

RESISTANCE TO VANCOMYCIN IN ENTEROCOCCUS FAECIUM AND FAECALIS CLINICAL ISOLATES

A. Aleyasin¹, A.M. Mobarez², M. Sadeghizadeh³, R. Hosseini Doust⁴, N. Khoramabadi⁵

ABSTRACT

The aim of this study was to investigate antibacterial resistance among enterococci species isolated in Tehran Baghyatallah Hospital. It consisted of 126 isolates of *E. faecalis* (86%), *E. faecium* (9%) and other *Enterococcus* Spp. (5%) isolated from urine (34.92%), blood (27.77%), wound swabs (19.84), stool (5%) endotracheal secretions (3.37%), abscess (3.4%), dialysis fluids (1.7%) and catheter (4%). Twelve (9.5%) isolates were resistant to vancomycin. The VRE isolates were resistant to ampicillin (75%), erythromycin (50%), tetracycline (58%), ciprofloxacin (41.6%), chloramphenicol (33.3%) and gentamicin (41.6%). Two (16.66%) of VRE isolates were multidrug resistant. Eight (66.6%) of the vancomycin-resistant strains and all of the MDR strains carried the *vanA* phenotype and genotype. The MIC of VRE isolates were between 32-512µg/ml. Our results show that most glycopeptide resistant *E. faecalis* and *E. faecium* carried *vanA*. It is also possible that frequency of infections caused by glycopeptide-resistant enterococci will increase in our geographical area.

KEY WORDS: Enterococcus, Clinical Samples, VanA, Vancomycin-resistant Enterococci, Iran.

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INTRODUCTION

Enterococci are important nosocomial pathogens recovered often from the patients with urinary tract infections, wounds, bacteremia, endocarditis or meningitis.¹ Detection of vancomycin-resistant enterococci (VRE) in the mid-1980s was therefore a major therapeutic concern. VRE were first isolated in France and England^{2,3} but the strains have subsequently

been reported on a worldwide scale.^{2,4,5} Acquired resistance to vancomycin by Enterococci greatly reduces the number of treatment options for disease management²⁻⁶ and the problem is further compounded by the fact that resistance genes can potentially be transferred to other pathogenic organisms, such as *Staphylococcus aureus* and *Streptococcus* species.^{2,3} To date seven genotypes (*vanA*, *vanB*, *vanC1*, *vanC2/3*, *vanD*, *vanE* and *vanG*) of vancomycin resistance have been reported for enterococci.⁷ Strains with the *vanA* genotype are characterized by high-level vancomycin and teicoplanin resistance, whereas, those with the *vanB* genotype exhibit moderate to high resistance to vancomycin.¹⁻⁴ The aim of the present study was to use PCR for detection of *vanA* isolates of *E. faecium* and *E. faecalis* from the patients in Tehran Baghyatallah hospital within a specific time-period in 2004-05.

MATERIAL AND METHODS

A total of 126 isolates were obtained from different clinical samples between March 2004

1. A. Aleyasin,
2. Dr. A. M. Mobarez,
3. Dr. M. Sadeghizadeh,
Department of Genetic, Faculty of Basic Sciences,
4. Dr. R. Hosseini Doust,
Dept. of Microbiology &
Research Center of Molecular Biology,
Baghyatallah University, Tehran - Iran.
5. N. Khoramabadi,
1,2,5: Department of Bacteriology,
Faculty of Medical Sciences,
1,3,5: University of Tarbiat Modares,
Tehran - Iran.

Correspondence

Dr. A. M. Mobarez,
E-mail: mobares80@yahoo.com

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and December 2005 in Bagyatallah hospital in Tehran. They were cultured from urine, wound swabs, blood, endotracheal secretions, dialysis fluids, abscess and stool. Stool samples were inoculated into enterococcal broth, incubated overnight at 35°C and subcultured onto brain-heart infusion agar (BHIA) containing 6µg vancomycin ml⁻¹^{8,9} and onto BHIA without vancomycin to recover vancomycin-susceptible isolates. The isolates were identified to the genus and species level by cultural characteristics, Gram's stain, catalase test, bile aesculin reactions and arabinose, sorbose and manitol fermentation.

