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Pheno- and Genotypic Epidemiological Characterization of Vancomycin-Resistant *Enterococcus faecium* Isolates from Intensive Care Unit Patients in Central Türkiye

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Abstract

Vancomycin-resistant *Enterococcus faecium* (VRE) has been detected in Türkiye. Only limited information is available on its dissemination in the central regions of the country. This study describes the first epidemiological characterization of VRE clinical isolates detected in patients in a hospital in the province of Aksaray. In this one-year study conducted between 2021 and 2022, stool samples from intensive care unit patients were screened for VRE using the phenotypic E-test method, and the antibiotic sensitivity test was analyzed by using the VITEK® 2 system. A molecular assay for confirmation of species level was carried out by 16S rRNA gene-based sequencing and testing for antibiotic resistance (*van*A or *van*B) and virulence factor-encoding genes (*esp*, *asa1*, and *hyl*). Further, genotypic characterization was determined by macro-restriction fragment pattern analysis (MRFPA) of genomic DNA digested with *Sma*I restriction enzyme. Of the total 350 *Enterococcus* positive patients from different hospital intensive care units, 22 (6.3%) were positive for VRE using the phenotypic E-test method. All isolates showed resistance to ampicillin, ciprofloxacin, vancomycin, and teicoplanin and positive amplification for the *van*A gene. However, none of the isolates was positive for the *van*B gene. The most prevalent virulence gene was *esp*. The results indicate that the isolates are persistent in the hospital environment and subsequently transmitted to hospitalized patients, thus representing challenges to an outbreak and infection control. These study results would also help formulate more effective strategies to reduce the transmission and propagation of VRE contamination in various hospital settings.

K e y w o r d s: *Enterococcus faecium*, antimicrobial resistance, vancomycin resistance, *van*A gene, *van*B gene

Introduction

Enterococci, normal gut commensals, have been recognized as an important causative agent of nosocomial infections. The impact of these infections is augmented by the high rates of acquired resistance, including first-line antibiotics such as vancomycin (Woodford et al. 1995; Mellmann et al. 2000). In the last decades, vancomycin-resistant enterococci, *Enterococcus faecium* (VRE) in particular, are the most common cause of multi-drug-resistant infections, including bacteremia, urinary tract infection, and surgical site infection (Pinholt et al. 2014). However, *E. faecium* has developed globally into a widespread nosocomial pathogen that can adapt to healthcare conditions and develop resistance to glycopeptides (Bonten et al. 2001; Willems et al. 2005; Arias and Murray 2012; Cattoir and Leclercq 2013). In addition to the high costs associated with treating VRE, high mortality and morbidity from infections have been reported in both the USA and Europe (Reyes et al. 2016). In the European Union, an increasing proportion of VRE has been reported, with the national percentages for vancomycin resistance among *E. faecium* isolates reaching up to 56% (WHO

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and ECDC 2022). More than 80% of *E. faecium* isolates in the USA were resistant to vancomycin and ampicillin (Levitus et al. 2022). A similar rate of prevalence of vancomycin-resistant *E. faecium* in different regions of Türkiye has been reported by the Turkish Health Ministry (USHIESA 2021); nonetheless, data on clinical and molecular characterization of vancomycin-resistant *E. faecium* in hospitals are not well documented.

Therefore, the aim of the present study was to determine the point prevalence rate of enterococci colonization among hospitalized patients as well as the clinical and clonal relatedness of the vancomycin-resistance *E. faecium* isolates recovered between 2021 and 2022. The purpose thereof was to identify the sources and spread of VRE between various hospital units.

