Intestinal Colonization of Vancomycin-Resistant Enterococci Isolates among Patients in an Iranian Hospital

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Vancomycin-resistant enterococci (VRE) remain as one of the important cause of nosocomial infections. Reports of the VRE carriers are increasing worldwide. In this study, a total of 220 patients was screened for vancomycin-resistant enterococci colonization, of which 36 (16.3%) were VRE carriers. Among the VRE isolates, 17 were *vanA* positive. The distribution of VRE carriers in different wards that pose high risks for healthcare infection to hospitalized patients emphasizes applying suitable infection control strategies to prevent the dissemination of the organism. This is the first report from Iran in which a *vanA*-containing enterococci were isolated from intestinal colonization of patients. Strict measures are required to control the further spread of VRE strains in the Iranian patients.

Keywords: Vancomycin-resistant enterococci, Antibiotic resistance, Colonization surveillance, Hospital infection control, Patients.

Vancomycin-resistant enterococci (VRE) have been known as one of the most important nosocomial pathogens worldwide, and their colonization has occurred in the hospital setting. The screening of patients at high risk of VRE colonization is recommended to prevent transmission of VRE¹. Carriage of VRE by patients is important from two aspects: (1) the source of Enterococci infections could be endogenous, and carriage of VRE may predispose the carrier to Enterococcal infections. (2) Carriers can serve as the reservoir for vancomycin-resistant enterococci and spread these to other hospitalized patients^{1,2,3}. In Iran, no study has been performed to evaluate the

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prevalence of VRE intestinal tract colonization in the hospitalized patients. We have tried to fill this knowledge gap and have undertaken this study to determine the prevalence of intestinal colonization and antibiotic resistance proûle of VRE strains in patients, in a hospital afûliated to Tehran University of Medical Sciences, in Iran.

METHODS

This cross-sectional study was performed among 220 admitted patients from January to December 2015. These patients admitted to hospital were screened for gastrointestinal carriage of VRE. A fecal sample was taken during the hospitalization. Stool specimens were inoculated onto the bile-esculin agar plates and into the bile-

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esculin broth. Colonies growing on bile-esculin agar with the dark brown halo were identified by Gram staining; absence of catalase; and growth on 40% bile, in 6.5% sodium chloride, and for further identification was based on the conventional biochemical tests. Antibiotic sensitivity of the enterococci isolates was investigated by disk diffusion and agar dilution methods. All tests were performed and interpreted according to Clinical and Laboratory Standards Institute guidelines. Vancomycin resistance was determined by using two methods, including vancomycin disk diffusion test (d"14 mm indicated VRE) and vancomycin MICs (e"32 mg/ mL indicated VRE)⁴. Additionally, vanA, vanB and vanC genes were determined by polymerase chain reaction ⁵. The protocol was approved by the local Ethical Committee of Tehran University of Medical Sciences, and informed consent was taken from all subjects. Data were analyzed using descriptive statistics.

RESULTS

A total of 220 patients (hospitalized more than ten days) out of 492 (the total number of hospitalized patients) participated in the study. The prevalence of intestinal carriage of enterococci was 25.4% (56), of which 9.1% (20) and 16.3% (36) were vancomycin-sensitive enterococci and VRE, respectively (Table 1). Polymerase chain reaction testing of 36 VRE isolates identified 17 (47.2%) positive for the *vanA* gene. The MICs for vancomycin was between 32 to 256 mg/mL and 0.25 to 1 mg/mL for VRE and VSE strains, respectively. Table 2 shows the distribution of VSE and VRE carriage in different wards. VRE carriers were seen in hematology, dialysis unit, internal medicine ward, infectious diseases ward, surgical ward and intensive care unit. Dialysis unit, intensive care unit and surgical ward showed the highest rate of VRE carriage. The HICPAC Guidelines recommended for the management of the infection control measures to reduce cross transmission among hospitalized patients: these included restriction of vancomycin use; education of hospital staff (including hand washing with an antiseptic soap or a waterless antiseptic agent); routine screening for vancomycin resistance among clinical isolates; contact isolation for patients with VRE²⁰. Table 3 represents the antibiotic resistance pattern of Enterococci isolates (VRE and VSE). All VRE isolates were sensitive to linezolid and tigecycline. 7 (35%) of 20 VSE and 29 (80.5%) of 36 VRE isolates were resistant to tetracycline. The MICs for teicoplanin against resistant VRE strains were >16 mg/mL and for the resistant VSE strains were >4 mg/mL.

