



## ارزیابی شیوع سویه‌های انتروکوک مقاوم به ونکومايسين جدا شده از بیماران بستری شده در بخش مراقبت‌های ویژه شهر کاشان

سیده مرضیه جباری شیاده<sup>۱</sup>، رضوان منیری<sup>۲\*</sup>، احمد خورشیدی<sup>۳</sup>، محمد علی صبا<sup>۴</sup>، سید غلامعباس موسوی<sup>۵</sup>، مهدی صالحی<sup>۶</sup>  
<sup>۱</sup> کارشناس ارشد، کمیته تحقیقات دانشجویی، دانشگاه علوم پزشکی کاشان، آستاد، گروه میکروبیولوژی و ایمنولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی کاشان، <sup>۲</sup> دانشیار، گروه میکروبیولوژی و ایمنولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی کاشان، <sup>۳</sup> استادیار، گروه داخلی، بیمارستان شهید بهشتی، دانشگاه علوم پزشکی کاشان، <sup>۴</sup> کارشناس ارشد، مرکز تحقیقات علوم آناتومی، دانشگاه علوم پزشکی کاشان، <sup>۵</sup> مربی، کمیته تحقیقات دانشجویی، دانشگاه علوم پزشکی کاشان.

### چکیده

**سابقه و هدف:** انتروکوک‌ها بخشی از فلور طبیعی روده انسان و حیوانات را تشکیل می‌دهند. در صورت خروج این باکتری‌ها از مکان طبیعی شان، عفونت‌هایی مانند باکتری، اندوکاردیت و عفونت دستگاه ادراری را در بیماران بستری شده در بیمارستان ایجاد می‌کنند. این پژوهش با هدف ارزیابی شیوع سویه‌های انتروکوک مقاوم به ونکومايسين جدا شده از نمونه‌های مدفوع بیماران بخش مراقبت‌های ویژه انجام شد. **مواد و روش‌ها:** این پژوهش به صورت مقطعی-توصیفی بر روی ۱۵۶ نمونه سواب مدفوع جمع‌آوری شده از ۳ بخش مراقبت‌های ویژه بیمارستان شهید بهشتی کاشان انجام شد. تشخیص انتروکوک‌ها با رنگ آمیزی گرم و آزمون‌های بیوشیمیایی انجام شد. تست سنجش مقاومت آنتی‌بیوتیکی با استفاده از معیارهای CLSI انجام شد. سپس شناسایی انواع ژن‌های مقاومت به ونکومايسين با استفاده از روش PCR چندگانه انجام شد.

**یافته‌ها:** انتروکوک‌ها در ۱۳۵ نمونه مدفوع (۸۶/۵٪) تشخیص داده شدند. میزان شیوع سویه‌های مقاوم به ونکومايسين ۴۹/۹٪ (۵۸ مورد) بود. فراوانی ژن‌های *vanA* و *vanC* به ترتیب ۶۹٪ و ۶۷/۹٪ بود. در این پژوهش هیچ کدام از ژن‌های *vanB*، *vanD*، *vanE* و *vanG* شناسایی نشد. ۵۹/۲٪ از بیماران دارای ژن مقاومت به ونکومايسين به ۳ تا ۴ آنتی‌بیوتیک و ۳۵/۴٪ از آن‌ها به ۱ تا ۲ آنتی‌بیوتیک مقاوم بودند. **نتیجه‌گیری:** نتایج این پژوهش نشان داد که مصرف آنتی‌بیوتیک‌ها می‌تواند منجر به افزایش فنوتیپ مقاومت گردد. میزان شیوع سویه‌های مقاوم به ونکومايسين ۳/۶ برابر در بیمارانی که این آنتی‌بیوتیک را مصرف کرده بودند بیشتر بود. همچنین مشخص شد که افزایش مصرف تعداد آنتی‌بیوتیک‌ها از ۱ تا ۲ به ۳ تا ۴ آنتی‌بیوتیک موجب افزایش ۲/۶۵ برابری مقاومت آنتی‌بیوتیکی در انتروکوک‌ها می‌شود.