Experimental

Materials and Methods

Sampling and isolation of *E. faecium*. The study was carried out at the Aksaray University Training and Research Hospital in central Türkiye in the province of Aksaray. The patients aged 18 or older were admitted to the hospital between July 2021 and July 2022 and had given informed consent before being included in the study. A total of 5,932 intensive ward patients were enrolled in the study to assess the occurrence of enterococci. Stool samples were taken from patients at possibly the exact times, usually at the beginning of the patients' stay in the intensive care unit and screened for rectal colonization of VRE using a sterile rectal swab. One sample was taken from each patient. Specimens were plated on selective Esculin Agar (Standard Media, Türkiye), and the plates were incubated at 35 ± 2°C for 24 to 72 h. Based on colony morphology and gram staining reaction, putative enterococci culture was further inoculated on Columbia blood agar (Standard Media, Türkiye) containing sheep blood 5% and incubated aerobically at 35 ± 2°C for 24 h. A characteristic isolate representing the dominant type of colony morphology was taken from each sample plated agar plate. Subsequently, isolates were identified using the VITEK® 2 GP ID card (bioMérieux, France). The purified culture isolates were further processed for pheno- and genotypic analyses.

Antimicrobial susceptibility testing. Resistance to vancomycin was determined by susceptibility testing using the E-test (Bioanalyse Ltd., Türkiye). *E. faeceium* cultures were inoculated in phosphate-buffered saline (PBS, pH 7.2), equivalent to 0.5 McFarland opacity standard (Bioanalyse Ltd., Türkiye). The breakpoint for vancomycin susceptibility was set at 4 mg/ml. The reference quality control strains *E. faecalis* ATCC® 29212™ and *Staphylococcus aureus* ATCC® 29213™ were used as controls. Minimum inhibitory concentrations (MICs) were interpreted by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and breakpoint standards for *Enterococcus* spp. (EUCAST 2018). In addition, the MICs of ampicillin, ciprofloxacin, gentamicin (high level), vancomycin, teicoplanin, tigecycline, and linezolid were determined using the VITEK® 2 AST-GP640 test card (bioMérieux, France). All protocols were conducted following the manufacturer's instructions.

Extraction of total DNA. The total genomic DNA was extracted from single colonies of each isolate using the DNeasy Blood and Tissue Kit (QIAGEN, Germany) according to the manufacturer's recommendations. The DNA purity and quantity were performed using the NanoDrop™ One^{C} Spectrophotometer (Thermo Scientific™, Thermo Fisher Scientific, Inc., USA).

16S rRNA gene sequencing. To confirm the putative *E. faecium* isolates to species level, 16S rRNA gene-based sequencing was conducted. The PCR amplification of a 1,400-bp region of the 16S rRNA gene was performed using the oligonucleotide forward 16SUNI-L (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse 16SUNI-R (5'-AAGGAGGTGATCCAGCCGCA-3') primers (Kuhnert et al. 1996). The PCR reaction (total volume 50 µl) consisted of 0.5 µl AmpliTaq™ DNA Polymerase (1 U/µl; Applied Biosystems™, Thermo Fisher Scientific, Inc., USA), 0.25 µl of each primer (10 pmol/µl), and 2.5 µl of each deoxynucleoside triphosphate (2 µmol/l; MBI Fermentas, Germany), 16.5 µl of sterile Aqua dest., and 2.5 µl DNA template. The cyclic amplification was performed using a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA) according to the following program: initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s with a final extension for 5 min at 72°C. The PCR amplification products were separated by gel electrophoresis on 1.5% agarose gels, stained with ethidium bromide and visualized under UV light using the GelDoc system (Bio-Rad Laboratories, Inc., USA). Positive PCR products were purified using QIAquick® Gel Extraction Kits (QIAGEN, Germany) according to the manufacturer's recommendation and sequenced bidirectionally by Sanger-sequencing by Microsynth Seqlab GmbH (Germany). The obtained DNA sequences were edited and combined in Molecular Evolutionary Genetics Analysis software version 10 (MEGA X) (Kumar et al. 2018). The sequences were analyzed using a BLAST search against the available global sequence database for species-level identification. The multiple sequence alignment, including *E. faecium* reference, was carried out using the MegAlign program within DNAStar software version 7.1.0 (DNASTAR Inc., USA).