Susceptibility testing: Disk diffusion method on Mueller–Hinton agar¹⁰ was used to detect resistance to tetracycline, erythromycin, chloramphenicol, ampicillin, ciprofloxacin, gentamicin, kanamycin, streptomycin, linezolid, vancomycin and teicoplanin. The MICs for vancomycin and teicoplanin were determined by the agar dilution method.

Detection of vancomycin-resistance determinants: The presence of the *vanA* resistance gene was assessed by PCR analysis describes by Khan et al.¹¹ The primers were (*vanA* Forward; 5'-AAT ACT GTT TGG GGG TTG CTC-3' and *vanA* Reverse, 5'-CTT TTT CCG GCT CGA CTT CCT-3'). The amplification mixture consisted of 5µl of 10 X PCR buffer (100mM Tris / HCl, pH 8.4, 500 mM KCl, 20mM MgCl₂, 220µM each dNTP, 22 U recombinant Taq DNA polymerase ml⁻¹, 5µl bacterial DNA and 5µl primer, 6µl H₂O. An Ependroff thermocycler was programmed for 30 cycles with the following parameters: denaturation at 97°C for 1 min, annealing at 52°C for 55 seconds, extension at 72°C for 1.5min and final extension at 72°C for 10 min. Amplified products were detected by agarose gel electrophoresis using 1.5% agarose (w/v) in TAE buffer for 2h at 70 V. *E. faecium*, ATCC 51559 (kindly provided by Dr M. feyzabady) were used for standardizing the PCR amplification of *vanA*. A vancomycin-sensitive strain, *Enterococcus faecalis* ATCC 29212, was used as the negative control.

RESULTS

One hundred twenty six bacterial isolates from urine (34.92%), blood (27.77%), wound swabs (19.84%), dialysis fluids (1.7%), stool (5%), abscess (3.4%) endotracheal secretions (3.37%), and catheter (4%) were studied in the years 2004 - 2005 in Tehran Baghytallah Hospital. Of the 126 samples, 59% collected in medical ward, 19% in intensive care facilities and 17% in surgical wards while 5% could not be classified. The strains with vancomycin MICs>16mg/ml were considered as vancomycin resistant. Special emphasis was given to detection of vancomycin resistance marker *vanA* (Fig1), because vancomycin is considered as one of the antibiotics to be used as a last resort¹² used. To test the presence of *vanA*, we set up a PCR assay¹¹ for detecting the *vanA* (734-bp) marker (Fig.1). When the individual PCR reactions were carried out, specific and predicted size amplicon of *vanA* (734bp) was observed (Fig-I, lane 1, 2, 3, and 5). No PCR product was observed when template DNA from a vancomycin-sensitive *E. faecalis* strain, ATCC 29212, was used (Fig.1, lane6). As expected, in strain with MICs < 16mg/ml, no

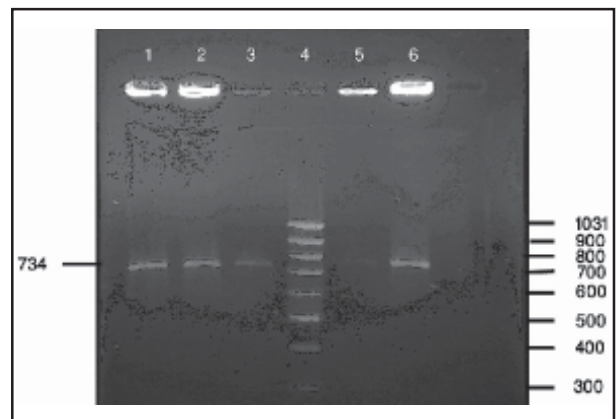


Fig-1: PCR amplification of *vanA* genes Lane1: *E. faecalis* isolated from urine, MIC= 512mg/ml. Lane 2: *E. faecium* isolated from blood, MIC= 128mg/ml. Lane 3: *E. faecalis* isolated from urine, MIC= 128mg/ml. Lane 4: 100bp marker. Lane 5: *E. faecium* isolated from ulcer, MIC= 64mg/ml. Lane6: *E. Faecium*, ATCC 15559, as a *vanA* positive control strain. Lane 7: *E. Faecalis*, ATCC 29212, as a sensitive and negative control strain. All of the *vanA* positive isolates generated a 734 bp PCR product.

