Prevalence of Vancomycin-Resistant Enterococcus in a Teaching Hospital in Kashan, Iran

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Abstract

Background: Enterococci bacteria are part of the intestinal microbial flora of humans and animals. However, the widespread use of antibiotics causes antibiotic resistance among these bacteria, making it necessary to identify effective antimicrobial agents against them.

Objectives: This study aimed to investigate the phenotypic prevalence of vancomycin-resistant enterococci (VRE) in the clinical samples of patients admitted to Beheshti hospital in Kashan.

Materials and Methods: This cross-sectional descriptive study was performed on 110 enterococci isolated from the clinical samples of hospitalized patients during 2017-2018. Vancomycin-resistant cases were identified and recorded after recording clinical and demographic information. Finally, the groups were statistically compared using chi-square and Fisher’s exact tests.

Results: The present study findings demonstrated that the prevalence of VRE was 37.3%. There was a significant association between the prevalence of VRE and older age, diabetes, history of antibiotic use, and more extended hospital stays. Conversely, no significant relationship was found between VRE prevalence and gender, blood pressure, heart disease, year of sampling, and type of clinical sample.

Conclusion: Overall, the incidence of vancomycin resistance in enterococci is increasing, which can be reduced by identifying effective antimicrobial agents and providing appropriate training to the medical staff and the general public.

Keywords: Enterococcus, Vancomycin, Resistance, Kashan

Background

Enterococci are important nosocomial pathogens. Enterococcus species, namely, Enterococcus faecalis and Enterococcus faecium, are opportunistic pathogens that can cause hospital-acquired infections. The drug of choice for treating enterococcal severe infections is commonly aminoglycoside plus vancomycin (anti-cell wall antibiotic). The use of broad-spectrum antibiotics causes remarkable challenges, including the emergence of vancomycin resistance in enterococci. Enterococci are considered low-grade pathogens; however, they can rapidly acquire virulence genes and exchange them among different species.

Approximately 30 years after the introduction of vancomycin, vancomycin-resistant enterococci (VRE) were first reported in 1986. The VanA and VanB operons are functionally similar operons that induce vancomycin resistance in enterococci. Person-to-person transmission and selective antibiotic pressure are the reasons for the emergence and spread of VRE in hospital settings and even VRE outbreaks caused by the spread of a single clone. Risk factors identified in other studies for VRE colonization include transplantation, long-term hospital stay, contact with intensive care units (ICUs), exposure to antibiotics, and hematologic malignancies.

The spread of enterococcal infections has been reported to be associated with different risk factors, including age, hemodialysis, central venous catheter placement, history of antibiotic use, surgery, catheterization, long duration of hospitalization, and underlying immunosuppressive diseases such as human immunodeficiency virus, cancer, and diabetics. Numerous studies have shown that nosocomial VRE infections are related to long-term hospital stays and high mortality rates.

Out of 66 000 enterococcal infections identified in the USA in 2013, 20 000 (30%) cases were VRE-related, and above 1300 deaths with VRE infections were recognized. In addition, a great VRE outbreak happened in Lyon France between December 2013 and July 2014. According to a global surveillance summary, the prevalence of VRE ranges from 1% to 9.8% in different areas, including Asia, the Pacific, Europe, and America. In comparison, the prevalence of antibiotic-resistant enterococci in Africa and Ethiopia ranges from 2.2% to 76% and 6.3% to 95.5%, respectively.

The overall prevalence of VRE in nursing facilities has been reported to be between 5% and 18%, and even 50% in one report. In a study, the E. faecium infection was
found to be related to increased mortality and antibiotic resistance. VRE show high resistance to vancomycin, ampicillin, and penicillin, generally the most appropriate anti-enterococci antibiotics. The spread of VRE is a challenge for clinicians, especially in controlling and treating severe infections. Thus, to prevent, control, and manage vancomycin-resistant enterococcal infections, it is important to investigate the prevalence and risk factors of infections caused by Enterococcus species.

The irregular use of antibiotics to treat or control infections in humans or animals causes the spread of antibiotic-resistant bacteria. Few studies have evaluated the risk factors of nosocomial VRE infections in Beheshti hospital in Kashan. Therefore, there is limited knowledge of the risk factors of nosocomial diseases produced by VRE. This study sought to investigate the prevalence and risk factors of nosocomial infections produced by VRE in Beheshti hospital in Kashan, Iran from 2017 to 2018.

Patients and Methods
Setting, Participants, and Data Sources
This cross-sectional descriptive-analytical study was conducted at Beheshti hospital in Kashan, Iran. All patients with Enterococcus-positive culture samples and complete medical records in Beheshti Hospital in Kashan during 2017-2019 were included in the study. However, patients with defects in their medical records were excluded from the study.