**واژگان کلیدی:** انتروکوک، ونکومايسين، مقاومت آنتی‌بیوتیکی

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\*آدرس برای مکاتبه: کاشان، دانشگاه علوم پزشکی، دانشکده پزشکی، گروه میکروبیولوژی و ایمنولوژی

تلفن: ۰۹۱۳۳۶۱۲۶۳۶، پست الکترونیک: moniri@kaums.ac.ir



## Evaluation of the prevalence of Vancomycin- resistant *Enterococci* strains isolated from patients in the ICU in Kashan

Seyedeh Marzieh Jabbari Shiadeh<sup>1</sup>, Rezvan Moniri<sup>2</sup>, Ahmad Khorshidi<sup>3</sup>, Mohammad ali Saba<sup>4</sup>,  
Seyed Gholam Abbas Mousavi<sup>5</sup>, Mahdi Salehi<sup>6</sup>

<sup>1</sup>M.Sc., Student Research Committee, Kashan University of Medical Science, Kashan, Iran.

<sup>2</sup>Professor, Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran.

<sup>3</sup>Associate Professor, Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran.

<sup>4</sup>Assistant Professor, Department of Internal Medicine, Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran.

<sup>5</sup>M.Sc., Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.

<sup>6</sup>Lecturer, Student Research Committee, Kashan University of Medical Science, Kashan, Iran.

### Abstract

**Background and Objectives:** *Enterococcus* is part of human and animal intestinal flora. The withdrawal of these bacteria from their original location causes infections such as bacteremia, endocarditis, and Urinary Tract Infection (UTI) in hospitalized patients. The aim of this study is to determine the prevalence of vancomycin-resistant *Enterococci* (VRE) strains and the phenotypes of the *Van* genes in *Enterococcus* isolated from rectal swabs of patients hospitalized in the Intensive Care Unit (ICU).

**Material and Methods:** In this cross-sectional study, 156 rectal-swab samples were collected from patients in three wards of ICUs in the Shahid Beheshti Hospital. *Enterococcus* was detected in samples with the Gram stain and biochemical tests. An antibiotic resistance test was done using CLSI criteria. Different types of vancomycin resistance genes were identified by the multiplex PCR technique.

**Results:** *Enterococcus* was detected in 135 rectal-swab samples (86.5%). The prevalence of VRE strains was 42.9% (58 cases). The frequency of *VanA* and *VanC* genes were 69% and 6.9%, respectively. In this study neither of *van B*, *D*, *E* and *G* genes were observed. 59.2% of patients who consumed 3 to 4 types of antibiotics, and 35.4% of those who consumed 1 to 2 types of antibiotics, had VRE.

**Conclusion:** Our findings highlight that antibiotic consumption can lead to increasing the resistance phenotypes. The prevalence of VRE was indicated 3.6 times more in patients who had consumed antibiotics. Also, with increasing number of antibiotic consumption of 1-2 to 3-4 types, risk of antibiotic-resistant *Enterococci* increases 2.65 times.

**Keywords:** *Enterococcus*, Vancomycin, Antibiotic Resistance.

### Introduction

*Enterococci* are part of the intestinal flora of humans and animals. They are the major cause of nosocomial infections in hospitalized patients (1,

2). Also they are the third most common cause of nosocomial bacteremia (3). More than 30 different species of *Enterococci* have been detected, but most of these infections are caused by *Enterococcus faecalis* and *Enterococcus faecium* (4). Many antibiotics, such as beta-lactams, macrolides, aminoglycosides and glycopeptides,

Correspondance to: Rezvan Moniri

Tel: +989133612636

E-mail: moniri@kaums.ac.ir

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are used for treatment of *Enterococcus* infections (5). But the optional drug for treatment of resistant *Enterococci* infections is vancomycin (6). This antibiotic is used as one of the last lines of treatment of resistant gram-positive bacterial infections (7). The first vancomycin-resistant *Enterococcus* (VRE) was found in France in 1986 (8, 9). Then VRE spread to other countries (10-13). VRE carry genes for resistance, and they are the main sources of infection in humans (14-17). There is an intrinsic resistance in *Enterococcus* to antibiotics, such as cephalosporins and some penicillins, and the prevalence of resistance to ampicillin and glycopeptide antibiotics such as vancomycin and teicoplanin has caused concern in recent years (18). This indicates a serious problem in the treatment of these infections (14, 19). Management of treatment will be in trouble because suitable antibiotics to treat these infections have decreased (1).