PCR-based detection of antibiotic resistance- and virulence factor-encoding genes. Gene-specific *van*A and *van*B-based PCR assays were carried out by amplifying 1030 bp of *van*A gene-specific primer pair (5'-CATGAATAGAATAAAAGTTGCAATA-3' and 5'-CCCCTTTAACGCTAATACGATCAA-3') and 433-bp of the *van*B gene using specific primer pair (5'-GTGACAAACCGGAGGCGAGGA-3' and 5'-CCGCCATCCTCCTGCAAAAAA-3') (Clark et al. 1993). The amplification reaction was performed with an initial incubation at 94°C for 10 min, followed by 30 cycles consisting of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s with a final extension at 72°C for 5 min. Virulence factors-coding genes for enterococcal surface protein (*esp*), aggregation substance (*asa1*), and hyaluronidase (*hyl*) were chosen for PCR amplification, following established methodologies as described previously (Vankerckhoven et al. 2004). As described above, PCR amplification products were subjected to agarose gel electrophoresis, visualized with ethidium bromide under UV light. DNA molecular size standards (100 bp) (Fermentas GmbH, Germany) were used to confirm the amplified product size.

Macro-restriction fragment pattern analysis (MRFPA). MRFPA of genomic DNA was performed, following the preparation of whole DNA in agarose gel plugs. Digestion was then carried out using *Sma*I restriction enzyme under previously described test conditions (Antonishyn et al. 2000). Subsequently, the pulsed-field gel electrophoresis was conducted using the CHEF-DR® II system (Bio-Rad Laboratories, Inc., USA). The fragment patterns were analyzed using BioNumerics version 6.6 software (Applied Maths, Belgium).

Statistical Analysis. The Fisher's exact test was used to compare the categorical variables. A probability $p < 0.05$ value was considered statistically significant.

Availability of GenBank 16S rRNA Gene Sequences and Accession Numbers. The partial 16S rRNA gene sequences are available in the NCBI GenBank with the accession numbers OP005485 to OP005506. The results of this study are supported by data available at reasonable request from the corresponding author.

Results

Isolation and identification of VRE. A total of 350 individual patients were positive for *Enterococcus*. The putative *Enterococcus* culture isolates were gram-positive cocci in chains and formed dark colonies on selective Esculine Agar medium (Standard Media, Türkiye). The isolates were catalase negative, and pyrase positive reactions were further confirmed by VITEK® 2 GP ID card. Of the total 350 putative *Enterococcus* culture isolates, 22 (6.3%) were positive for vancomycin resistance as confirmed by the phenotypic E-test method. The data showed that VRE culture isolates were recovered from 12 male and 10 female patients from various age groups. A high frequency (n = 14, 64%) was detected among patients in the age group between 61 and 80 years, followed by the age groups between 81 and 90 years (n = 3, 14%), and 51 and 60 years (n = 2, 9%) (Table I). However, one VRE isolate for each age group ranging between 21 and 30, 31 and 40, and 90 and 100 years was detected. The majority of patients (95.5%) positive for VRE infection were from intensive care unit (ICU) and internal medicine intensive care unit (IICU) wards.

Antimicrobial susceptibility testing. Antibiotic sensitivity profiling results mainly showed two antibiotic resistance patterns (I and II), with a total of four isolates having pattern I, whereas 18 had pattern II (Table I). Both antibiotic resistance patterns were detected on different hospital wards. As presented in Table I, of the total seven antibiotics tested, the isolates showed maximum resistance to four antibiotics, including 100% for ampicillin (MIC > 32), ciprofloxacin (MIC > 8 μg/ml), vancomycin, and teicoplanin (MIC > 256 μg/ml). A high (81.8%) frequency of gentamycin resistance (MIC ranging from < 128 to > 128 μg/ml) was recorded. However, none of the isolates was resistant to linezolid (MIC 2 μg/ml) and tigecycline (MIC ≤ 0.12 μg/ml). Based on these results, all isolates showed vanA phenotype (vancomycin-resistance with MIC ≥ 256 μg/ml and teicoplanin-resistance with MIC ranging from 8 to 56 μg/ml).

