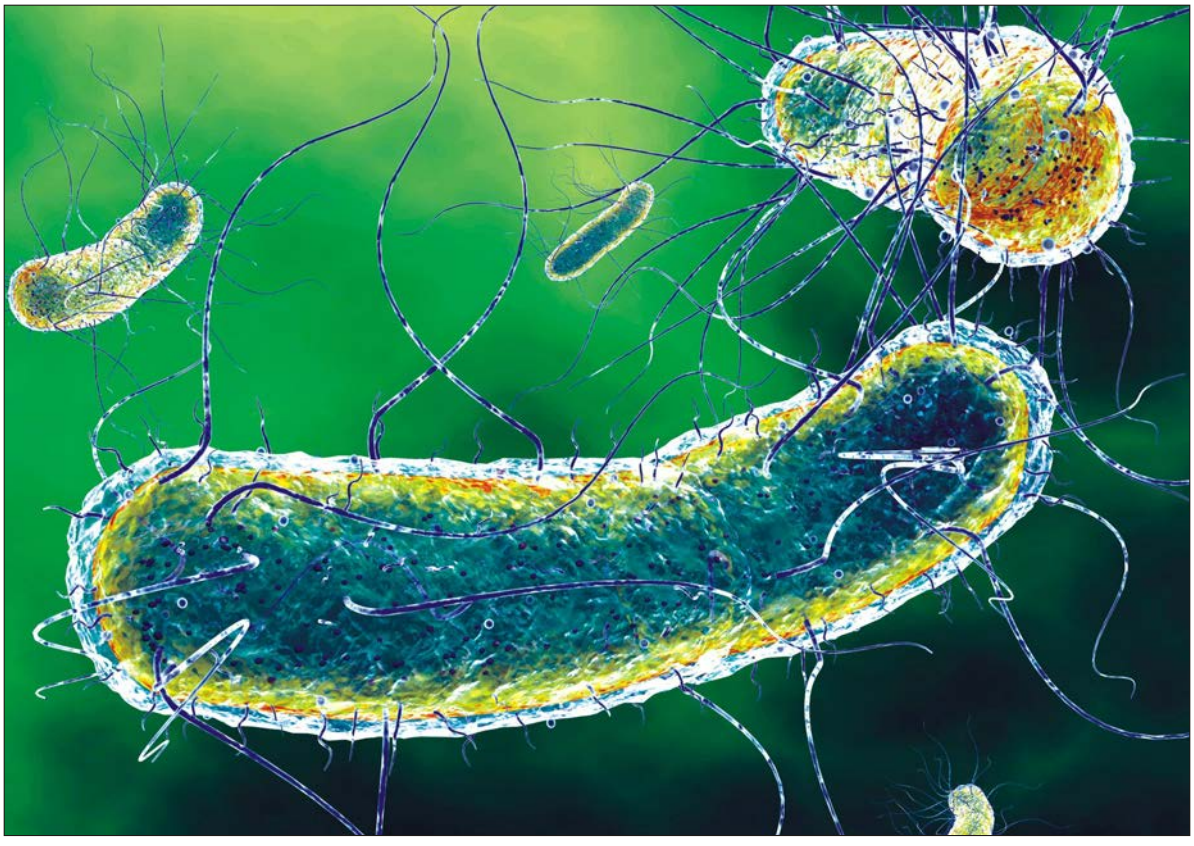

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Studies of Vancomycin Resistant *Enterococcus faecium* Isolated from Clinical Samples in Tehran, Iran

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ABSTRACT

Antibiotic resistance pattern and the plasmid profiles of Vancomycin Resistance *Enterococcus faecium* (VREF) isolated from hospitalized patients were analyzed. Four hundred and fourteen enterococcal isolates from the clinical specimens including Urine, wound and respiratory tract secretions were obtained from patients hospitalized in three major hospitals in Tehran. Antibiotic susceptibility test, plasmid extraction and Polymerase Chain Reaction (PCR) for *van A* and *van B* were done. It was shown that most of the isolates (95%) were resistant to four antibiotics. The results showed 15 different plasmid profiles among the 19 VREF isolates. The number of plasmids in each of the VREF isolates varied from 2-7. Analysis of plasmid profiles revealed that none of the isolates from three hospitals had identical patterns, indicative of lack of inter-hospital transmission. On the other hand, six isolates from different wards from a single hospital showed identical plasmid pattern, suggestive of intrahospital transmission of VREF isolates.