Resistance occurs by mutation or acquisition of related genes and allows *Enterococci* to survive in areas such as hospitals, where the anti-microbial agents are many. In addition, these genes are transferred from *Enterococcus* to the other gram-positive bacteria, making conditions worse. And if the infection becomes endemic in many hospitals, great challenge in treatment would result (20-22). On the other hand, other bacteria, including Gram-negative or anaerobic bacteria in the Gastrointestinal tract which live with *Enterococci*, are killed by consuming antibiotics, and this leads to the proliferation of resistant *Enterococci* and intestinal flora imbalance, thereby causing disease (6). Van A type resistant to both antibiotics (vancomycin and teicoplanin) and Van C has low level resistance to vancomycin and sensitivity to teicoplanin (23-25).

The aim of this study was to determine the prevalence of VRE strains and the phenotypes

of the *Van* genes in *Enterococci* isolated from rectal-swabs of patients hospitalized in the ICU in Shahid Beheshti Hospital, Kashan, Iran.

### Methods and Materials

**Isolation of bacteria:** In this cross-sectional study, 156 rectal-swab samples were collected from patients in three ICUs (Surgical ICU, Neurosurgery ICU and Medical ICU) in the Shahid Beheshti Hospital from November 2011 to April 2012. After getting permission from patients, rectal-swab samples were taken from them. All the samples were put in 5 ml sterilized containers with a 6.5% NaCl solution. Sampling was done after coordinating with ICU nurses and changing the position of the patients during a specific time in compliance with the project. All patients hospitalized in ICU with different reasons, were evaluated as to the presence of *Enterococcus* as the rectal flora and VRE. All the collected samples after 6 hours incubation at 37°C, were cultured on Bile Esculin Azide Agar (Merck Co.). After 24 hours incubating at 37°C, black colonies were detected due to the ability to hydrolyze Esculin and growth in the presence of Bile. These colonies were identified with gram-stain and biochemical tests, such as catalase reaction and the presence of *Pyro-Lidonyl* Arylamidase (PYR).

**Determining the VRE strain sensitivity:** All *Enterococcus* samples were cultured on Mueller-Hinton agar (Merck Co.) with the *McFarland 0.5 standard* dilution. For determining sensitivity, vancomycin (30 µg) and teicoplanin (30 µg) disks (Mast Co.) were used. The sensitivity was determined after a 24-hour incubating period of plates at 37 °C, based on the halo of lack of growth and according to the Clinical and Laboratory Standards Institute (CLSI-2012). This standard defines the halo of

lack of growth, which is a resistance indicator, equal to/or less than 14 for vancomycin and 10 for teicoplanin. Data were analyzed and evaluated by using the Software SPSS 11.5 version. Also a comparison between groups was performed by using Chi-square and Fisher exact tests. Also, a *p* value <0.05 was considered as significant.

**Determining Van genes with Multiplex PCR**  
 For Multiplex PCR, the reaction mixture was prepared with a final volume of 25 µl. For this purpose, 12 µl of deionized distilled water, 2.5 µl PCR buffer, 0.75 µl MgCl<sub>2</sub>, 0.5 µl dNTP, 1 µl of each primer (Reverse and Forward primers included a total of 4 µl) (Table 1), 0.25 µl of *Taq* DNA Polymerase and 5 µl of sample DNA, were mixed in a 1.5 microtube. Reactions were run in thermocycler (Eppendorf) using the following cycling parameters: 3 min of denaturation at 94°C, followed by 30 cycles of 1 min at 94°C (denaturing), 1 min at 55°C (annealing), and 1 min at 72°C (elongation), with a final extension at 72°C for 7 min. The presence of amplicons was confirmed by gel electrophoresis on a 1.8 % agarose gel and staining with ethidium bromide.