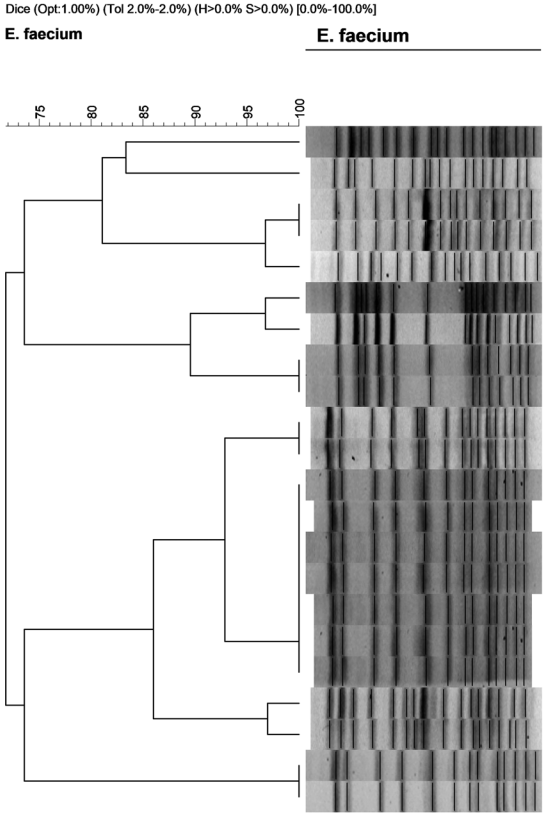
Partial 16S rRNA gene sequencing and amplification of antibiotic resistance and virulence genes. Of the 22 VRE positive culture isolates, one characteristic isolate representing the predominant type of colony morphology per sample selected was further identified by 16S rDNA sequencing. The BLAST search results of 16S rRNA gene analysis revealed that all isolates showed 100% similarity to *E. faecium*. The *E. faecium* sequences were deposited in the NCBI GenBank with the accession numbers OP005485 to OP005506. Similar to the phenotypic results, all 22 VRE isolates were positively amplified for the *van*A gene. However, the *van*B gene was not detected in these isolates. Regarding the targeted genes encoding enterococcal virulence factors, the *esp* gene was commonly detected in isolates (n = 15/22), while the *asa1* gene was only found in three isolates. Finally, none of the isolates tested positive for the *hyl* gene.

Macro-restriction fragment pattern analysis (MRFPA). For strain-level variation, the 22 isolates were further characterized by macro-restriction fragment pattern analysis using pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested genomic DNA. Digestion with the *Sma*I restriction enzyme produced six to 12 distinct and distinguishable DNA bands for the various isolates. The isolates were analyzed using

Table I
Relationship between MRFP types and antimicrobial susceptibility profiles of vancomycin-resistant *Enterococcus faecium* isolates (n = 22).