Key words: Antibiotic, plasmid, *E. faecium*, vancomycin resistance *Enterococcus*

INTRODUCTION

Nosocomial infections are important sources of morbidity and mortality in hospital settings (McGowan, 2001). Furthermore, hospital-acquired infections with multidrug-resistant pathogens represent a major public health problem. Major antibiotic resistance problems are typically associated with gram-positive nosocomial pathogens which include glycopeptide-resistant enterococci and Methicillin-resistant *Staphylococcus aureus* (MRSA) (Malani *et al*., 2002; Smith *et al*., 1999). Enterococci nosocomial infections have been reported widely throughout the world (Raad *et al*., 2005). One major reason for long-term survival of enterococci in the hospital is their intrinsic resistance to several commonly used antibiotics and the potential to acquire resistance to newer generation of antibiotics. For example, resistance to vancomycin has been reported through either gene mutation or transfer of plasmids and transposons (Cetinkaya *et al*., 2000). The importance of various extrachromosomal genomes has been extensively studied. It has been shown that *Enterococcus* can pass the vancomycin resistance genes to other species as well as to other genus. The transfer of vancomycin resistant gene from *Enterococcus* to *S. aureus* has been shown in the laboratory (Noble *et al*., 1992). Moreover, in the in clinical setting vancomycin resistant *S. aureus* (VRSA) strains have been reported in several countries (Hiramatsu, 2001). The

appearance of VRSA can generate a significant problem in the treatment of *S. aureus* because vancomycin has been considered as the drug of choice for treatment of enterococcal and MRSA infections (Kuhn *et al.*, 2005).

Enterococcus faecium has also become a major hospital infection worldwide. With emergence of vancomycin resistant strains additional burden has been placed on the patients, physicians and health authorities. Proper usage of antibiotics against Vancomycin Resistant *Enterococcus faecium* (VREF), their distribution and relatedness are essential for determining the epidemiology of nosocomial infections which in turn, would aid in designing methods in pathogen control (Singh *et al.*, 2006).

Therefore, the present study was done to confirm the distribution of VREF and determination of plasmid content in the isolates.

MATERIALS AND METHODS

Sample collection and identification of isolates: Four hundred and fourteen enterococcal isolates were collected during the period of June to December 2005, VREF were isolated by standard culture methods from clinical specimens. Urine, wound and respiratory tract secretions were obtained from patients hospitalized in three major hospitals in Tehran.

Identification of strains to the genus level was performed by detecting the following characteristics: growth and blacken of bile-esculin agar, growth in the presence of 6.5% NaCl, absence of catalase, presence of pyrrolidonyl arylamidase, 0.04% tellurite reduction, arabinose acidification, arginine dehydrolase activity, methyl-α-D-glucopyranoside acidification, motility and pigmentation using Facklam's recommendations (Facklam and Collins, 1989; Ameri *et al.,* 2009). The final identification of *Enterococcus* species was based on PCR results as described previously (Kariyama *et al.*, 2000; Pourshafie *et al.,* 2008).

Plasmid extraction and PCR: Plasmid DNA was extracted with QIAprep Miniprep kit (Qiagen GmbH, Hilden, Germany) in accordance with the manufacturer's recommendations. Plasmid DNA separated by 0.8% agarose gel electrophoresis and stained with ethidium bromide. The patterns clustered by the unweighted pair group method with arithmetic averages (UPGMA) with Gelcompar II version 4.0 (Applied Maths, Sint-Matens-latem, Belgium). All VREF were tested for *van* genes using plasmid DNA. Identification of *van* genotype (*vanA* and *vanB*) for each isolate of VREF was performed by PCR with specific primers.

Primer sequences (vanA: 50 CATGAATAGAATAAAAGTTGCAATA-30, 50-CCCCTTTAACGCTAATACGATCAA-30 vanB : 50-GTGACAAACCGGAGGCGAGGA-30, 50-CCGCCATCCTCCTGCAAAAAA-30) were from the published sequences of the genes (Talebi *et al.*, 2008).

Determination of antibiotic susceptibility: Antibiotic susceptibility test was done by standardized disc diffusion method (NCCLS, 2000) using the following antibiotics; vancomycin (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), erythromycin (15 μg), ciprofloxacin (5 μg), gentamicin (120 μg) (BioRad, Hercules, CA, USA), quinupristin-dalfopristin (15 μg) and linezolid (30 μg) (Mast Diagnostics Ltd., Bootle, Mersey Side, UK). Quality control strains were *E. faecium* ATCC 29212 and *E. faecium* ATCC 51299.