**Results**

In the present study, the prevalence of *Enterococcus* was 86.5% (135 of 156 cases). The mean and standard deviations of age in patients were 55.2 ± 25.6. Eighty seven samples were male (64.4%), and 48 samples were female (35.6%). The mean and standard deviations of age in male and female were 53.03 ± 26.1 and 59.06 ± 24.5, respectively (*p*=0.19).

Also, the prevalence of VRE strain was 42.9% (58 samples). The results of multiplex-PCR showed that 79.3% of strains were belong to *Enterococcus faecium*, followed by *Enterococcus faecalis* (15.5%), *Enterococcus gallinarum* (3.4%), and *Enterococcus casseliflavus* (3.4%). Frequency of *van A* and *van C* genes were 69% and 6.9%, respectively. However, other genes, such as *van B, D, E, G*, were not found to be resistant to vancomycin in isolated VRE. From vancomycin-resistant strains, 25 (39.7%), 16 (36.4%) and 17 (60.7%) samples were isolated from Surgical ICU, Neurosurgery ICU and Medical ICU, respectively, so that the highest percentage belongs to medical ICU (Table 2). In this study of 58 patients who had VRE, 55 patients (94.8%) had consumed antibiotics and 77 patients whose VRE was detected, 64

Table 1: Primers, target genes, sequences and length of the genes used in the study.

Target	Serological group	Sequence (5'→3')	Size (bp)	Genes
Confirmation of species	DD	CACCTGAAGAAACAGGC ATGGCTACTTCAATTTACAG	475	<i>ddl (E. faecalis)</i>
	FAC	CGCTGATGGTATCGATTTCAT GAGTAAATCACTGAACGA	1091	<i>ddl (E. faecium)</i>
	EC	ATGGATTGGTAYTKGTAT TAGCGGGAGTGMICYMGTA	815/827	<i>vanC1/2</i>
Determining type of <i>van</i> gene	EA	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	723	<i>vanA</i>
	EB	TGCACCCGATTTCGTTTC ACGGAATGGGAAGCCGA	647	<i>vanB</i>
	ED	TGTGGGATGCGATATTCAA TGCAGCCAAGTATCCGGTAA	500	<i>vanD</i>
	EE	ATAGTTTAGCTGGTAAC TGTGGTATCGGAGCTGCAG	430	<i>vanE</i>
	EG	CGGCATCCGCTGTTTTTGA GAACGATAGACCAATGCCTT	941	<i>vanG</i>

**Table 2:** The frequency distribution of vancomycin resistant *Enterococcus* by type of ICUs.

VRE	Surgery	Neurosurgery	Medical
Positive	25 (37.9%)	16 (34.4%)	17 (60.7%)
Negative	38 (60.3%)	28 (63.6%)	11 (39.3%)
Total	63 (100%)	44 (100%)	28 (100%)

patients (83.1%) had not consumed antibiotics ( $p=0.037$ ). It means that in patients who had consumed antibiotics, their VRE was 3.7 times more than those who did not consume antibiotics. In 54 patients who consumed 3 to 4 types of antibiotics, 32 patients (59.2%) had VRE, and in 65 patients who consumed 1 to 2 types of antibiotics, 23 patients (35.4%) also had it ( $p=0.009$ ). In other words, in patients who consumed 3 to 4 types of antibiotics, their VRE was 2.6 times more than patients who consumed 1 to 2 types of antibiotics. Also, from 66 patients hospitalized more than 1 week, 42 patients (63.6%) had VRE and from 69 patients hospitalized less than a week, 16 patients (23.2%) had VRE ( $p<0.01$ ). It shows that patients hospitalized more than 1 week, their VRE was 5.7 times more than patients hospitalized less than a week (Table 3). In this study a significant association was not pinpointed by using corticosteroids, the presence or absence of diabetes, history of hospitalization and type of diseases.

## Discussion

Antibiotic resistance rises globally due to

increased usage of antibiotics that causes resistance in pathogenic bacteria and spreads it to other bacteria. The indiscriminate use of antibiotics increases drug resistance in normal flora bacteria and resistance in pathogenic bacteria which have the potential to spread; thus, pathogenic bacteria will be resistant to antibiotics and cause problems in treatment (26).

