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Detection of vancomycin-resistant enterococci and vancomycin-resistance genes in patients hospitalized in the pediatric intensive care unit

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Abstract

Detection of vancomycin-resistant enterococci and vancomycin-resistance genes in patients hospitalized in the pediatric intensive care unit

Objective: Vancomycin-resistant enterococci (VRE) infection and colonization are seen increasingly frequently, especially among intensive care unit (ICU) patients. In this study, the aim was to detect VRE in swab samples taken from patients hospitalized in the Pediatric ICU (PICU), colonization, and to investigate the clonal relationship between isolates.
Method: In the present study, swab samples were taken from the external auditory canal (EAC), umbilical region, and rectal region from 82 patients hospitalized in the Çukurova University Balcalı Hospital PICU. The 246 swab samples from patients were inoculated on Kanamycin-Esculin-Azide agar. Isolates were identified with the help of the BBL Crystal Gram-Positive identification system. The susceptibility of the isolates to vancomycin (30 µg) was investigated by Kirby-Bauer disk diffusion method according to CLSI criteria. VanA-VanB genes in phenotypically defined vancomycin-resistant enterococci were investigated by Polymerase Chain Reaction (PCR) method. The clonal relationship between vancomycin-susceptible (VSE) and -resistant enterococci was determined by the SmaI-PFGE method.
Results: A total of 49 (20.3%) enterococcal strains were isolated from 246 swab samples from the patients, of which 14 (28.5%) were VRE. Of the enterococci isolates, 27 (55.10%) were *E. faecium* and 13 (26.53%) were *E. feacalis*. While VanA type resistance was detected in 11 of the vancomycin-resistant *E. faecium* and *E. feacalis* isolates, VanB type resistance was not detected in any sample. There was no significant clonal relationship between the isolates.
Conclusion: Although the prevalence of VRE in the PICU was high throughout the study, no enterococcal infection was observed.
Keywords: Vancomycin-resistant Enterococci, VanA, VanB, PCR, SmaI-PFGE

Öz

Çocuk yoğun bakım ünitesinde yatan hastalarda vankomisine dirençli enterokok ve vankomisine dirençli genlerin tespiti

Amaç: Vankomisine dirençli enterokok (VRE) enfeksiyonu ve kolonizasyonu, özellikle yoğun bakım ünitesi (YBÜ) hastalarında giderek artan sıklıkta görülmektedir. Bu çalışmanın amacı, Pediatrik YBÜ'de (PYBÜ) yatan hastalardan alınan sürüntü örneklerinde VRE'yi saptamak, kolonizasyonu ve izolatlar arasındaki klonal ilişkiyi araştırmaktı.
Yöntem: Çalışmada, Çukurova Üniversitesi Balcalı Hastanesi PYBÜ'nde yatan 82 hastadan dış kulak yolu (DKY), göbek bölgesi ve rektal bölgeden sürüntü örnekleri alındı. Hastalardan alınan 246 sürüntü örneği Kanamisin-Esculin-Azide agara inoküle edildi. İzolatlar, BBL Crystal Gram-Positive tanımlama sistemi yardımıyla tanımlandı. İzolatların vankomisine (30 µg) duyarlılıkları Kirby-Bauer disk difüzyon yöntemi ile CLSI kriterlerine göre araştırıldı. Fenotipik olarak tanımlanmış vankomisine dirençli enterokoklarda VanA-VanB genleri, Polimeraz Zincir Reaksiyonu (PCR) yöntemi ile araştırıldı. Vankomisine duyarlı (VSE) ve dirençli enterokoklar arasındaki klonal ilişki SmaI-PFGE yöntemi ile belirlendi.
Bulgular: Hastalardan alınan 246 sürüntü örneğinden 14'ü (%28.5) VRE olan toplam 49 (%20.3) enterokok suşu izole edildi. Enterokok izolatlarının 27'si (%55.10) *E. faecium* ve 13'ü (%26.53) *E. feacalis* idi. Vankomisine dirençli *E. faecium* ve *E. feacalis* izolatlarının 11'inde VanA tipi direnç tespit edilirken, hiçbir örnekte VanB tipi direnç tespit edilmedi. İzolatlar arasında önemli bir klonal ilişki yoktu.
Sonuç: Çalışma boyunca PYBÜ'de VRE prevalansı yüksek olmasına rağmen enterokok enfeksiyonu gözlenmedi.
Anahtar Kelimeler: Vankomisine Dirençli Enterokoklar, VanA, VanB, PCR, SmaI-PFGE.