MRFP pattern	Number of isolates/patients[a]	Isolate code	Hospital ward[b]	Gender	Age	Date of admission	Diagnostics	Short-term outcome	Susceptibility profile[c]							Resistance pattern
									AMP	CIP	GN	TEC	VA	LNZ	TGC	
P1	2	EN2	ICU	female	79	25.08.2021	pneumonia, cerebral infarction	died	R	R	S	R	R	S	S	I
		EN5	IICU	male	57	06.10.2021	pneumonia, chronic obstructive pulmonary disease	died	R	R	R	R	R	S	S	II
P2	7	EN1	ICU	female	40	20.08.2021	pneumonia septicemia, encephalopathy	died	R	R	S	R	R	S	S	I
		EN4	ICU	female	82	22.10.2021	pneumonia, cerebrovascular disease, heart failure	recovered	R	R	R	R	R	S	S	II
		EN9	ICU	female	63	11.11.2021	cerebrovascular disease, coronary artery disease	died	R	R	R	R	R	S	S	II
		EN10	ICU	male	70	16.02.2022	pneumonia chronic obstructive pulmonary disease, heart failure	died	R	R	R	R	R	S	S	II
		EN14	ICU	male	29	26.03.2022	pneumonia, epilepsy	recovered	R	R	R	R	R	S	S	II
		EN15	NU	male	71	03.04.2022	pneumonia, cerebrovascular disease, acute renal failure	died	R	R	S	R	R	S	S	I
		EN20	ICU	female	52	26.06.2022	pneumonia, septicemia, urinary tract infection	died	R	R	R	R	R	S	S	II
P3	2	EN21	ICU	male	71	16.09.2021	acute renal failure, hypertension	died	R	R	R	R	R	S	S	II
		EN22	ICU	male	68	22.09.2021	pneumonia, atherosclerotic heart disease	died	R	R	R	R	R	S	S	II
P4	1	EN3	IICU	female	77	22.10.2021	pneumonia, cerebrovascular disease	died	R	R	R	R	R	S	S	II
P4a	1	EN6	ICU	female	87	27.11.2021	pneumonia, abdominal pain	recovered	R	R	R	R	R	S	S	II
P5	1	EN7	IICU	female	84	20.09.2021	chronic kidney failure, liver cancer	recovered	R	R	R	R	R	S	S	II
P6	1	EN16	ICU	male	79	11.06.2022	chronic obstructive pulmonary disease, heart failure	died	R	R	R	R	R	S	S	II
P6a	1	EN8	IICU	female	62	15.08.2021	diabetes mellitus, urinary tract infection	recovered	R	R	R	R	R	S	S	II
P7	2	EN18	IICU	male	62	24.02.2022	syncope, cerebrovascular disease	recovered	R	R	R	R	R	S	S	II
		EN19	IICU	male	64	29.02.2022	syncope, diabetes mellitus, hypertension	recovered	R	R	R	R	R	S	S	II
P8	2	EN11	ICU	male	64	11.02.2022	pneumonia, cerebrovascular disease, heart failure	died	R	R	R	R	R	S	S	II
		EN12	ICU	male	93	23.02.2022	pneumonia, chronic obstructive pulmonary disease	died	R	R	S	R	R	S	S	I
P8a	1	EN13	ICU	female	77	10.01.2022	pneumonia, cerebrovascular disease	died	R	R	R	R	R	S	S	II
P9	1	EN17	ICU	male	74	20.06.2022	liver failure, chronic obstructive pulmonary disease	died	R	R	R	R	R	S	S	II

[a] – isolates with identical antimicrobial sensitivity and macro-restriction fragment pattern (MRFP)
[b] – ICU – intensive care unit, IICU – internal medicine intensive care unit, NU – neurointensive care unit
[c] – breakpoints to discriminate between susceptible (S), intermediate (I), and resistant (R) were ≤ 4, > 8 μg/ml for ampicillin (AMP), ≤ 4, > 4 μg/ml for ciprofloxacin (CIP), ≤ 128, > 128 for gentamycin (GN), ≤ 2, > 2 μg/ml for teicoplanin (TEC), ≤ 4, > 4 μg/ml for vancomycin (VA), ≤ 4, > 4 μg/ml for linezolid (LNZ), ≤ 0.25, 0.25 μg/ml for tigecycline (TGC) (EUCAST 2018)



