# *Enterococcus faecalis* as multidrug resistance strains in clinical isolates in Imam Reza Hospital in Kermanshah, Iran

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**ABSTRACT**

The current study aimed to investigate the prevalence of vancomycin-resistant *Enterococcus* in *E. faecalis* and *E. faecium* and antimicrobial susceptibility patterns, then dominant genes responsible for vancomycin resistance were determined. For this propose, 180 clinical isolates of *Enterococcus* were subjected for identification and antibiotic susceptibility assay. Then, the gene responsible vancomycin resistant strains were determined. The results demonstrated the *E. faecalis* as a dominant *Enterococcus*. Resistance to erythromycin was dominant and multidrug resistance strains observed in *E. faecalis*. *vanA* was responsible for vancomycin resistance. In conclusion, a high rate of resistance to antibiotics in *Enterococcus* is clearly problematic, and a novel strategy is needed to decrease resistance in *Enterococcus*.

KEY WORDS: Drug resistance, microbial. Enterococcus. vanA

## Introduction

Although considered benign and opportunistic pathogens, enterococci have long been known to cause endocarditis in hospitalised patients.[1,2] Recently, vancomycin-resistant enterococci (VRE) and multidrug-resistant (MDR) enterococci (MDRE) have emerged as causes of nosocomial super infections. Now around 40% of the enterococcal nosocomial infections worldwide are caused by *Entercoccus faecium*. *E. faecalis* and *E. faecium* are ranked as third and fourth nosocomial pathogenic infections.[3]

Infections caused by VRE in Iran, as in many other countries, have been associated with high morbidity and mortality rates, especially in immunocompromised patients.[4,5] There are several reports on the endemic vancomycin resistance of enterococci in Iran, and also several small short-term VRE prevalence studies from Iranian institutions in international and Iranian medical journals.[6,7] Despite the sporadic reports of VRE isolation from Iranian medical centres, morbidity and mortality caused by enterococcal infections in Iran are on the rise.[8] This is primarily because appropriate antimicrobial therapy for enterococcal infections has become progressively more difficult for Iranian physicians due to the lack of adequate information regarding the prevalence of VRE.

The current study aims to investigate the prevalence of vancomycin-resistant *Enterococcus* in *E. faecalis* and *E. faecium*, and also antimicrobial susceptibility patterns. In addition, dominant genes responsible for vancomycin resistance in *Enterococcus* are determined.

## Materials and methods

### *Bacterial isolates*

A total of 180 clinical isolates of *E. faecalis* ($n$=128) and *E. faecium* ($n$=52) were identified during the period April 2009 to February 2010 from Imam Reza Hospital in Kermanshah in the west of Iran. The isolates were in collected urine.

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### *Bacterial identification*

Enterococcal genus identification was performed based on the following microbiological tests: Gram staining, catalase reaction, presence of pyrrolidonyl arylamidase (PYR), growth on bile-aesculin agar and 6.5% NaCl medium. A previously published scheme[9,10] was used in this study to identify the enterococcal species. This scheme utilised a motility test, arginine decarboxylation in Moeller decarboxylase medium, pyruvate utilisation, and fermentation of carbohydrates (arabinose, raffinose, mannitol, ribose).

### *Antibiotic susceptibility testing*

Vancomycin susceptibility testing of enterococcal species was performed by screening of microorganisms on brain heart infusion (BHI) agar (Difco, Detroit, Michigan, USA) containing 6 μg/mL vancomycin (Sigma). The other antibiotic susceptibility analysis was performed by a disc-diffusion method on Mueller Hinton agar (Difco). The antibiotics were erythromycin, ampicillin, cefotaxime, gentamicin, imipenem, streptomycin, teicoplanin and ciprofloxacin.

### *DNA extraction of* **E. faecium** *and* **E. faecalis**

DNA was extracted using a DNA extraction kit (Gene ALL, South Korea).

***Evaluation of distribution of different vancomycin resistant genes***

All the isolates were subjected to the amplified *vanA* and *vanB* genes using specific primers as listed in Table 1. PCR amplification was carried out in a DNA thermocycler (Bio-Rad) using the amplification parameters with initial denaturation at 95˚C for 2 min, followed by 35 cycles of denaturation at 95˚C for 20 sec, annealing at 58˚C for 10 sec, and extension at 72˚C for 20 sec with a final extension at 72˚C for 5 min. PCR amplified products were analysed by 1% agarose gel electrophoresis.

***Statistical analysis***

Statistical analysis was performed using the SPSS statistical package software for determination of frequency of different antibiotic resistance and vancomycin-resistant genes (Version 16.0; SPSS, Chicago, IL).