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INTRODUCTION

Enterococci, which are normal flora of the gastrointestinal tract in humans, are usually associated with gastrointestinal and urinary tract infections, bacteremia, and endocarditis. The *Enterococcus faecalis* and *Enterococcus faecium* species commonly found in human intestines are generally responsible for nosocomial enterococcal infections (1, 2). Increasing antimicrobial resistance in enterococci has caused difficulties for treatment in recent years (3). Vancomycin-resistant *E. faecium*, which is on the World Health Organization's list of antibiotic-resistant bacteria, has been defined as the most common cause of nosocomial infection (4). Since enterococci showing vancomycin resistance usually also show penicillin and aminoglycoside resistance, treatment options are limited (5). Vancomycin-resistant enterococci (VRE) infection and colonization are seen increasingly frequently, especially among hemodialysis patients and intensive care unit (ICU) patients. Since nosocomial infections due to VRE are widespread problem, VRE colonization should be investigated, especially among ICU patients (6).

The cause of antibiotic resistance in enterococci is mutation and horizontal gene transfer with transposons and plasmids (7). Glycopeptide resistance is mediated by nine different genes called Vancomycin resistance (Van) gene operons. Vancomycin resistance in enterococci is mainly provided by the VanA or VanB genes. Phenotypically, the VanA gene shows a high level of resistance to vancomycin and teicoplanin, while the VanB gene only provides a lower level of resistance to vancomycin (8). Among the nine Van genotypes reported to date, VanA (80–90%) and VanB (10–20%) are predominant (9). VanC is responsible for internal resistance found in *E. gallinarum* and *E. casseliflavus* (10).

Infections caused by VRE have significant effects on morbidity and mortality, length of stay in hospital, and total costs. Because asymptomatic VRE colonization acts as a reservoir for spread and subsequent infections, monitoring and prevention of colonization can reduce transmission of VRE. Therefore, rapid detection of VRE is important for the control and prevention of nosocomial infections (11). Primary colonization sites in hospitalized patients are usually the gastrointestinal tract, skin, and soft tissues (12). Therefore, in this study, it was aimed to determine the incidence of enterococci and VRE colonization, the presence of VanA and VanB genes, and the clonal relationship between all isolates in swab samples taken from body parts such as the rectum, external ear canal (EAC), and umbilical region of patients hospitalized in a tertiary pediatric ICU (PICU).

METHOD

Approval was obtained for this study with the decision of the Çukurova University Clinical Research Ethics Committee (20.01.2011/ 19). The study was carried out between 10.11.2010 and 3.01.2012 in the PICU of Çukurova University Balcalı Hospital (a tertiary teaching hospital). Between the specified dates, a total of 246 swab samples were taken from the external auditory canal (EAC), umbilical, and rectal regions from 82 patients who were admitted to the PICU within the first 24 hours and on the third, fifth, and seventh days after hospitalization. The swab samples taken were left to incubate at 37°C for 24 hours in Brain Heart Infusion Broth (BHIB), then passaged into Kanamycin-Esculin-Azide agar and incubated under the same conditions. Colonies that hydrolyze esculin in the medium were accepted as suspicious of enterococci, and their pure cultures were obtained by passages on blood agar. Pure cultures were identified at the species level with the help of the BBL Crystal Gram-Positive identification system (catalog number: 245240, BD Diagnostic System). The enterococci isolated in pure culture were kept at -20°C in BHIB medium containing 10% glycerol and 10% blood.

Vancomycin Susceptibility Test

The susceptibility of enterococci to vancomycin (30 µg) (bioMérieux, France) was evaluated according to the Kirby-Bauer disk diffusion method. Results were analyzed according to the Clinical Laboratory Standards Institute (CLSI) criteria.

Vancomycin Resistance Gene Detection by Real-Time PCR

PCR method was used to determine VanA/VanB genes in vancomycin-resistant enterococci. Specific primers for VanA (5'-TCT GCA ATA GAG ATA GCC GC-3 '\ 3'-GGA GTA GCT ATC CCA GCA TT-5') and for VanB (5'-GTG ACA AAC CGG AGG CGA GGA-3 '\ 3'-CCG CCA TCC TCC TGC AAA AAA-5') were used. Amplicons were subjected to electrophoresis under 120 V current for 30 minutes in 2% agarose gel containing 0.5% ethidium bromide. The DNA fragment in the gel was imaged using the Gel Logic 1500 imaging system (Kodak Company, NY, USA).

