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Original Article

Evaluation of Screening Results for Vancomycin-Resistant Enterococci: Three-Year Surveillance

Zeynep Ayaydın [1], Ayşe Batgi Azarkan [1], Özge Alkan Bilik [2], Ali Cem Tekin [1]

1. *Department of Medical Microbiology, Diyarbakır Gazi Yaşargil Training and Research Hospital, Health Sciences University, Diyarbakır, Turkey*
2. *Department of Medical Microbiology, Selahaddin Eyyubi State Hospital, Diyarbakır, Turkey*

***Corresponding Author:** Email: zeynepayaydin@gmail.com

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Abstract

Background: Enterococci are facultative anaerobic, binary, or chained Gram-positive cocci. The gastrointestinal colonization of hospitalized patients is the most important reservoir of vancomycin-resistant enterococci. We aimed to evaluate retrospectively the screening results of vancomycin-resistant enterococci, studied by the simultaneous (real-time) polymerase chain reaction method on rectal swabs of adult and pediatric patients hospitalized in our hospital in 2019-2021.

Methods: Adult and pediatric patients were included in our study between Jan 2019 and Dec 2021. The results of vancomycin-resistant enterococci, studied with the real-time polymerase chain reaction method from rectal swabs sent from intensive care units and services, were analyzed retrospectively. Isolation of the samples was performed using the Fluorion VRE QLP 1.0 real-time polymerase chain reaction kit (Iontek, Turkey), and detection was performed with the Fluorion Detection System (Iontek, Turkey) real-time polymerase chain reaction device.

Results: Overall, 31,725 patients were included in our study. When evaluated in order of years, in 2019, 379 (7%) of 5,389 adults, 322 (7.4%) of 4,003 children, 234 (5.5%) of 4,185 adults in 2020, 157 (2.4%) of 6,499 children, and in 2021, vancomycin-resistant enterococci were detected in 469 (7.5%) of 6,232 adults and 224 (4.1%) of 5,417 children.

Conclusion: The prevalence of vancomycin-resistant enterococci is greater in adults, particularly in intensive care units, compared to children. Infection control precautions and training be augmented in high-risk clinics, while the unnecessary utilization of glycopeptides should be limited.

Keywords: Vancomycin-resistant enterococci; Vancomycin-resistant enterococci (VRE); Rectal swab; Real-time PCR

Introduction

Enterococci, found widely in nature, forming facultative anaerobes, double or short chains, are Gram-positive cocci. These microorganisms are predominantly found in the gastrointestinal tract flora. Due to their resilience to environmental factors, these organisms are capable of enduring extended periods in outdoor settings (1). *Enterococcus faecalis* and *E. faecium* are the most important

species, and glycopeptide antibiotics are used in the treatment of infections caused by these microorganisms. Vancomycin resistance, caused by the frequent use of glycopeptide antibiotics, was first detected in the UK in 1988 in an *E. faecium* strain. The emergence of vancomycin-resistant enterococci (VRE) in Turkey was initially observed in a university hospital located in Antalya in 1998. Subsequently, the prevalence of VRE detection expanded to encompass a greater number of medical centers (2-4). Spread of VRE is a clinical concern. Vancomycin resistance is a major problem in the group of patients hospitalized for a long time, use antibiotics for a long time, have any underlying chronic disease, immunosuppressed, oncological or poor general condition. The gastrointestinal (rectal) colonization of hospitalized patients is the most important reservoir of VRE. For this reason, contaminated hands-on hospital staff can cause nosocomial infections and epidemics because of rapid transmission. They frequently cause intra-abdominal infections, urinary tract infections, endocarditis, and bacteremia. They live easily in the hospital environment because they have natural resistance or the potential to develop resistance to many antibiotics (5-7).

Enterococci exhibit two primary phenotypes of acquired resistance to vancomycin, namely VanA and VanB. The predominant sources of these two resistance phenotypes are *E. faecium* and *E. faecalis*. Real-time polymerase chain reaction (PCR) provides expedited outcomes in the detection and validation of resistance phenotypes in comparison to standard culture and traditional PCR methods. Conducting rectal swab cultures periodically for identifying gastrointestinal (rectal) colonization is considered the preferred method (8-10). In our hospital, rectal swab scans are conducted utilizing real-time PCR testing as a means of expediting the diagnostic process and facilitating prompt implementation of isolation protocols. This method is preferred over traditional culture-based testing due to its superior speed and efficiency. According to reports, the prevalence of rectal carriage of vancomycin-resistant enterococcus (VRE) in our region ranged from 3.6% to 11.6% during the period spanning from 2016 to 2021 (11-14). The timely and appropriate implementation of VRE screening is crucial to mitigating the transmission of nosocomial VRE. Regular rectal swabs of patients admitted to high-risk clinical settings, particularly intensive care units, must be conducted promptly and at designated intervals to achieve this objective.