## Results

The results demonstrated that, of 180 clinical isolates of *Enterococcus* spp from Kermanshah (the largest province in the west of Iran), 70.3% ($n$=128) were *E. faecalis* and 28.75% ($n$=52) were *E. faecium*.

***Erythromycin resistance was dominant in enterococcal clinical isolates***

The antibiotic susceptibility assay demonstrated the most resistance observed for erythromycin, which was 59.9% ($n$=109) follow by ampicillin, streptomycin, teicoplanin, ciprofloxacin, cefotaxime, imipenem, vancomycin and gentamicin, with 58.2% ($n$=106), 33% ($n$=60), 32.4% ($n$=59), 26.4% ($n$=48), 24.4% ($n$=4), 18.1% ($n$=33), 17.6% ($n$=32) and 6% ($n$=11), respectively. The $\chi^2$ analysis demonstrated there was significant difference between vancomycin resistance and *Enterococcus* spp. ($P$=0.049). While 27 *E. faecalis* were resistant to vancomycin, only five *E. faecium* showed resistace to vancomycin. In addition, a significant difference was observed between erythromycin, ampicilin, gentamicin and ciprofloxacin with *E. faecalis* and *E. faecium* ($P$≤0.05). Teicoplanin, streptomycin, imipenem and cefotaxim did not show any significant difference with *E. faecalis* and *E. faecium* ($P$≥0.05) (Fig. 1).

**Table 1.** Characteristics of the primers used in the current study.

| Primer | Sequence (5'-3') | Size (bp) | Accession No. |
|---|---|---|---|
| *vanB* | ATTCAGCTGTACTCTCGCCG<br>CCCAGCATTTTTCGCAACGA | 250 | YP_009076352 |
| *vanA* | AAAGTCAATAGCGCGGACGA<br>TCAGTACAATGCGGCCGTTA | 377 | YP_006961941 |

**vanA *is responsible for vancomycin resistance in* Enterococcus faecalis *and* Enterococcus faecium**

The vancomycin-resistant strains were examined for the presence of *vanA* and *vanB* by PCR. The results demonstrated that all *E. faecalis* and *E. faecium* harboured the *vanA* gene, and *vanB* was observed in *E. faecalis* strain 82, which harboured both *vanA* and *vanB* (Fig. 2).

***Multidrug-resistant* Enterococcus *observed in* E. faecalis**

Two strains of *E. faecalis* showed MDR (strains 15 and 82). They showed resistance to all tested antibiotics. *E. faecium* did not show MDR. Sequencing analysis revealed 99% identity between vancomycin-resistant *E. faecalis* and *E. faecium*.

## Discussion

The spread of antimicrobial resistance among *Enterococcus* spp. in Iran has presented a serious challenge for the Iranian medical community.[4] Unfortunately, treatment failures in enterococcal infections are on the rise because of the lack of adequate information regarding glycopeptide resistance among endemic enterococci. Such information is required for appropriate treatment of patients with enterococcal infections, which rank among the third most common cause of bacteraemia and the second most frequent cause of urinary tract infection (UTI).[1,2]

This investigation indicates a severe problem of antimicrobial resistance among enterococci in Imam Reza