Pulsed-field gel electrophoresis (PFGE)

A single colony was grown from bacteria identified at the species level on blood agar medium. Cells in 2% low melting temperature agarose (Bio-Rad Low-Melt Agarose 161-3113EDU) plates were lysed with 1 ml of cell lysis solution (10 mM Tris-HCl, 50 mM NaCl, 50 mM EDTA-0.2% sodium deoxycholate-0.5% sarcosyl) and 150 µg/ml proteinase K. Next, the chromosomal DNA was digested with the SmaI restriction enzyme (Fermentas, Lot: 00015137). The fragmented DNA samples were subjected to electrophoresis with a pulse duration 3.5-20 seconds at 6 V/cm^2 at 12 °C for 12 hours and a pulse duration of 1-5 seconds at 6 V/cm^2 at 12 °C for 8 hours using 1.2% agarose (Pulsed Field Certified Agarose, Bio-Rad Laboratories; CHEF-DR II system Bio-

Rad Laboratories, Nazareth, Belgium). Gels were stained with 1 mg/ml ethidium bromide and photographed under ultraviolet illumination. Band profiles were analyzed using the GelCompar II software system (version 5.0 Applied Maths, Sint-Martens-Latem, Belgium). First, normalization between pictures was performed with the help of three standards (carried out in wells 1, 7, 15) in each picture. The dendrogram of PFGE profiles was created using the "unweighted pair group method with mathematical averaging (UPGMA)" and cluster analysis was performed. The relationship between the strains was determined according to the "Dice" similarity coefficient depending on the bands. In the calculation of the similarity coefficient, the band and profile tolerance was taken as 1.5%. Isolates with 80% similarity in band profiles were evaluated in the same cluster and named with capital letters. Subtypes within the same cluster are shown with numbers.

Statistical Analysis

SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program was used to evaluate the data of the participants. The distribution of the variables were expressed as percentage.

RESULTS

In the study, 49 (20.3%) enterococci strains were isolated from 246 swab samples taken from the patients, and it was determined that the rectal region was most frequently colonized. Colonization was detected in only 5 (6%) of the EAC samples (Table 1). E. faecium was the most common species with 27 (54%) isolates in the species identification of enterococci isolates using the BBL Crystal Gram-Positive identification system (Table 1).

VRE were isolated from a total of 14 (28.5%) swab samples according to the vancomycin resistance distribution determined by the Kirby-Bauer disk diffusion method (Table 1). When the VRE distribution was examined according to the sample material, eleven strains were isolated from the rectal area, two from the umbilical swab specimen, and one strain from the EAC. Eight of the rectal swab samples were *E. faecium* (57.14%), three strains were *E. feacalis* (21.42%), and three strains isolated from EAC and umblical were *E. gallinarum* (21.42%). All of the 14 (100%) samples with VRE and 30 (51%) samples with VSE were obtained within the first 24 hours following admission (Table 2).

Table 1: Characteristics of enterococci isolates isolated from swab samples.

Isolates	Rectal	Navel	External auditory canal	Total	%
E. faecium	17	5	5	27	55.10
E. feacalis	11	2	0	13	26.53
E. gallinorum	3	2	0	5	10.20
E. casseliflavus	2	2	0	4	8.16
VRE	11	2	1	14	28.6
VSE	22	9	4	35	71.4

VRE, Vancomycin-resistant enterococci; VSE, Vancomycin-susceptible enterococci

Table 2: Distribution of enterococci isolates obtained from samples taken at different times according to vancomycin resistance.

	First 24 hours		>24 hours	
Isolates	n	%	n	%
VRE (14)	14	100	0	0
VSE (35)	30	85.7	5	14.3

VRE, Vancomycin-resistant enterococci; VSE, Vancomycin-susceptible enterococci

In the investigation of the VanA and VanB resistance genes of 14 vancomycin-resistant strains with specific primer-PCR method, VanA type resistance was detected in 11 of the vancomycin-resistant *E. faecium* and *E. feacalis* isolates, while VanB type resistance was not found in any sample. VanA-VanB genes were not detected in vancomycin-resistant *E. gallinarum* isolates (Figure 1).

Clonal relationships of VREs and VSEs were evaluated as separate groups at the species level by SmaI-PFGE method. In the clonal similarity study of eight *E. faeceum* isolates with VRE to provide evidence for cross contamination, it was determined that the isolates were distributed into six clusters, two (C-E) two-membered and four single-membered (A-B-D-F). The strains with cluster C sub-members (c1-c2) were isolated from the EAC and rectal swab samples of the same patient, and the similarity rate was found to be 82.4%. Subset strains (e1-e2) forming the E cluster were isolated from rectal swab samples of two unrelated patients hospitalized at different times (Figure 2A).