Key	Species	Gender of patient	Age of patient	Date of admision	Hospital ward	virulence genes	MRFP type
EN17	E.faecium	male	74	June 26, 2022	ICU	esp	P9
EN7	E.faecium	female	84	September 25, 2021	IICU	-	P5
EN11	E.faecium	male	64	February 11, 2022	ICU	esp, asa	P8
EN12	E.faecium	male	93	February 23, 2022	ICU	esp, asa	P8
EN13	E.faecium	female	77	January 10, 2022	ICU	esp, asa	P8a
EN16	E.faecium	male	79	June 11, 2022	ICU	-	P6
EN8	E.faecium	female	62	August 15, 2021	IICU	-	P6a
EN18	E.faecium	male	62	February 24, 2022	IICU	-	P7
EN19	E.faecium	male	64	September 29, 2022	IICU	-	P7
EN2	E.faecium	female	79	November 8, 2021	ICU	esp	P1
EN5	E.faecium	female	57	October 06, 2021	IICU	esp	P1
EN1	E.faecium	female	40	August 20, 2022	ICU	esp	P2
EN10	E.faecium	male	70	February 16, 2022	ICU	esp	P2
EN14	E.faecium	male	29	March 26, 2022	ICU	esp	P2
EN15	E.faecium	male	71	April 03, 2022	NU	esp	P2
EN20	E.faecium	female	52	August 24, 2022	ICU	esp	P2
EN4	E.faecium	female	82	October 22, 2021	ICU	esp	P2
EN9	E.faecium	female	63	November 11, 2021	ICU	esp	P2
EN3	E.faecium	female	77	December 12, 2021	IICU	esp	P4
EN6	E.faecium	female	87	November 27, 2021	ICU	esp	P4a
EN21	E.faecium	male	71	September 16, 2021	ICU	-	P3
EN22	E.faecium	male	68	November 04, 2021	ICU	-	P3

Fig. 1. Dendrogram showing the MRFP patterns of 22 vancomycin-resistant *Enterococcus faecium* isolates, denoted MRFP types P1 to P9, obtained from 22 patients digested with the *Sma*I restriction enzyme. The dendrogram was constructed with the BioNumerics v6.6 (Applied Maths, Belgium) program, with the Dice coefficient setting tolerance and optimization at 1.0% and 2.0% with 95% similarity cut-off value. Hospital ward: ICU – intensive care unit, IICU – internal medicine intensive care unit, NU – neurointensive care unit.

dendrogram analysis to estimate the degree of similarity of the different DNA restriction profiles. The MRFPA revealed the clustering of isolates' types and origins, confirming the isolates' non-identical status (Fig. 1). The dendrogram resulted in nine distinct profiles with an overall similarity of approximately 72% between the clusters. However, MRFP subtypes P4 and P4a, P6, and P6a, and P8 and P8a shared identical MRFP subtypes with two or three isolates (Fig. 1).

Discussion

This research is the first study on *E. faecium* vancomycin-resistant isolates recovered from patients hospitalized in a medium-sized Anatolian research and training hospital. Of the 350 putative *E. faecium* culture isolates, 6.3% were positive for vancomycin resistance. The frequency of VRE colonization was similar to previous studies carried out in Türkiye. In a neonatal intensive care unit of a hospital in the province of Izmir, a prevalence rate of 8% was found based on rectal swab samples from 506 patients (Kılıç et al. 2012). Kacar et al. (2022) reported a prevalence rate of 3.4% within five years in the intensive care unit of a training and research hospital in the Konya province based on swab cultures of the wounds of 177 patients. However, a comparatively high frequency (15%) of VRE colonization was also reported in other regions of Türkiye. Yiş et al. (2011) reported a 14.6% prevalence rate at a pediatric hospital in province Gaziantep based on perirectal swab samples obtained 123 patients. In another report, Binici et al. (2022) showed that VRE colonization was detected in rectal swab samples in 9% of 600 patients. According to the Turkish Health Ministry report (USHIESA 2021), the national rate of prevalence of VRE ranged from 17 to 20%. This report is mainly based on a single institution. However, multi-institutional or regional-level reports have been rarely published. Therefore, data comparison is difficult since different populations with different age groups, a different methodology, and different antibiotic sensitivity testing were studied. Similarly, VRE also varies widely across European countries, where seven of the 38 countries/territories (Finland, France, Iceland, the Netherlands, Norway, Sweden, and Ukraine) reported < 1% compared to Italy (2.5%) and Germany (1.2%) (Peta et al. 2006; Bui et al. 2021). On the other hand, a high rate (25%) of frequency of VRE was reported in Bosnia and Herzegovina, Lithuania, Northern Macedonia, and Serbia (WHO and ECDC 2022).