The sample size was calculated based on a prevalence of 14.6% for Enterococcus-positive culture samples according to a previous study, a 95% confidence interval, and a 7% acceptable error.

The study participants were all patients admitted to different hospital wards (e.g., female, male, internal, surgical, ICU, and infectious wards). The clinical samples were collected from the trachea, urine, blood, wound, pleura, abscess, and ascites.

Sample Processing and Culture Identification
The samples collected from each patient were streaked on bile esculin agar and sterile 6.5% NaCl broth media and incubated at 37°C for 24-48 hours. The grown colonies were selected to characterize Enterococcus species. Each isolate was assessed using colony morphology, gram staining, and catalase tests. An antibiotic susceptibility test (vancomycin: 30 µg) was performed for the isolates by the disc diffusion method on Mueller-Hinton agar plates; then, the plates were incubated at 37°C for 24 hours. The results were reported according to Clinical and Laboratory Standards Institute (2018) guidelines.

Data Collection Procedures
The medical files of the study participants were reviewed, and all their clinical and demographic data were extracted and collected in a checklist especially designed for this study.

The data extracted from the patient’s medical records included the sample type, sampling date, file number, age, gender, history of underlying diseases, duration of hospitalization, and history of antibiotic use in the last month.

Statistical Analysis
The obtained data were analyzed by SPSS software, version 16 (SPSS, Chicago, IL, USA). A P value of <0.05 was considered statistically significant, and data were analyzed using descriptive and chi-square test statistics.

Results
Prevalence of Enterococcus Species
During the study period, 110 enterococci isolated from the clinical samples of hospitalized patients with enterococcal infections underwent investigation (2017-2019). Moreover, most Enterococcus species were isolated from urine samples, followed by endotracheal tube specimens (Figure 1).

The incidence of VRE isolated from the clinical samples of patients admitted to Beheshti hospital in Kashan was 41 (37.3%) cases, and 69 (62.7%) cases were sensitive to vancomycin. Other details are presented in Table 1.

Analysis of Factors Related to Vancomycin-resistant Enterococcus spp
There was no statistically significant relationship between the prevalence of vancomycin-resistant Enterococcus spp. in the clinical samples of patients admitted to Beheshti Hospital in Kashan and their gender (P > 0.05).

Further, no significant difference was detected in the frequency of Enterococcus spp. isolated from different clinical specimens. However, a significant relationship was found between the age of over 60 years and the prevalence of VRE (P = 0.016).

Diabetes mellitus was the only underlying condition that was significantly related to the prevalence of VRE.

The analysis of the results revealed that a history of hospitalization for more than 7 days was a significant risk factor for the prevalence of VRE in the clinical samples of patients hospitalized in Beheshti Hospital in Kashan. A history of antibiotic use in the last month was also identified as a significant risk factor for VRE prevalence.

Demographic data and clinical characteristics of patients with infections caused by Enterococcus spp. are provided in Table 2.

Discussion
The present study results showed that the frequency of VRE was 37.3%, which is comparable with the findings of previous studies conducted in Mexico (55.7%), Ethiopia (45.5%), Gondar (41.5%), and Nigeria (42.9%).
However, this result is higher than those reported in other studies conducted in southern Ethiopia (7.5%), Canada (4%), India (13.7%), and Iran (16.9% and 13.1%). The differences between the results of these studies may be attributed to differences in the sample size, geographical area, study time, sampling method, socioeconomic position, daily habits, drug prescription pattern, and the use of media with different vancomycin concentrations for strain screening. The increasing incidence of transfer resistance genes in enterococci in hospitals to other bacteria is a major threat to human health worldwide.

Based on the findings of this study, a significant association was found between the prevalence of VRE and older age, diabetes, history of antibiotic use, and length of hospitalization. Contrarily, there was no significant association between VRE prevalence and gender, blood pressure, heart disease, year of sampling, and type of clinical sample.

Some studies focused on the use of antibiotics and reported its link with VRE colonization. In the current study, the risk of presenting with a positive VRE sample increased with the number of received antibiotics. This is easily explainable because bacterial resistance is favored by the pressure of antibiotic selection, thus enabling cross-transmission. Such results have previously been reported by Mathis et al and McEvoy et al, describing a significant association between the number of applied antibiotics and VRE colonization.

Due to genetic changes among the strains, drug resistance to vancomycin antibiotics has a different frequency in Iran and other parts of the world. The resistance of these bacteria to glycopeptide antibiotics is related to \( \text{van A}, \text{B}, \text{C}, \text{D}, \text{and E} \) genes, reducing the effect of these antibiotics on the wall of these bacteria.