DISCUSSION

Hospitalized patients are at higher risk of the acquisition of VRE. Risk factors for VRE colonization included vancomycin use, hospitalization, ICU stays, receipt of antibiotic, anemia, leukocytosis, diabetes mellitus, gastrointestinal procedures and acute renal failure ¹⁶. In this study, out of 220, 56 (25.4%) of subjects were enterococci carriers, of which 36 (16.3%) were VRE carriers. Askarian et al., in Shiraz Namazi Hospital reported that 99 out of 700 patients (14%) were colonized with VRE 6. The estimated prevalence in this study correlates with reported ranges. The rate of VRE colonization varies widely in different studies. In the study of Wisplinghoff *et al.* was shown that the prevalence of vancomycin resistant enterococci in hospitalized patients was 2 % and 60 % for E. fecalis and E. faecium, respectively¹². The prevalence of intestinal colonization of VRE in patients at Rawson Hospital (12.20%) was similar to that reported by Coque et al.¹³ in hospitals in the USA, and was higher than that reported by Endz et al. ¹⁴ in Europe (4.9%). Zanella et al. 15 reported vanA Enterococcus clinical isolates from colonized patients obtained during a nosocomial outbreak in a hospital in São Paulo, Brazil.

The important ûnding of this study is that the VRE carriers were mainly those who hospitalized prolonged in the wards in which the risk of nosocomial infection is relatively high (Table-1). Similar ûndings have been reported in other studies ⁷.

VRE strains isolated in the present study shown three patterns of MIC values for vancomycin. Five VRE isolates were *vanA*-gene positive and had MIC values of 256 mg/mL. One VRE isolate was *vanA*-gene positive and had the MIC value of 32 mg/mL for vancomycin. Two isolates were *vanA*-gene-positive (MIC: 16 mg/ mL) enterococci strains.

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28Septic arthritis $E. gallinarum$ $ 14$ 29Chronic mycloid leukemia $E. faecium$ $ 16$ 30Septic arthritis $E. gallinarum$ $ 15$ 31Septic arthritis $E. gallinarum$ $ 16$ 32Diabetes mellitus $E. faecalis$ $ 19$ 33Chronic mycloid leukemia $E. faecalis$ $ 11$ 34Septic arthritis $E. faecalis$ $ 12$ 35Septic arthritis $E. faecalis$ $ 12$ 36Ischemic heart disease $E. hirroe 10$		Chronic myeloid leukemia	E. faecium		10	Vancomycin, azithromycin, amoxicillin-clavulanate
29Chronic myeloid leukemia $E. faecium$ $ 16$ 30Septic arthritis $E. gallinarum$ $ 15$ 31Septic arthritis $E. gallinarum$ $ 20$ 32Diabetes mellitus $E. faecalis$ $ 19$ 33Chronic myeloid leukemia $E. faecalis$ $ 11$ 34Septic arthritis $E. faecalis$ $ 12$ 35Septic arthritis $E. herococcus Spp$ $ 24$ 36Ischemic heart disease $E. hirae$ $ 10$		Septic arthritis			14	None
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31Septic arthritis $E. gallinarum$ $ 20$ 32Diabetes mellitus $E. faecalis$ $ 19$ 33Chronic myeloid leukemia $E. faecalis$ $ 11$ 34Septic arthritis $E. faecalis$ $ 12$ 35Septic arthritis $Enterococcus Spp$ $ 24$ 36Ischemic heart disease $E. hirae$ $ 10$		Septic arthritis			15	Ciprofloxacin, gentamicin
32Diabetes mellitus $E. faecalis$ -1933Chronic myeloid leukemia $E. faecum$ -1134Septic arthritis $E. faecalis$ -1235Septic arthritis $Enterococcus Spp$ -2436Ischemic heart disease $E. hirae$ -10		Septic arthritis	E. gallinarum		20	None
33Chronic myeloid leukemia $E. faecium$ -1134Septic arthritis $E. faecalis$ -12135Septic arthritis $Enterococcus Spp$ -24336Ischemic heart disease $E. hirae$ -100		Diabetes mellitus	E. faecalis		19	None
34 Septic arthritis E. faecalis - 12 1 35 Septic arthritis Enterococcus Spp - 24 2 36 Ischemic heart disease E. hirae - 10 0		Chronic myeloid leukemia	E. faecium		11	Vancomycin
35 Septic arthritis Enterococcus Spp - 24 36 Ischemic heart disease E. hirae - 10 0		Septic arthritis	E. faecalis		12	Piperacillin-tazobactam, ciprofloxacin
36 Ischemic heart disease E. hirae - 10		Septic arthritis	Enterococcus Spp		24	Vancomycin, imipenem
		Ischemic heart disease	E. hirae	ı	10	Ciprofloxacin, gentamicin

Table 1. Type of antibiotic(s) received and days of hospitalization among Vancomycin-resistant enterococci strains isolated from intestinal tract of patients

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Morris *et al.*, ¹⁷ found, that the restriction of vancomycin use, cannot lead to reducing the rate of colonization with VRE in a hospital where VRE were endemic. Nonetheless, use of vancomycin was probably the crucial factor in the initial emergence of VRE, and use of vancomycin, cephalosporins, and other antibiotics probably maintains the selective pressure for VRE.