We retrospectively analyzed the results of VRE scans conducted via real-time PCR on rectal swabs obtained from adult and pediatric patients hospitalized in our medical facility during the years 2019, 2020, and 2021. The investigation aimed to evaluate the distribution of VRE positivity rates across different clinics and years.

Materials and Methods

Overall, 31,725 adults and children hospitalized in the intensive care units and different clinics of our hospital in 2019, 2020, and 2021 were included in our study. A retrospective analysis was conducted on the outcomes of rectal swab-based VRE scanning, carried out using the real-time PCR technique, on samples obtained from intensive care units and different clinics.

Rectal specimens were collected and placed in a suitable transport medium containing buffered phosphate solution (PBS) before being dispatched to our laboratory. After isolation from the rectal swab using the Fluorion VRE QLP 1.0 real-time PCR test kit (Iontek, Turkey), VRE scanning was performed with the real-time PCR device of the Fluorion Detection System (Iontek, Turkey). Antibiogram tests were not used to detect vancomycin resistance. Resistance gene-based vancomycin resistance was detected using commercial real-time PCR kits.

The obtained data were analyzed in the SPSS ver. 20.00 (IBM Corp., Armonk, NY, USA) package program. The Chi-Square test was applied to determine whether there is a relationship between categorical variables. While performing the statistical analysis, the confidence interval was accepted as 95% and $P \leq 0.05$.

Ethics Committee Approval

This study was approved by the Clinical Research Ethics Committee of SBU Diyarbakir Gazi Yaşargil Training and Research Hospital (05/26/2023 date and 423 decision no.). Informed consent form was taken from the patients.

Results

Overall, 31,725 patients hospitalized in different clinics of our hospital during the three-year period covering the years 2019-2021 were included in our study. In order to compare the positivity in age groups, patients were divided into three groups: those aged 0-1 year, those aged 2-17 yr, and those aged 18 yr or older.

Table 1 displays the distribution of VRE positivity across age groups and years. Upon examination of Table 1, there exists a statistically significant difference in the prevalence of VRE positivity rates across age groups over the years as well as in the overall population ($P \leq 0.05$). This situation shows that the low VRE positivity rate in the 2-17 ages group is statistically significant according to the other age groups in terms of both years and total number of patients. The high total VRE positivity rate in 2019 is statistically significant according to both years and total number of patients ($\chi 2=139.825$, $P=0.00$). Infection control precautions and strict contact isolation in the pandemic years (2020 and 2021) have been effectively implemented.

Table 1: Distribution of VRE positivity by age groups and years

Age groups (yr)	***2019***			***2020***			***2021***			***Total***		
	n	VRE (+) n (%)	*P*	n	VRE (+) n (%)	*P*	n	VRE (+) n (%)	*P*	n	VRE (+) n (%)	*P*
0-1	320	272	0.02	550	144	0.00	448	178	0.00	131	594	0.00
	8	(8.5)	$\chi^2=7.$	2	(2.6)	$\chi^2=7$	2	(4.0)	$\chi^2=6$	92	(4.5)	$\chi^2=8$
2-17	795	50	822	997	13	6.944	935	46	0.786	272	109	9.242
		(6.3)			(1.3)			(4.9)		7	(4.0)	
≥18	538	379		418	234		623	469		158	1082	
	9	(7.0)		5	(5.6)		2	(7.5)		06	(6.9)	
Total	939	701		106	391		116	693		317	1785	
	2	(7.5)		84	(3.7)		49	(6.0)		25	(5.6)	

Table 2 displays the distribution of VRE positivity among pediatric patients aged 0-17 yr, categorized by clinics and years. Table 2 indicates that the COVID-19 intensive care unit, pediatric intensive care unit, and neonatal clinic exhibited the highest rates on a total patient basis, with percentages of 27.7%, 10.5%, and 9.3%, respectively. The pediatric clinic exhibited the smallest proportion, measuring 0.9%. The prevalence of vancomycin-resistant enterococci (VRE) in pediatric patients exhibits variability across different clinical settings. However, a statistically significant reduction in the overall incidence of VRE positivity has been observed over the years ($\chi 2=187.434$, $P=0.00$). The observed reduction in percentage is believed to be attributable to infection control precautions and strict contact isolation implemented in response to the COVID-19 outbreak.

