High prevalence of diverse vancomycin resistance Enterococcus faecium isolates in clinical and environmental sources in ICU wards in southwest of Iran

Maniya Arshadi a, Masoumeh Douraghi a, Leili Shokoohizadeh b, Seyed Mojtaba Moosavian c, Mohammad Reza Pourmand a, *

a Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
b Department of Microbiology, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran
c Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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ABSTRACT

This study aimed at determining the prevalence, antibiotic resistance patterns, and genetic linkage of Vancomycin Resistant Enterococcus faecium (VREfm) from different sources in the southwest of Iran. A total of 51 VREfm isolates were obtained and subjected to antibiotic susceptibility testing, carriage of virulence genes, and pulsed-field gel electrophoresis (PFGE) method. All the VRE isolates exhibited a high level of resistance to teicoplanin, ampicillin, erythromycin, ciprofloxacin, and gentamicin, also carried the vanA gene. A total of 59% and 34% of the VREfm strains harbored esp and hyl genes, respectively. The results from PFGE showed 31 PFGE patterns including 10 common types (CT) and 21 single types (ST) among the VRE isolates. Furthermore, isolates from different sources in each common type revealed cross transmission between clinical and environmental sources. Overall, the study showed a high prevalence of diverse VRE faecium strains with threatening resistance phenotypes in the environment and clinical sections among different ICU wards of Ahvaz hospitals.

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1. Introduction

Enterococci are common inhabitants of the gastrointestinal tract in human and animals and have a relatively low virulence compared to other gram-positive pathogens. However, they have been proposed as pathogens responsible for urinary tract infections, endocarditis, septicemia, meningitis, and wound infection. Most enterococcal infections are caused by Enterococcus faecalis and Enterococcus faecium, while the majority of VRE infections are attributed to the latter [1].

There are many problems caused by this pathogen when treating infections due to their intrinsic resistance to some available antibiotics such as aminoglycosides, cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole (SXT) [2]. Moreover, they can acquire antibiotic resistant genes through mutation, plasmids, or chromosomal exchanges [3]. These problems are mainly due to multi-drug resistant (MDR) and vancomycin-resistant Enterococcus faecium (VREfm) strains. Vancomycin resistance genotypes vanA followed by vanB are known as the most prevalent genotype in enterococcus strains [4].

VREfm may lead to nosocomial infections in hospitalized patients particularly ICUs inpatients [5]. Several studies have confirmed the higher mortality rate in patients infected with VREs [6–8]. Prolonged hospitalizations, extensive use of antibiotics, and increased ICU stay, especially among immunocompromised patient’s condition, are the main risk factors of increasing the VREs in hospitals [9,10]. The capability of VRE to colonize and cause infections in high-risk patients, especially in ICU wards, or in contaminating the hospital’s environment, healthcare workers, and medical equipment is the main causative agent of hospital-associated infection [11]. Epidemiological studies have shown the key role of the environment surrounding the patient in the spread of E. faecium, especially VRE strains [12,13].

The majority of hospital-associated strains of VREfm acquire virulence traits, including esp and hyl, thereby potentially increasing bacterial fitness in hospital settings.

* Corresponding author. Department of Pathobiology, School of Public Health and Biotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
E-mail address: mpourmand@tums.ac.ir (M.R. Pourmand).

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Evidence regarding the VRE infection's prevalence in Iranian hospitals indicates an increasing VRE infection rate [14,15]. In this regard, there are limited data on the molecular epidemiological study of VREfm in hospitals environment as well as in ICUs in Iran. Ahvaz is a metropolis and the capital city of Khuzestan Province in the southwest of Iran. The university hospitals in Ahvaz serve as major referral centers in southwest Iran where there is no information regarding the prevalence of the VRE in ICU wards of its hospitals and no study on VRE strains and the clonal relationship between clinical and environmental samples in ICU wards of Ahvaz hospitals. Therefore, the present study was set to identify: 1. VREfm prevalence in clinical, rectal swabs, environmental and hands of healthcare workers (HCW) samples; 2. Antibiotic resistance patterns of VREfm strains; 3. Prevalence of two major virulence genes including esp and hyl and genetic linkage and the clonal relationship between VRE isolates applying pulsed-field gel electrophoresis (PFGE) in different ICU wards from three major university hospitals in Ahvaz.