RESULTS

Patient population and bacterial strains: Out of 414 enterococcal isolates, 19 (4.6%) VREF were isolated from the clinical specimens including 17 (89%) from urine and one (11%) from each of wound and respiratory tract secretion. All isolates were positive for VANA and negative for VANB. Of these 19 patients, 1 (5.2%) was obtained from Bone Marrow Transplant (BMT) ward, 3 (16%) from Intensive Care Unit (ICU) and Critical Care Unit (CCU), 3 (16%) from surgery ward, 5 (26%) from internal ward, 1 (5.3%) from emergency, 2 (10.5%) from dialysis ward and the remaining 4 (21%) isolates from other wards which could not be traced.

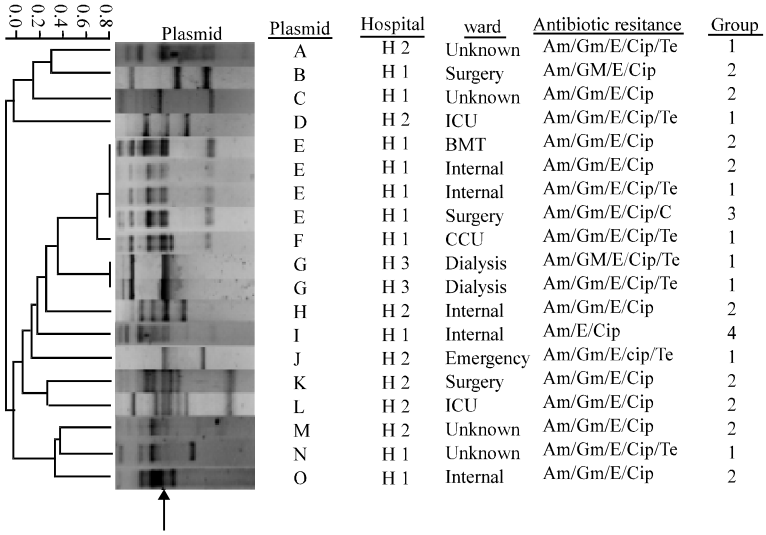
Antibiotic susceptibility tests: The isolates were divided into four distinct antibiotic resistance groups (Fig. 1). The isolates in three groups (group 1, 2, 4) were susceptible to linezolid, quinupristin-dalfopristin and chloramphenicol. Most of the VREF isolates (95%) were resistant to ampicillin, gentamicin, ciprofloxacin and erythromycin and only one isolate was susceptible to gentamicin (group 4). Forty two percent of the isolates were resistant to tetracycline (Table 1).

Plasmid profile analysis: All 19 VREF isolates were shown to contain plasmid DNA. In total, 15 different plasmid profiles (A to O) were observed among the isolates. Thirteen isolates (68%) showed

Table 1: Percentage of antibiotic resistance pattern of VR *E. faecium* strains isolated from clinical samples in Tehran, Iran

Antibiotic resistance pattern	No.	%
Am/Gm/E/Cip/Te	8	42
Am/Gm/E/Cip/C	1	5.5
Am/Gm/E/Cip	9	47
Am/Gm/E	1	5.5

Gm: Gentamicin, Cip: Ciprofloxacin, Te: Tetracycline, E: Erythromycin, Am: Ampicillin, C: Chloramphenicol



Plasmid	Hospital	ward	Antibiotic resitance	Group
A	H 2	Unknown	Am/Gm/E/Cip/Te	1
B	H 1	Surgery	Am/GM/E/Cip	2
C	H 1	Unknown	Am/Gm/E/Cip	2
D	H 2	ICU	Am/Gm/E/Cip/Te	1
E	H 1	BMT	Am/Gm/E/Cip	2
E	H 1	Internal	Am/Gm/E/Cip	2
E	H 1	Internal	Am/Gm/E/Cip/Te	1
E	H 1	Surgery	Am/Gm/E/Cip/C	3
F	H 1	CCU	Am/Gm/E/Cip/Te	1
G	H 3	Dialysis	Am/GM/E/Cip/Te	1
G	H 3	Dialysis	Am/Gm/E/Cip/Te	1
H	H 2	Internal	Am/Gm/E/Cip	2
I	H 1	Internal	Am/E/Cip	4
J	H 2	Emergency	Am/Gm/E/cip/Te	1
K	H 2	Surgery	Am/Gm/E/Cip	2
L	H 2	ICU	Am/Gm/E/Cip	2
M	H 2	Unknown	Am/Gm/E/Cip	2
N	H 1	Unknown	Am/Gm/E/Cip/Te	1
O	H 1	Internal	Am/Gm/E/Cip	2

Fig. 1: A UPGMA dendrogram showing the plasmid profile of VR *E. faecium* strains. Gm: Gentamicin, Cip: Ciprofloxacin, Te: Tetracycline, E: Erythromycin, Am: Ampicillin, C: Chloramphenicol, H: Hospital. The arrow indicates the most common plasmid DNA in the isolates

distinct plasmid profiles whereas 4 (profile E) and 2 isolates (profile G) showed identical plasmid pattern (Fig. 1). All of the isolates harbored 2-7 plasmids. A unique plasmid DNA band (Fig. 1, arrow) was found in 80% of the isolates.