Transmission of VRE is a major concern because the pathogen may develop phenotypic resistance during a course of antibiotic therapy ^{8,9}. VRE can colonize the gastrointestinal tract and the skin, thus producing an epidemiological risk similar to that of nosocomial gut flora (eg, antibiotic-resistant gram-negative bacilli) and nosocomial colonizers (eg, methicillin-resistant *Staphylococcus aureus*). Moreover, because environmental contamination occurs frequently, it is associated with an epidemiological risk similar to that of *Clostridium difficile*.

Furthermore, because colonization seems to be persistent in the gastrointestinal tract, persistently colonized patients can be a reservoir from which VRE can be continually spread¹⁹.

The high resistance rate for most used antibiotics (eg, Ciprofloxacin) was observed

 Table 2. Prevalence of intestinal carriage of VRE and VSE strains among patients in relationship with hospital wards

Ward	Carriage, n (%)	With VRE, n (%)	With VSE, n (%)
Hematology	7 (12.5)	2 (28.6)	5(71.4)
Dialysis	13 (23.2)	9 (69.2)	4(30.8)
Internal	4 (7.1)	4 (100)	-
Infectious	4 (7.1)	2 (50)	2(50)
Operating room	-	-	-
Clinic	-	-	-
Surgery	10(17)	8(80)	2(20)
Angiography	1 (1.7)	-	1(100)
Cardiology	-	-	-
Cardiac care unit	-	-	-
Emergency	-	-	-
Intensive care unit	17(30.3)	11 (64.7)	6(35.3)
Total	56 (25.4)	36 (16.3)	20 (9.1)

Table 3. Antibiotic susceptibility proûles of enterococci strains isolated from the intestinal tract of patients by disk diffusion method

Antibiotic	VRE (N = 36), n (%)			VSE (N = 20), n (%)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Erythromycin	6 (16.6)	4 (11.2)	26 (72.2)	12 (60)	6 (30)	2 (10)
Ampicillin	23 (63.8)	4 (11.2)	9 (25)	9 (45)	6 (30)	5 (25)
Vancomycin	-	-	36 (100)	20 (100)	-	-
Teicoplanin	-	1 (2.8)	35 (97.2)	20 (100)	-	-
Tetracycline	6 (16.6)	2 (5.5)	28 (77.7)	10 (50)	2 (10)	8 (40)
Doxycycline	13	1	22	16 (80)	1 (5)	3 (15)
Ciprofloxacin	6 (16.6)	9 (25)	21 (58.4)	7 (35)	4 (20)	9 (45)
Levofloxacin	14 (39)	6 (16.6)	16 (44.4)	11 (55)	2(10)	7 (35)
Nitrofurantoin	34 (94.4)	-	2 (5.6)	20 (100)	-	-
Rifampicin	3 (8.3)	2 (5.5)	31 (86.1)	2 (10)	9 (45)	9 (45)
Linezolid	36 (100)	-	-	20 (100)	-	-
Tigecyclin	36 (100)	-	-	20 (100)	-	-
Quinupristin/ dalfopristin	12 (33.3)	1 (2.7)	23 (63.8)	8 (40)	5 (25)	7 (35)

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among VRE isolates. None of the isolates tested in our study were resistant to tigecycline and linezolid. However, other studies have reported the colonization of linezolid-resistant enterococci strains among patients ¹⁰.

Unexpectedly, in our study, resistance to quinupristin/dalfopristin was observed in both VSE and VRE isolates. Werner et al., ¹⁸ suggested an association of quinupristin/dalfopristin resistance with the use of virginiamycin as a feed additive and indicate the possibility of transfer to humans via the food chain.

Our study shows that VRE colonization among patients in a University hospital in Iran is around 16.3%. Secondly, this unrecognized silent carriage of VRE could be one of the most important factors leading to spread of VRE to others in the hospital. Therefore, patients who were colonized with the VRE strain in our hospital were subjected to isolation precautions, even in the case of repetitive negative follow-up cultures.

In conclusion, the distribution of VRE carriers is more likely in certain high risk wards like ICU hence suitable application infection control strategies is very important to prevent the spread of the organism. The occurrence of the VRE strains demonstrates the need for using suitable approaches for treatment and diagnosis of VRE infections. More studies are necessary to reveal the relative contributions of patient-related factors, antibiotic treatment, and characteristics of VRE colonization ¹¹. The routine surveillance cultures are one way to identify asymptomatic VRE-colonized patients. Infection prevention and control strategies include improving compliance with hand hygiene, enhancing environmental cleaning, ensuring antimicrobial stewardship, and identifying and isolating VRE carriers to interrupt transmission and reduce VRE infections. Strict measures will be required to control the further spread of VRE pathogens in hospital settings. More attention should be paid to the efficacy of prevention of VRE colonization in patients.

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