The 19 *E. faeceum* isolates with VSE were distributed in 12 clusters. The largest cluster was the H cluster with three members. Clusters A, E, G, J, and L formed two-membered clusters, and the other isolates formed single-membered specific clusters. The 26G-26K isolates constituting the h1-h2 subsets showing 92.3% clonal similarity in the H cluster were isolated from the umbilical and EAC samples of the same patient (Figure 2E).

In the analysis of the three vancomycin-resistant *E. faecalis* isolates by the smaI-PFGE method, it was seen that all strains were distributed in three clusters (A-B-C) consisting of clonally

unrelated specific strains (Figure 2B).

Ten *E. faecalis* isolates susceptible to vancomycin were distributed in a total of seven clusters. The two isolates (17G-17R) constituting the a1 subset were isolated from samples belonging to the same patient and showed 100% similarity, and the 16R isolate in the a2 subset was 94.1% similar to the isolates of the a1 cluster. It was determined that the patient from whom the 16R isolate was taken and the patient from whom the a1 cluster isolates were taken were admitted to the hospital on the same day (Figure 2F).

All *E. gallinorum* isolates obtained from the swab samples that were phenotypically vancomycin-resistant but lacking VanA-VanB resistance genes were distributed into three specific, clonally unrelated clusters (A-B-C) (Figure 2C).

The vancomycin-susceptible *E. gallinorum* isolates were distributed into four clusters, one of which was the A cluster with three members, and three specific clusters with one member each. It was determined that 10G and 14R isolates belonging to the a1 and a2 subsets of cluster A were associated with each other at a rate of 94.7%, and the 14G isolates in the

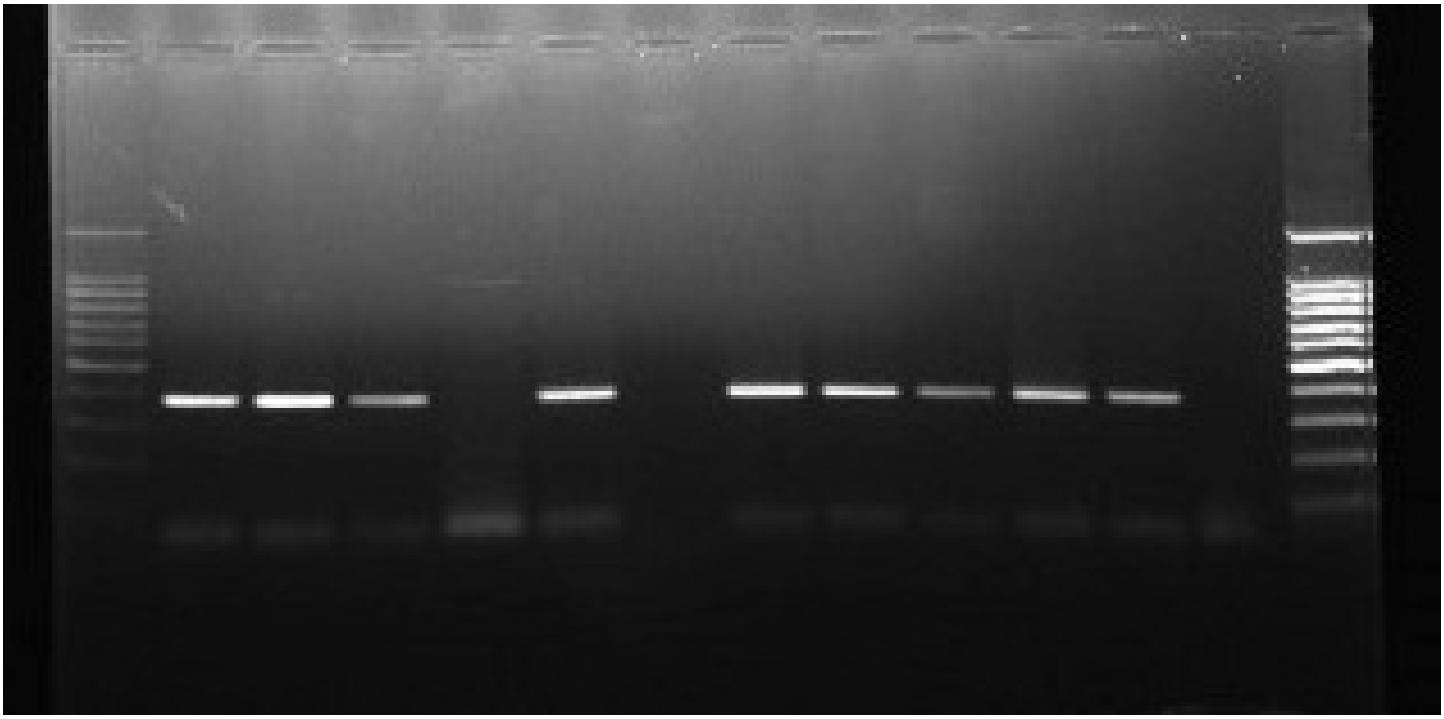

Figure 1: Gel image of amplicons of VanA gene cluster of vancomycin resistant isolates.