DISCUSSION

The persistence of VREF in hospitals or communities for months or years has been accompanied with spread of plasmids or transposons to many different VREF clones (Cetinkaya *et al.*, 2000). The presence of 4.6% *van A* gene in our isolates indicates a moderate percentage of our *E. faecium* isolates carried resistance to vancomycin. This is in contrary to the reports by other countries where higher percentages of VREF have been reported (Bouchilon *et al.*, 2004). Comparison of plasmid profiles could be a useful method for assessing relatedness of the clinical isolates with resistance to antibiotics for epidemiological studies (Singh *et al.*, 2006). In the present study, some of the plasmids were detected in most of the isolates, suggesting high rate of persistence of these plasmids in the VREF isolates. The maintenance of plasmids within the bacterial species is dictated by many environmental and genetic factors. Widespread usage of antibiotics in Iran may cause significant variations in plasmids patterns amongst the enterococcal species. This could, in turn, explain the presence of 15 patterns amongst the 19 VREF isolates.

Analysis of plasmid profiles of the VRE isolates showed different patterns in different hospitals. On the other hand, some isolates from different wards contained the same plasmids. Fig. 1 shows that 4 isolates from hospital 1 (H1) and 2 isolates from hospital 3 (H3) contained identical plasmid DNA profile which were grouped into profiles E and G, respectively. Type E contained isolates from internal ward (2 isolates) and BMT (1 isolate) and surgery (1 isolate) wards. Isolates with type G plasmid profile were obtained from patients with chronic renal failure in dialysis ward only.

The microorganisms can be transmitted by health care workers in particular via hands which are probably the most common mode of nosocomial transmission (Boyce *et al.*, 1994). The rapid spread and multiplication of VREF have been reported in the hospital's environment. Transmission of VREF by the way of contaminated medical equipments and health care carriers has been investigated to occur in different hospital sections especially in the dialysis ward (Kalocheretis *et al.*, 2004). This could explain the reason that our two isolates obtained from the dialysis ward carried similar plasmid profile.

It has been reported that among the hospital wards, higher prevalence of VREF has been observed in ICU due to prolonged stay of the patients (Boyce, 1995). Moreover, in this study most of the VREF isolates were collected from the internal ward. The prolong antibiotic treatment of the patients in the internal ward could explain higher prevalence of VREF.

In the present study, 80% of the VREF were isolated from urinary tract infections. This number is significantly higher than what has been reported in North America and Europe which is around 40% in 2007 (Deshpande *et al.*, 2007). There are several factors for such a difference, (1) due to the fact that this study was carried with limited number of cases and only in city of Tehran, it may not represent the whole country and (2) the use of vancomycin started much earlier in the western countries. Enterococci have intrinsic or acquired resistance to many commonly used antibiotics. The resistances that cause the most severe therapeutic problems include high level resistance to ampicillin, aminoglycosides and vancomycin which have been reported frequently in the isolates of *E. faecium*. This, in turn, would limit the choice of antibiotic use (Nichol *et al.*, 2006). In the present study, 95% of the isolates were found resistant to ampicillin, gentamicin, ciprofloxacin,

erythromycin and vancomycin. On the other hand, the VREF isolates were found to be susceptible to Linezolid (100%), quinupristin-dalfopristin (100%) and chloramphenicol (95%). The two new antibiotics, linezolid and quinupristin-dalfopristin, have been used successfully in the treatment of VREF whereas the use of chloramphenicol is limited due to side effects (Lim and Webb, 2005).

In conclusion, the plasmid profiling did not show any linkage among VREF isolates from different hospitals. Moreover, the results may suggest a possibility of patient to patient transmission and spreading of VREF isolates between different wards in a single hospital as shown by the similar plasmid bands. In addition, this study suggests the use of simple and cost effective plasmid profiling which could be used easily in tracking inter- and intra-hospital distribution of VREF.

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