The results of this study showed that more than 40% of patients in the ICU, carrying VRE and in patients had vancomycin-resistant *Enterococci*, 69% had Van A phenotype, and 6.9% had Van C phenotype. Vancomycin is a glycopeptide antibiotic used to treat infections caused by *Enterococci* which are not treated with common antibiotics. The high prevalence of resistance to these antibiotics can be challenging to the treatment of infections caused by these bacteria. In another study on stool samples in different parts of this hospital in 2007, the prevalence of VRE was reported as 34% in hospitalized patients (27). According to our study done in the ICUs of the hospital, there is an association of high usage of antibiotics and increased prevalence of VRE. The importance of evaluating resistant strains prevalent in the ICU is that colonization with VRE in sensitive patients increases after hospitalization in this ward. Because VRE are colonized in ICU more than other wards of the hospital (28). While subsequent infections of

**Table 3:** The frequency of distribution of VRE strains based on the antibiotic therapy and the duration of hospitalization in ICU.

Risk factors for colonization		Positive	Negative	Total	p value	OR	CI 95%
Antibiotic consumption		55 (94.8)	64 (83.1)	119	0.037	3.7	(1.09 -13.7)
Number of antibiotic consumption	1-2	23 (35.4)	42 (64.6)	65	0.009	2.65	(1.26 – 5.58)
	3-4	32 (59.2)	22 (40.7)	54			
Duration of hospitalizing	Less than a week	16 (23.2)	53 (76.8)	69	<0.01	5.7	(2.7 –12.28)
	More than a week	42 (63.6)	24 (36.4)	66			

VRE occur more in patients colonized with this bacteria (29), likewise the risk of infections from these strains in ICU patients is greater. Prevalence of VRE in patients in our study was greater than in other studies done in Tehran City, Iran. Two studies, done in 2004 and 2007, reported the prevalence as 7% and 12% in Tehran city hospitals, respectively (30).

Antibiotics can increase the resistance phenotype. Our study showed a significant association between antibiotic consumption and increased prevalence of VRE and this ascending of resistance had significant association with using 3 to 4 types of antibiotics. In general, patients who are hospitalized in ICU are more susceptible to infections due to underlying factors such as immune deficiency, suffering multiple diseases simultaneously and using invasive devices. These factors, combined with the use of antibiotics, make easier the transmission of VRE from the patient to other patients (31). This study showed that the prevalence of Van A phenotype was more than Van C phenotype. Due to resistance to vancomycin and teicoplanin antibiotics, Van A phenotype is created and Van C has low level resistance to vancomycin and sensitivity to teicoplanin (8). According to a study done in Shiraz city, Iran, in 2009, the prevalence of VRE was evaluated 35% and Van A phenotype was the greatest (32). The presence of Van A in the majority is consistent with our study. In Australia during 2003, the prevalence of VRE in rectal-swab samples was 45% that Van B was more common (4). This finding is not comparable with our study. In another study done in two periods of time in the same year,

the VRE prevalence in Brazil in February and June of 2006 was estimated at 49.9% and 9.7%, respectively. This decrease was the result of treatment control measures. Also the Van A phenotype was observed, but Van B was not (33). In the past, *Enterococcus* was considered as bacteria with low *pathogenicity* power, but the rapid spread of VRE strains was causing serious attention to them. Currently VRE infections are considered to be a major cause of nosocomial infections in many countries. This can be dangerous without suitable medication and can cause limitation in choosing suitable antibiotics to treat the infection (21, 34). Risk of nosocomial infections caused by VRE is associated with delaying proper treatment, treatment insufficiency and extended hospitalization which can lead to increased mortality in patients (35).

### **Conclusion**

Due to the high prevalence of VRE in the ICU and the importance of its survival in the hospital and its spread in the population, making guidelines for infection control in hospitals and continuing education for ICU personnel are necessary for effective control of VRE colonization and infection in hospital's ICUs.

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