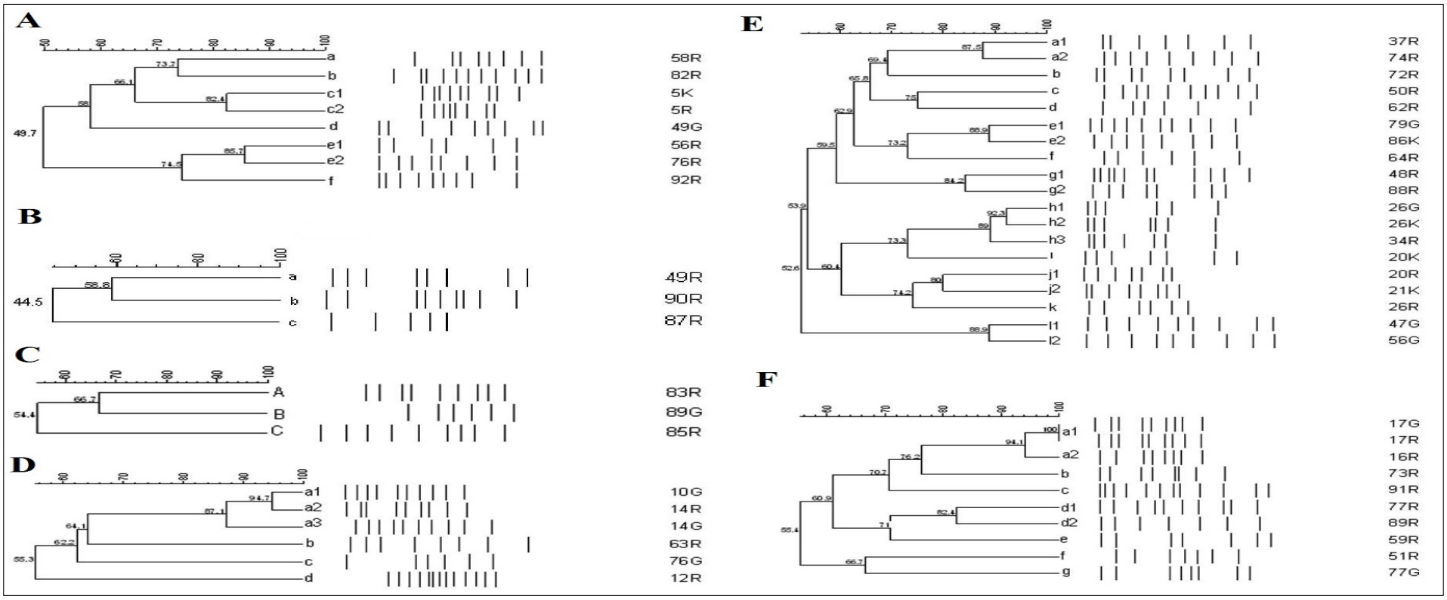

Figure 2: PFGE analysis and dendrograms of VRE isolates. (A) Vancomycin-resistant *E. faecium* isolate, (B) Vancomycin-resistant *E. faecalis* isolate, (C) Vancomycin-resistant *E. gallinorum* isolate, (D) Vancomycin-susceptible *E. gallinorum* isolate, (E) Vancomycin-susceptible *E. faecium* isolate, (F) Vancomycin-susceptible *E. faecalis* isolate.

a3 cluster were associated with the a1 and a2 isolates at a rate of 87.1% (Figure 2D).

DISCUSSION

Since vancomycin-resistant enterococci were first reported in 1986, VRE has continued to spread as a result of the widespread use of vancomycin and broad-spectrum cephalosporins in hospitals. Severe enterococcal infections have become difficult to treat in the last 20 years (13). Despite scientific reports on the reported incidence of VRE in PICUs in Türkiye different regions (14-18), there has not been a study on the prevalence of VRE in PICUs in Türkiye South.