vanA was detected. Four of Twelve VRE isolates did not possess any vanA genes, even though they were resistant to vancomycin (MICs between 16-32mg/ml) (Table-I). Most of the VRE strains were resistant to 6-8 other antibiotics (Table-II) indicating either previous exposure to these antibiotic or the acquisition of resistance markers. Vancomycin resistance was detected in 12 (9.5%) isolates consisting of five (41.6%) *E. faecium* and seven (58.33%) *E. faecalis* (Table-I). Most of the VRE isolates studied were also resistant to teicoplanin (91.6%), ampicillin (75%), erythromycin (50%), tetracycline (58%), ciprofloxacin (41%), chloramphenicol (33%), and gentamicin (41%) (Table-II). Two (16.66%) of the VRE isolates were multidrug resistant. Eight (66.6%) of the vancomycin-resistant and all multidrug resistant strains carried the vanA phenotype and genotype. The MIC of multidrug resistance isolates were between 128-512mg/ml. One of the seven vancomycin-resistant *E. faecalis* and two vancomycin-resistant *E. faecium* isolates had MIC values >128µg ml⁻¹ for vancomycin (Table-II). Two multidrug resistant *E. faecium* from urine and blood, with MICs >128-512µg/ml were isolated.

DISCUSSION

Vancomycin-resistant enterococci have been increasingly reported worldwide since first described in 1987, although the epidemiology of these microorganisms varies widely in different geographical areas.^{13,14} The present study

Table-I: VRE isolated from different clinical samples, using disk diffusion, Broth dilution and PCR.

Species	Specimen	MIC µg/ml	Van A
<i>E. faecalis</i>	Ulcer (1)	64	+
<i>E. faecalis</i>	Urine (2)	32	-
<i>E. faecium</i>	Ulcer (1)	64	+
<i>E. faecalis</i>	Trachea(1)	64	-
<i>E. faecalis</i>	Blood (1)	128	+
<i>E. faecalis</i>	Dialysis fluid(1)	16	-
<i>E. faecium</i>	Urine (1)	64	+
<i>E. faecium</i>	Urine (1)	512	+
<i>E. faecium</i>	Catheter (1)	128	+
<i>E. faecium</i>	Urine (1)	64	+
<i>E. faecium</i>	Blood (1)	128	+

Table-II: Resistance to antimicrobial agents of VRE strains of clinical origins

AB	No of resistance
Vancomycin	12 (100%)
Ampicillin	9 (75%)
Erythromycin	6 (50%)
Tetracycline	7(58.33%)
Ciprofloxacin	5(41.66%)
Chloramphenicol	4 (33.3%)
Gentamicin	5(41.66%)
Teicoplanin	11(91.66%)
MDR	2(16.66%)

documents the phenotypic and genotypic characterization of 12 vancomycin-resistant enterococci, isolated over 1-year period (2004-2005) from different clinical samples at Tehran Baghyatallah Hospital. The predominant species were *E. faecium* (9%) and *E. faecalis* (85%). The prevalence of *E. faecium* in this study was 9% lower than 29% prevalence reflected in a similar study from Cincinnati.¹⁵ Of the 12 VRE isolates, 75, 50 and 58% were resistant to ampicillin, erythromycin and tetracycline respectively. All of the isolates were susceptible to linezolid. High-level resistance to glycopeptide antimicrobials was first reported in Europe in 1986 and the United states in 1987. Between the years 1989 and 1993, the rate of vancomycin resistance in the United States increased from 0.3% to 7.9%.¹⁶ In this study, we found that 9.5 % of the enterococcal isolates were resistant to vancomycin. Of the 12 VRE isolates, 7(58.3%) were *E. faecalis* and 5(41.7%) were *E. faecium*, respectively. Vancomycin-resistance phenotypes in enterococci were classified as vanA, vanB, vanC, vanD and vanE based on levels of resistance.¹⁷ The vanA determinant was carried on the transposon Tn1546 or its close relatives that are transferable in conjugation experiments.¹⁷ Eight of the 12 vancomycin-resistant isolates in this study expressed vancomycin-resistance patterns compatible with the vanA phenotype and all the multidrug resistant strain gave positive results in PCR experiments for the vanA genotype. The vanA phenotype is responsible for approximately 70% of the VRE isolates. In our study, vanA was found in 66.6% of the

