.[6,18,19] For the first time in Turkey, Aktaş et al.[20] reported resistance to linezolid at a rate of 2% in *E. faecium* isolates in rectal swab samples taken from hospitalized patients at Istanbul University Faculty of Medicine. Although linezolid is an antibiotic that is widely used in resistant enterococcal infections and has high clinical success, it is not the antibiotic of first choice in bacteremia due to its bacteriostatic nature. This may be considered as one of the reasons for the high linezolid sensitivity rates detected in blood cultures.

CONCLUSION

Although nosocomial infections due to VRE species are increasing, vancomycin is still an effective antibiotic against enterococci. In addition, linezolid, to which all strains including VRE are susceptible, is a good alternative, Knowing the current susceptibility rates of enterococci isolated from blood culture samples in our hospital will contribute to clinicians' empirical treatment planning.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date 08.06.2023, Decision No: 8/27)

Informed Consent: Because the study was designed retrospectively, no written informed consent from was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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