In a meta-analysis involving adult patients, the VRE colonization rate was documented in the range of 0–42% with a mean of 12.5% (19). However, when data from ICUs (neonatal, pediatric, and adult) were evaluated, it was reported that the VRE colonization rate ranged from 0% to 66% (19-22). These differences in colonization rates may be due to hospital infection control policies, methodologies followed to detect colonization, geographic variation, and differences in personal care applied to health care (23). In a surveillance study from two ICUs in Brazil, the rectal swabs of patients had a VRE strain in 32.6% (24). Rectal colonization for VRE was identified in 29.3% of patients in the ICU and postoperative ward of a hospital in Iran (25). In another study conducted in Iran, intestinal colonization was observed in 33/47 (70.2%) of the patients with VRE-related infection. In the same study, the majority of VRE was reported to be *E. faecium* (63.3%) and the remainder as *E. faecalis* (36.7%) (26). In the study conducted by Amberpet et al., they reported that the VRE colonization rate was 18.6% in rectal swab samples of 198 patients admitted to the PICU and that the majority of isolates were *E. faecium* (75.6%) and *E. faecalis* (24.4%) (23).

In a study conducted in northwest Türkiye (Istanbul), fecal VRE colonization was documented in 72 (31.4%) of 229 children admitted to the hematology/oncology service. 32 patients whose VRE types could be identified among these patients, *E. faecium* was isolated in 28, *E. gallinarum* was isolated in 2, and untypable enterococcus was isolated in 2 (15). In studies conducted in the same region, it was reported that rectal colonization was detected in 200 (12%) of 1671 patients admitted to the NICU [14] and 9.5% of all patients admitted to the PICU [17]. In a study conducted in a children's hospital in the southeast of Türkiye, 18 (14.6%) of 123 perirectal swab samples were found to have VRE colonization. It was observed that VRE colonization rates were high, especially in wards with long hospitalization and antibiotic use (72.2% (13/18) in oncology service, 27.8% (5/18) in ICU). Three of the 13 VRE isolates isolated from patients in the oncology service were identified as *E. faecalis* and ten as *E. faecium*, and all five VRE isolates isolated from patients hospitalized in the ICU were identified as *E. faecium* (18). In this study, It was determined an asymptomatic VRE colonization rate of 28.5% in the tertiary PICU located in the southern region of Türkiye. In addition, the most common VRE isolates isolated in rectal swab samples were *E. faecium* at a rate of 57.14% and then *E. feacalis* at a rate of 21.4%. The frequency of VRE observed in this study and the types of enterococcus isolated are similar to the results of the studies conducted both in Türkiye and in the nearby geography.

VanA or VanB genes are mainly responsible for vancomycin resistance in enterococci. In their study, Amberpet et al. found that they confirmed the VanA gene in all 37 VRE isolates by PCR method, but none of these isolates had VanB and VanC genes (23). Lee et al. (27) showed that all 54 isolates of vancomycin-resistant *E. faecium* that were isolated as colonizing and infectious agents in a VRE outbreak carried the VanA gene. Similarly, Kim et al. (28) found in their study that all VRE strains isolated from the swab cultures of 184 patients carried the VanA gene. Studies conducted in Türkiye have also reported that the VanA gene is dominant. Cilo et al. (16) identified the VanA resistance gene in all swab samples in an outbreak in the NICU in 2013-2014. Ongut et al. (29) reported that all of the VRE positivity they identified in 20 samples in their study included *E. faecium* carrying the VanA gene. Yis et al. (18) showed that all 18 VRE strains they isolated in their study had the VanA gene. In this study, 11 (78.5%) of 14 VRE isolates had the VanA gene and none of these isolates had the VanB gene. Unlike other studies, it was observed that VanA-VanB resistance genes were not found in the other three isolates.

The PFGE method is frequently preferred in the detection of clonal relationships of VRE outbreaks and nosocomial infections. Jahansepas et al. (26) showed the small clonal distribution of VR *E. faecium* and VR *E. faecalis* species in different wards of the same hospital and in different hospitals and different cities. Dendrogram profiles in the studies of Cilo et al. (16) revealed two different strains, most of them (22/24) with the same clonal origin. In this study, as a conclusion of the clonal examination of enterococcal isolates with the PFGE method, no finding indicating clonal similarity of the isolates and cross contamination between the hospitalization dates of the patients was found.

CONCLUSION

In conclusion, the findings emphasize the high prevalence of VRE in the clinical setting. In addition, *E. faecium* and *E. feacalis* strains were the most common VRE strains. The most common glycopeptide resistance phenotype distinguished in this study was the VanA gene. Detection of VREs, determination of phenotypic-genotypic antibiotic resistance profiles, and active surveillance studies are important due to the prevalence of VREs.

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Peer-Review

Externally peer reviewed.

Conflict of interest

The authors declare that they have no conflict of interests regarding content of this article.

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Thesis study was prepared by rearrangement of the specialty thesis by the first author, dated 2012, entitled as "Vancomycin rezistans Enterococcus colonization, moleculer epidemyology and risc factors in pediatric intensive care unit".