The most crucial important gene is \( \text{van A} \), which with the highest prevalence, causes high-level resistance, while with a lower prevalence, it causes resistance to vancomycin, vancomycin, and teicoplanin; \( \text{van B} \) gene does not cause resistance to teicoplanin. Both of the mentioned genes are located on transferable plasmids, which, under favorable conditions, can transfer between different strains of enterococcus and even other bacteria. The \( \text{van C} \) gene is also located on the chromosome, causes
low-level resistance to vancomycin, and has a lower prevalence than the other two genes. The \textit{van E} and \textit{D} genes also cause low-level resistance to vancomycin and are extremely rare.\textsuperscript{21}

The results demonstrated that \textit{Enterococcus} species mainly were mostly isolated from urine samples, followed by endotracheal tube specimens. Compared to other studies performed all over the world, this amount is different and variable. This result is similar to the findings of previous studies conducted in India, Iran, and Addis Ababa, Ethiopia. This is probably because of the nearness of the anal opening to the urethra. Because enterococci are commensal bacteria in the gastrointestinal tract and could attack the bladder through the urethral opening. In a research conducted in Ethiopia, 5% of VRE was reported in the feces of hospitalized patients.\textsuperscript{27} In a study performed in the United States, 30.4% of VRE was reported from clinical samples.\textsuperscript{8,28}

Goudarzi \textit{et al} conducted a study on 690 enterococcal isolates, among which 439 (64%), 228 (33%), and 23 (3%) cases were \textit{E. faecalis}, \textit{E. faecium}, and other enterococci, respectively. Of the 667 collected isolates, the highest and lowest numbers were collected from urine and catheter samples, respectively. The prevalence rate of vancomycin-resistant \textit{E. faecium} isolates was 51%, and there was no correlation between the prevalence of resistant isolates and the sampling location. However, in the current study, the prevalence of vancomycin-resistant isolates collected from pleural fluid samples was 100%, and no significant difference was obtained due to the small number of isolates.\textsuperscript{29}

In line with the results of the present study, some studies confirmed the relationship between the length of hospitalization and the increased risk of VRE colonization \textit{(P < 0.05)}.\textsuperscript{17-19,29}

The length of hospitalization is one of the important factors in increasing the prevalence of hospital infections and VRE colonization. The use of invasive monitoring and holding devices and various types of vascular and urinary catheters has increased the risk of VRE colonization and hospital infections.\textsuperscript{29}

In another study by Moghimbeigi \textit{et al}, the prevalence of VRE in Iran was 16% and 1% in hospitalized patients and outpatients, respectively. The prevalence of VRE among hospitalized patients in the mentioned study was half of the result found in the current study. This variance could be due to differences in the sample size, sampling method, inclusion and exclusion criteria, and antibiotic prescription pattern.\textsuperscript{20}

Goel \textit{et al} reported that out of 115 enterococci isolated from patients’ urine samples, the overall prevalence of VRE was 11.3%. In their study, a significant association was found between the prevalence of VRE and age. The majority of VRE in the mentioned study was extremely lower (3 times lower) than the result found in the present study.\textsuperscript{31} This contradiction may be due to differences in the sample size, sampling location, and study location and time.

In their study, Amberpet \textit{et al} found that older age was associated with increased resistance.\textsuperscript{24} These findings are in line with the present study results. However, the two studies used different methods in this regard. They employed the polymerase chain reaction method and concluded that totally the VRE isolates were positive \textit{van A} gene.

Amberpet \textit{et al} reported that diabetes was associated with increased resistance.\textsuperscript{24} The results of the present study are consistent with those of our study. Conversely, Jabbari-Shiade \textit{et al}\textsuperscript{25} and Mathis \textit{et al}\textsuperscript{26} observed no significant association between VRE colonization and diabetes. The contradiction in the results of the present studies with those of our study is probably due to the difference in the gender, age of the people under investigation, and the type of department under investigation.

\textbf{Conclusion}

The findings represented that there was a significant relationship between the prevalence of VRE (37.3%) and older age, diabetes, history of antibiotic use, and length of hospitalization. However, there was no significant association between VRE prevalence and gender, blood pressure, heart disease, year of sampling, and type of clinical sample. The increase in VRE reported a serious problem in treating enterococcal infections. VRE have become a vital hospital pathogen due to their fast spread, associated high mortality rates, and the possibility of transferring vancomycin resistance genes to other pathogens such as \textit{Staphylococcus}. Therefore, much attention should be paid to preventing the transmission and spread of VRE. Therefore, applying nosocomial infection control guidelines, continuing personnel training, reducing the indiscriminate prescription of antibiotics, and effectively controlling colonization and infections caused by VRE in different hospital departments seem necessary. The study on antibiotic resistance and the obtained results may be a warning to improve the public health situation in the current society.

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Competing Interests
The authors declare that they have no competing interests.

Ethical Approval
The research began after obtaining the approval of the Medical Ethics Committee of Kashan University of Medical Sciences, Kashan, Iran (Code Number: IR.KAUMS.NUHEPM.REC.1399.048).

References