Of the total 22 VRE-positive patients in the present study, the ratio between male and female patients was equally distributed, and the difference in the frequency rate was insignificant ($p > 0.05$). VRE culture isolates recovered from patients of various age groups, with the majority (95.5%) of patients testing positive for VRE infection originating from both wards, the intensive care unit (ICU) and the internal medicine intensive care unit (IICU). This indicates the critical condition of the patients in these units. Nonetheless, the prognosis was more strongly correlated with their clinical conditions at the time of infection (Table I), and it is difficult to verify whether patients succumb to VRE-associated infection. Furthermore, it is essential to undertake effective infection control measures for intensive care patients to prevent transmission of VRE (Lee et al. 2016; Erdem et al. 2020).

VRE isolates are generally multi-drug resistant to many antimicrobial agents, including cephalosporins, aminoglycosides, and trimethoprim-sulfamethoxazole. Furthermore, the ability of enterococci to acquire resistance to other agents like erythromycin, rifampin, chloramphenicol, ciprofloxacin, high concentrations of aminoglycosides, and vancomycin is well recognized (Lee et al. 2016; Erdem et al. 2020). Consequently, treating VRE infections is a clinical challenge and of great concern. In our study, all isolates showed a high level of resistance to vancomycin and teicoplanin, which is characteristic of the vanA phenotype (Table I). The results are in congruence with a previous study where an inducible high-level resistance to both vancomycin and teicoplanin was reported (Depardieu et al. 2004).

All isolates were confirmed to possess the *van*A gene. Our findings are consistent with previous studies conducted in various regions of Türkiye, which also reported a predominant presence of the *van*A gene. Gozalan et al. (2015), Sakin et al. (2019), and Erdem et al. (2020) found that all vancomycin-resistant *E. faecium* strains (55, 23, and 71 VRE strains, respectively) from in-patients treated at university or training hospitals in seven various hospitals harbored only the *van*A gene. Similarly, Asgin and Otlu (2020) examined 47 vancomycin-resistant *E. faecium* strains isolated from in-patients at a tertiary hospital in Karabük, and all strains carried only the *van*A gene. Zer et al. (2011) studied 81 vancomycin-resistant *E. faecium* strains from in-patients at a research and application hospital in Gaziantep, finding that 93.8% of the strains exhibited resistance via the *van*A gene type, 2.5% via the *van*B gene type, and 3.7% via the nonA-nonB type. However, VRE isolates exhibiting *van*B or both *van*A/*van*B resistance have been reported in various countries. The *van*A resistance is prevalent in the United States and Europe, while *van*B resistance is common in Australia and Southeast Asia (Coombs et al. 2014; Guzman et al. 2016). On the other hand, the *van*B gene was not detected in any of the VRE isolates in the present study. In Türkiye, the occurrence of the *van*B resistance type has been reported to range from 2.9% to 13.5%. Coşkun et al. (2012) were the first to report the occurrence of *van*B resistance in Türkiye, finding it in 16.6%, 5 out of 30 vancomycin-resistant and teicoplanin-negative *E. faecium* strains examined by PCR amplification. Uludağ Altun et al. (2014) detected 76 vancomycin-resistant *E. faecium* isolates from perirectal swab samples of patients admitted to internal medicine and surgical intensive care units using the PCR method; only 2.9% of these isolates were *van*B-positive. The *van*A resistance is mainly inducible and shows a high level of resistance to vancomycin and teicoplanin (Fisher and Philips 2009). Furthermore, the *van*A gene is localized on transposons (Arthur et al. 1993) and is transmissible to susceptible enterococci and other environmental pathogens. Thus, a high frequency of *van*A genes among the isolates also poses a risk to public health in the central region of Türkiye.