Ethical Declaration

Ethical permission was obtained from the Çukurova University, Medical Faculty Clinical / Human Research Ethics Committee for this study with date 20.01.2011 and number 19, and Helsinki Declaration rules were followed to conduct this study.

Authorship Contributions

Concept: NA, FK, ÖO, Design: ÖO, SK, FK, NA; Supervising: NA, Financing and equipment: None, Tools: NA, FK, ÖOi, Data collection and entry: SK, FK, ÖO, Analysis and interpretation: SK, FK, ÖO, Literature search: ÖO, NA, Writing: ÖO, SK, CÖG, Critical review: ÖK, SK, CÖG

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REFERENCES

1. Cotton MJ, and Packer CD. Vancomycin-resistant *Enterococcus faecium* empyema in an asplenic patient. Cureus .2018;10(8):3227. https://doi.org/10.7759/cureus.3227.
2. Kim HS, Kim DH, Yoon HJ, Lee WJ, Woo SH, et al. Factors associated with vancomycin-resistant enterococcus colonization in patients transferred to emergency departments in Korea. Journal Korean. Medical Science. 2018;33(48):295. https://doi.org/10.3346/jkms.2018.33.e295.
3. Farhadi R, Saffar MJ, Monfared FT, Larijani LV, Kenari SA et al. Prevalence, risk factors and molecular analysis of vancomycin-resistant enterococci colonization in a referral neonatal intensive care unit: A prospective study in northern Iran. Journal of Global Antimicrobial Resistance. 2022;30:474-479. https://10.1016/j.jgar.2022.05.019.
4. Gouliouris T, Raven KE, Ludden C, Blane B, Corander J, et al. Genomic surveillance of *Enterococcus faecium* reveals limited sharing of strains and resistance genes between Livestock and humans in the United Kingdom. mBio. 2018;9. ttps://doi.org/10.1128/mBio.01780-18.
5. Deshpande VR, Karmarkar MG, and Mehta PR. Prevalence of multidrug-resistant enterococci in a tertiary care hospital in Mumbai, India. The Journal of Infection in Developing Countries. 2013;7:155-158. https://doi.org/10.3855/jidc.3018.
6. Lee SC, Lee CW, Shih TC, See LC, Chu CM, et al. Identification of subclinical transmission of vancomycin-resistant enterococcus within an intensive care unit in Taiwan. Journal of Microbiology, Immunology and Infection. 2016; 49:749-759. https://doi.org/10.1016/j.jmii.2015.11.002.
7. Jafari, S, Abdollahi A, Sabahi M, Salehi M, Asadollahi-Amin A, et al. An Update to enterococcal bacteremia: epidemiology, resistance, and outcome. Infectious Disorders Drug Targets. 2022;22(2):170322187568. htt ps://10.2174/1871526520999201103191829.
8. Huh HJ. Evaluation of the intron vre vanA/vanB real-time PCR assay for detection of vancomycin-resistant enterococci. Annals of Laboratory Medicine. 2015;35:76-81. https://doi.org/10.3343/alm.2015.35.1.76.
9. Kuo AJ, Shu JC, Liu TP, Lu JJ, Lee MH, et al. Vancomycin-resistant Enterococcus faecium at a university hospital in Taiwan, 2002-2015: Fluctuation of genetic populations and emergence of a new structure type of the Tn1546-like element. Journal of Microbiology, Immunology and Infection. 2018;(51):821-828. https://doi.org/10.1016/j.jmii.2018.08.008.
10. Levitus M, Rewane A, and Perera TB. Vancomycin-resistant enterococci, in StatPearls. 2022:Treasure Island (FL).
11. Dutka-Malen S, Mohnas C, Arthur M and Courvalin P. The vanA glycopeptide resistance protein is related to D-alanyl-D-alanine ligase cell wall biosynthesis enzymes. Molecular and General Genetics. 1990;224:364-72. https://doi.org/10.1007/BF00262430.
12. Purohit G, Gaind R, Dawar R, Verma PK, Aggarwal K.C, Sardana R, et al. Characterization of vancomycin resistant enterococci in hospitalized patients and role of gut colonization. Journal of Clinical Diagnostic Research. 2017;11(9):01-05. https://doi.org/10.7860/JCDR/2017/25988.10548.
13. Salem-Bekhit MM, Moussa IMI, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian Journal of Medical Microbiology . 2012;30:44-51. https://doi.org/10.4103/0255-0857.93032.
14. Akturk H, Sutcu M, Somer A, Acar M, Karapınar BA, et al. Vancomycin-resistant enterococci colonization in a neonatal intensive care unit: who will be infected? The Journal of Maternal-Fetal & Neonatal Medicine. 2016;29:3478-82. https://doi.org/10.3109/14767058.2015.1132693.
15. Akturk H, Sutcu M, Somer A, Karaman S, Acar M, et al. Results of four-year rectal vancomycin-resistant enterococci surveillance in a pediatric hematology-oncology ward: from colonization to ınfection. Turkish Journal Haematology. 2016;33:244-7. https://doi.org/10.4274/tjh.2015.0368.
16. Cilo BD, Ağca H, Efe K, Sınırtaş M, Çelebi S, et al. Investigation of vancomycin resistant Enterococcus faecium outbreak in neonatal intensive care unit. International Journal of Clinical and Experimental Medicine. 2014; 7:5342-7.
17. Sutcu M, Akturk H, Acar M, Salman N, Aydın D et al. Impact of vancomycin-resistant enterococci colonization in critically ill pediatric patients. American Journal of Infection Control. 2016;44:515-9. https://doi.org/10.1016/j.ajic.2015.11.026.
18. Yi R, Selda Aslan S, Cıtak Ç, Değirmenci S. Evaluation of vancomycin-resistant enterococcus colonization at Gaziantep Children's Hospital, Turkey. Mikrobiyoloji Bülteni. 2011;45:646-54.
19. Ziakas PD, Thapa R, Rice LB, and Mylonakis E. Trends and significance of VRE colonization in the ICU: a meta-analysis of published studies. PLoS One. 2013;8:75658. https://doi.