Table 2: Distribution of VRE positivity in children (0-17 yr) by clinics and years

Clinics	*2019*		*2020*		*2021*		*Toplam*	
	n	VRE (+) n (%)	n	VRE (+) n (%)	n	VRE (+) n (%)	n	VRE (+) n (%)
COVID-19 ICU	0	0	4	2 (50.0)	43	11 (25.6)	47	13 (27.7)
Neonatal clinic	108	14 (13.0)	114	6 (5.3)	68	7 (10.3)	290	27 (9.3)
Neonatal ICU	2775	217 (7.8)	2579	97 (3.8)	2931	117 (4.0)	8285	431 (5.2)
Infant clinic	124	6 (4.8)	306	11 (3.6)	97	8 (8.3)	527	25 (4.7)
Pediatric clinics	221	10 (4.5)	3110	8 (0.3)	1898	28 (1.5)	5229	46 (0.9)
Pediatric ICU	775	75 (9.7)	386	33 (8.6)	380	53 (14.0)	1541	161 (10.5)
Total	4003	322 (8.0)	6499	157 (5.7)	5417	224 (4.1)	15919	703 (4.4)

ICU: Intensive Care Unit

Table 3 displays the distribution of VRE positivity among adult patients aged 18 yr or older, categorized by clinics and years. Table 3 indicates that the COVID-19 clinic and the internal medicine intensive care unit exhibited the highest rates on a total patient basis, with 11.4% and 10.4%, respectively. The surgery clinic and internal medicine clinic exhibited the lowest percentages, with 3.1% and 3.8%, respectively. Statistically significant findings were observed regarding the reduction in the rate of VRE positivity in the COVID-19 clinic and COVID-19 intensive care unit over time ($P \leq 0.05$). There is a significant increase in anesthesia and surgical intensive care units over the years ($P \leq 0.05$). In 2020, there is a serious decrease in internal medicine clinics and internal medicine intensive care units ($P \leq 0.05$). Although the rate of VRE positivity in adults showed a statistically significant decrease in 2020 compared to 2019, it increased again in 2021 ($\chi 2=15.139$, $P=0.0005$).

Table 3: Distribution of VRE positivity in adults (≥18 yr) by clinics and years

Clinics	*2019*		*2020*		*2021*		*Total*	
	n	VRE (+) n (%)	n	VRE (+) n (%)	n	VRE (+) n (%)	n	VRE (+) n (%)
COVID-19 clinic	0	0	370	79 (21.4)	995	77 (7.7)	1365	156 (11.4)
COVID-19 ICU	0	0	87	12 (13.8)	970	47 (4.9)	1057	59 (5.6)
Anesthesia ICU	1117	66 (5.9)	822	57 (6.9)	1336	119 (8.9)	3275	242 (7.4)
Surgical clinics	171	4 (2.3)	93	3 (3.2)	227	8 (3.5)	491	15 (3.1)
Surgical ICU	834	14 (1.7)	353	26 (7.4)	529	41 (7.8)	1716	81 (4.7)
Internal medicine clinics	1522	80 (5.3)	1887	17 (0.9)	1027	70 (6.8)	4436	167 (3.8)
Internal medicine ICU	1745	215 (12.3)	573	40 (7.0)	1148	107 (9.3)	3466	362 (10.4)
Total	5389	379 (7.0)	4185	234 (5.6)	6232	469 (7.5)	15806	1082 (6.9)

ICU: Intensive Care Unit

Discussion

The management of infections associated with VRE poses a significant challenge due to the co-existence of severe comorbidities, a severe clinical course, and the potential for cross-resistance to non-vancomycin antibiotics. Enterococci's ability to survive for extended periods in hospital environments is primarily attributed to their intrinsic resistance to numerous antibiotics, coupled with their capability to acquire resistance through mutation or transposon/plasmid transfer to most of the antibiotics commonly employed. Given the high prevalence of resistance to beta-lactam and glycopeptide antibiotics among enterococci, the use of aminoglycosides in combination therapy is recommended to achieve bactericidal synergism in the treatment of enterococcal infections (15). VRE has been observed to result in clinical presentations such as infective endocarditis, bacteremia, and urinary tract infections, particularly in the setting of intensive care units. The transmission of VRE is attributed to indirect contact with the contaminated hands of healthcare personnel, direct contact with colonized or infected patients, and proximity to contaminated care equipment or environmental surfaces (16). Factors such as the presence of serious underlying diseases, prolonged hospitalization, and long-term use of broad-spectrum antibiotics increase the risk of VRE colonization. Colonization periods ranging from 7 wk to 3 yr have been reported in the literature (17).

The likelihood of VRE colonization is elevated among patients who undergo hospitalization to high-risk units, including intensive care units, oncology, transplantation, or sugical clinics. This is particularly true for patients who had undergone intra-abdominal or cardiothoracic surgery, had prolonged hospital stays, subjected to extended courses of multiple antibiotics, or had an indwelling catheter. Factors contributing to colonization by VRE include prolonged hospitalization in the intensive care unit exceeding three days, chronic dialysis, and a history of two or more hospitalizations within the past year (18). Extended periods of hospitalization are associated with an elevated risk of transmission among patients. There was no significant difference in the duration of hospitalization between patients colonized with VRE and those not (19). In contrast, Bulut et al. (12) discovered a statistically significant increase in VRE colonization with the extension of hospitalization.