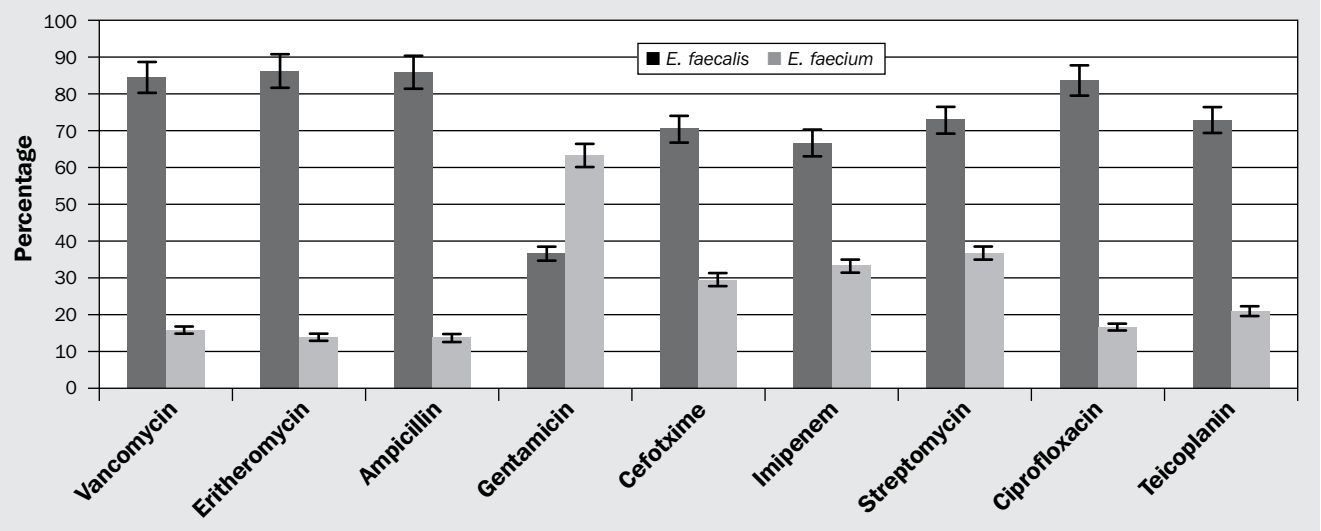


**Fig. 1.** Antibiotic resistant results for *E. faecalis* and *E. faecium*.

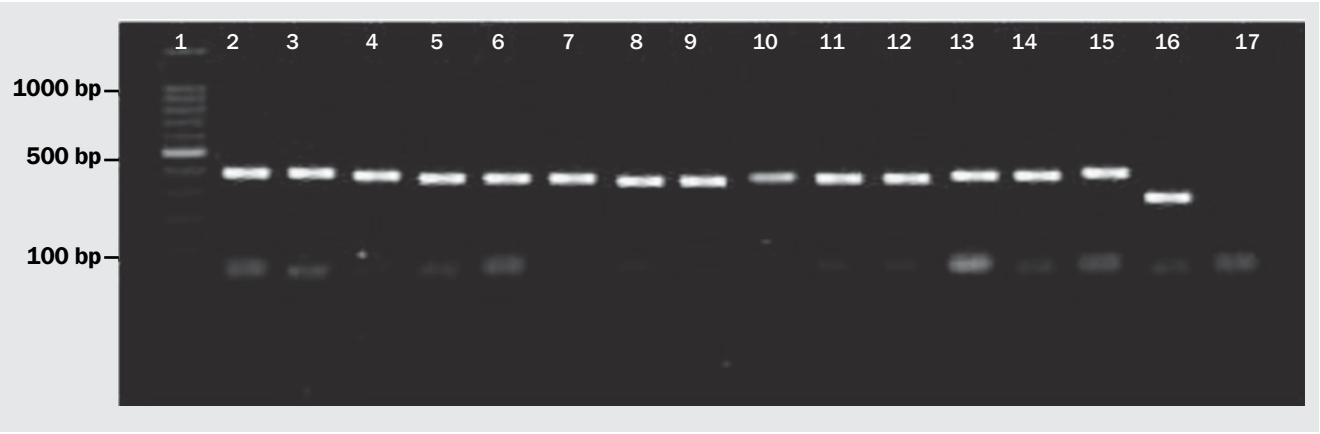


**Fig. 2.** *vanA* and *vanB* in *Enterococcus*. Lane 1: Marker, lane 2: positive control (*vanA*, *E. faecium* ATCC 29212), lanes 3–15: *vanA* (377 bp), lane 16: *vanB* (250 bp), lane 17: negative control.

Hospitals in Kermanshah. The 18.1 % rate of VRE prevalence in the present study is in disagreement with reports of VRE with prevalence of 7% in Tehran.[4] Despite the recent isolation of a single *vanB* genotype in an enterococcal strain of *E. faecalis*, the finding that all VREs isolated in this investigation were of the *vanA* genotype illustrates that *vanA* is the predominant type of enterococcal vancomycin resistance in Kermanshah, as reported in other countries.[2,11]

The results obtained by Sharifi and colleagues in Tabriz in North West Iran,[12] in which UTIs were the subject of investigation, 73.4% of *Enterococcus* isolates were *E. faecalis* and 26.6% were *E. faecium*. These findings are consistent with our results . Shrifi and colleagues in 2012, in another study,[13] showed that *vanA* is dominant in vancomycin-resistant *Enterococcus*, as found in the present study.

A high percentage of antibiotic-resistant *E. faecalis* and *E. faecium* isolates, and MDR *E. faecalis* strains, contribute to the challenge of selecting therapeutic measures.

In conclusion, our data indicate that the relaxed usage of antimicrobials has created a large pool of resistance genes which may result in the dissemination of resistant bacteria into the environment and could pose a health threat to humans in the future. □

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