Moreover, the VRE isolates also harbored virulence genes that have the potential to contribute to infections in immune-suppressed patients. Among these genes, the *esp* gene was identified as the most frequently occurring virulence gene in this study. The presence of the *asa1* gene was in three isolates. Similar results were previously reported among European hospital isolates of *E. faecium* (Vankerckhoven et al. 2004).

Among several molecular typing methods used for subtyping of bacterial species, MRFPA has proven to be a highly reproducible and accurate typing method, which can distinguish clonal populations, and hence, is considered for subspecies discrimination of *E. faecium* clinical isolates (Abele-Horn et al. 2006). Therefore, further genotypic characterization was performed in the present study to genetically compare and identify strain-level clonal relatedness and genetic variability among 22 isolates using MRFP analysis. The dendrogram analysis of MRFP patterns revealed nine clusters with an overall approximately 72% similarity among strain clusters, and a genotypic variability was found among isolates. Among the nine MRFP clusters identified, seven unique MRFP types and multiple isolates were observed across all hospital wards. Notably, most patients carrying VRE strains in MRFP cluster P2 were hospitalized within a consecutive 10-month period spanning 2021 and 2022, primarily within one ICU ward. After the initial discovery of the first VRE isolate in mid-August 2021, an six additional isolates were identified by the end of June 2022. The isolates recovered from the ICU were mainly types P2, P3, P8, and P9. However, some examples of isolates within the same type (P1) and subtypes (P4 and P4a, P6 and P6a) from two different wards (ICU and IICU) were found. In addition, the VRE isolates belonging to clusters P1, P3, P4, P6, P7, and

P8 were detected in each of two patients hospitalized together during an approximately two-month period, and a spread of isolates belonging to the same cluster seems to have mainly taken place on two hospital wards (ICU and IICU). This suggests that there was a spread of patients' isolates between care unit facilities.

Interestingly, MRFP type P2 was accounted for in seven isolates (Fig. 1, Table I) undergoing the same intensive procedure, suggesting the ward-associated clonal spread (Güldemir et al. 2015). This indicates that the transmission of VRE may also occur through contaminated medical equipment, although this is probably not as critical as staff-to-staff transmission (Cetinkaya et al. 2000). Consistencies of antibiotic resistance patterns were observed within several MRFP types. Most isolates were determined within types P2 and P8, and these isolates were resistant to ampicillin, ciprofloxacin, teicoplanin, gentamycin, and vancomycin (resistance pattern II). MRFP types P1, P2, and P8 were uniformly sensitive to high-level aminoglycosides (Table I).

Conclusions

In conclusion, this study reports on the rate of prevalence of VRE isolates in patients admitted to two different hospital wards. To our knowledge, this is one of few studies reporting on clonal relatedness between clinical VRE isolates detected in Türkiye. This study reveals the emergence of VRE carrying the *van*A gene from this geographic region of Türkiye. Based on MRFPA, we found genetically identical and related clusters of isolates from patients on different wards. This finding is alarming since it suggests a possible transfer of this plasmid-borne *van*A gene to other bacteria and plasmid-free enterococci in the hospital environment. It is therefore imperative to maintain strict measures and develop further strategies to eliminate the potential spread of these pathogenic isolates within hospitals. Further investigation will be needed to determine the direction of the spread of such isolates.

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Author contributions

All authors contributed to the conceptualization and study design. Material preparation, data collection, and analysis were performed by A. Akineden, C. Çicek, S. Türkel, and I.U. Khan. The first draft of the manuscript was written by A. Akineden and A. Abdulmawjood. All authors reviewed, edited, and approved the final manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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