Kutlu et al. (20) conducted an investigation into the antibiotic histories of patients who exhibited VRE colonization, revealing that the administration of beta-lactam group antibiotics was ubiquitous among them. The findings indicate a correlation between the incidence of VRE colonization and the administration of vancomycin and third generation cephalosporins. Units that exhibited reduced usage of vancomycin and third-generation cephalosporins were associated with a lower incidence of VRE colonization.

The detection of VRE in high-risk patients is contingent upon the performance of surveillance screenings, as colonized patients are typically devoid of symptoms. The earlier the colonization is determined; the sooner possible infections will be prevented. Therefore, it is important to carry out screenings at regular intervals (21). Each hospital should establish a screening protocol for the follow-up of VRE colonization, and continuous surveillance should be performed in units with a VRE carriage rate of more than 20%. In units with a low VRE carriage rate, point prevalence screening will be more accurate in risky patients (8). Hospital administrations should establish a regular active surveillance policy for high-risk patients (22).

The microbiology laboratory has an important role in isolation practices due to VRE colonization and/or infection. Identification of bacteria and rapid and accurate determination of vancomycin resistance by the microbiology laboratory will contribute to the prevention of intra-hospital spread by enabling the initiation of strict contact isolation in the early period (23).

In a pediatrics hospital, 17.2% of the samples were found to be positive with VRE agar and

23% with real-time PCR (24). A concordance rate of 93% was observed between the two methods. In our hospital, real-time PCR method is used for VRE scanning in rectal swabs, and colonized patients are promptly identified and isolated.

The initial screening for VRE in a pediatric hospital revealed a prevalence rate of 14.6%, which decreased to 3.3% in the subsequent screening following the implementation of infection control measures (23). In their retrospective studies, the prevalence of VRE positivity was 6% in rectal swab samples and 4% in media cultures (11). Following the study, the hospital implemented a rigorous contact isolation program, resulting in the absence of VRE during the subsequent screening. During an epidemic period in the neonatal intensive care unit, VRE colonization was present in 8.1% of the samples obtained during screening (25). Following the implementation of infection control measures, the second screening revealed a VRE rate of 0.6%. The findings highlight the significance of implementing effective infection control strategies and stringent contact isolation protocols to mitigate the dissemination of colonization.

Bulut et al. (12) conducted a retrospective study and reported that the culture method revealed a 4.3% rate of VRE colonization in rectal swab samples of adult patients. In a retrospective study spanning seven years (13) a rise was observed in vancomycin resistance in rectal swab samples from 5.5% in 2013 to 11.6% in 2019. The rate for seven years was ascertained to be 6%. The prevalence of VRE in rectal swabs of adult individuals was 8.1% (14).

4.4% of patients were colonized with VRE during the screening process prior to their admission to the intensive care unit (26). The prevalence of VRE in the intensive care unit was 7.2% (27). 2.8% of patients were colonized during hospitalization, while 4.4% were colonized after hospitalization.

The rate of VRE positivity we discovered in our study (5.6%) is consistent with the findings of related investigations, according to our analysis of the literature. Upon examining the data across the three-year period, the rates of positivity are comparatively greater in the adult population (6.9%) compared to the pediatric population (4.0% for 0-1 yr old; 4.5% for 2-17 yr old).

The emergence of the COVID-19 pandemic in Mar 2020, marked by the initial detection of the first case in our country, has had a discernible impact on the VRE positivity rate. Specifically, while the rate decreased in 2020, it has since experienced an upward trend in 2021, coinciding with the resumption of routine patient admissions. The observed decline in the year 2020 is hypothesized to be attributable to the implementation of infection control measures and stringent contact isolation protocols in response to the COVID-19 pandemic.

Conclusion

Patients with a positive VRE test should be rapidly identified and isolated, rectal screening samples should be taken, and screening should be continued until a negative culture result is obtained. Early detection of VRE colonizations and rapid taking of necessary isolation measures will largely prevent in-hospital spread and infections. As a method, molecular real-time PCR is very important in rapid detection of cases and colonization screening. Additionally, due to the increasing prevalence of vancomycin resistance, caution should be exercised in the use of unnecessary and inappropriate glycopeptides. In order to reduce VRE carriage, colonization and infections, we recommend increasing infection control measures, providing more frequent in-service training to employees, performing rectal VRE screenings more regularly, and especially restricting the use of glycopeptides.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors did not declare any conflicts of interest

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