org/10.1371/journal.pone.0075658.

20. Dutka-Malen S, Evers S, and Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. Journal of Clinical Microbiology. 1995;33:1434. https://doi.org/10.1128/JCM.33.5.1434-1434.1995.
21. Nourse C, Murphy H, Byrne C, O'Meara A, Breatnach F, Kaufmann M, et al. Control of a nosocomial outbreak of vancomycin resistant Enterococcus faecium in a paediatric oncology unit: risk factors for colonisation. European Journal of Pediatrics. 1998;157:20-7. https://doi.org/10.1007/s004310050760.
22. Zhang YZ and Singh S. Antibiotic stewardship programmes in intensive care units: Why, how, and where are they leading us. World Journal of Critical Care Medicine. 2015;4:13-28. https://doi.org/10.5492/wjccm.v4.i1.13.
23. Amberpet R, Sistla S, Parija SC, Rameshkumar R. Risk factors for intestinal colonization with vancomycin resistant enterococci' A prospective study in a level III pediatric intensive care unit. Journal of Laboratory Physicians. 2018;10:89-94. https://doi.org/10.4103/JLP.JLP_32_17.
24. Furtado GHC, Martins ST, Coutinho AP, Wey SB, Medeiros E. Prevalence and factors associated with rectal vancomycin-resistant enterococci colonization in two intensive care units in Sao Paulo, Brazil. The Brazilian Journal of Infectious Diseases. 2005;9:64-9. https://doi.org/10.1590/s1413-86702005000100011.
25. Javadi A, Behrooz A, Farzin K, Saied T, Sina M, et al. Prevalence of vancomycin resistant enterococci colonization in gastrointestinal tract of hospitalized patients. Iran Journal of Clinical Infectious Diseases. 2008;3:317-141.
26. Jahansepas A, Rezaee MA, Hasani A, Sharifi Y, Farzami MR, et al. Molecular epidemiology of vancomycin-resistant Enterococcus faecalis and Enterococcus faecium ısolated from clinical specimens in the Northwest of Iran. Microbial Drug Resistance. 2018;24:1165-1173. https://doi.org/10.1089/mdr.2017.0380.
27. Lee SC., Wu MS, Shih HJ, Huang SH, Chiou MJ, Bakın LC et al. Identification of vancomycin-resistant enterococci clones and inter-hospital spread during an outbreak in Taiwan. BMC Infectious Diseases. 2013;13:163. https://doi.org/10.1186/1471-2334-13-163.
28. Kim YJ., Kim SI, Kim YR, Lee JY, Parkı YJ, Kang AW. Risk factors for vancomycin-resistant enterococci infection and mortality in colonized patients on intensive care unit admission. American Journal of Infection Control. 2012;40:1018-9. https://doi.org/10.1016/j.ajic.2012.01.009.
29. Ongut G, Kilinckaya H, Baysan BO, Ogunc D, Colak D, et al. Evaluation of Brilliance VRE agar for the detection of vancomycin-resistant enterococci in rectal swab specimens . Journal of Medical Microbiology. 2013; 62:661-662. https://doi.org/10.1099/jmm.0.052845-0.