2. Materials and methods

2.1. Study design and sampling

In this cross-sectional study, 600 samples were collected from various clinical specimens, rectal swabs, (intestine colonization), hands of HCW, environmental surfaces, and medical equipment of three hospitals in the southwest Iran from March to September 2015. These hospitals, here designated as H1, H2, and H3, are major university hospitals that admit patients from other hospitals throughout the Khuzestan province and neighboring provinces. The clinical samples (n = 100) including specimens from urine (n = 65), blood (n = 10), wound (n = 15), and catheter tips (n = 10) obtained from hospitalized patients in ICU wards. Rectal swab samples (n = 130) were collected from all ICUs inpatients after gaining patient’s consent. One sample was taken from each patient. Environmental samples (n = 320) were obtained from patients’ beds, pillows, ward sinks, tables, door handles, and medical devices such as suction tubes, ventilator monitors, blood pressure cuffs, nebulizer, and also swabs (n = 50) from hands of HCW. All samples were obtained from different ICU wards in three hospitals including general ICUs, internal ICU, surgical ICU, and neurosurgery ICU. Ethical approval to conduct the study was granted by the Research Ethics Committee of Tehran University of Medical Sciences.

2.2. Species identification

All samples were initially examined for the presence of enterococci strain. Enterococci isolates were identified by gram staining, catalase test, growth in the presence of 6.5% NaCl, bile-esculin test, and fermentation of arabinose, arginine, and sorbitol [16]. The genomic DNAs of enterococci isolates were extracted by boiling method [17]. The presence of E. faecium and E. faecalis isolates were confirmed by PCR using species-specific primers (Table 1) as described previously [17].

2.3. The antimicrobial susceptibility pattern

Enterococcus faecium isolates were tested for resistance to vancomycin by disk diffusion method and growth in bile-esculin agar containing 6 μg/ml vancomycin (Sigma Aldrich, Germany). The antimicrobial susceptibility of the VREfm isolates to eight antimicrobial agents were previously determined by disk diffusion method [In press, IJPH]. Vancomycin and teicoplanin MICs (Minimum inhibitory concentration) of VREfm isolates were determined by E-test (Liofilchem, Italy) according to the manufacturer’s instructions [18].

2.4. Detection of glycopeptide-resistance genes

Multiplex PCR assay was performed to detect vancomycin-resistance genes (vanA and vanB), as described previously [17].

2.5. Pulsed-field gel electrophoresis

Molecular typing of 44 VREfm strains was performed by PFGE as described by Talebi et al. [19]. Extracted genomic DNA was digested with restriction enzyme Smal (Thermo Scientific, Lithuania) and separated by electrophoresis with ramped pulse times from 5 s to 35 s at 6 V/cm with a run time of 24 h at 14 °C in the Bio-Rad CHEF-DRIII system. Salmonella choleraesuis serotype Brandeup H9812 was used as the molecular size marker. The gels were stained with ethidium bromide. The banding patterns were clustered by the unweighted pair group method with arithmetic averages (UPGMA) using Gelcompar II version 6.6 (Applied Maths, Sint-Matens-Latem, Belgium) and interpreted using the guidelines proposed by Tenover et al. [20].

2.6. Detection of virulence genes

Two major virulence genes of enterococci (esp, hyl) in the VREfm isolates from different sources were previously detected by PCR using the primers listed in Table 1. PCR amplification was carried out as described previously [21,22]. The PCR products were analyzed by 1% agarose gel electrophoresis (SinaClon, Iran).

2.7. Statistical analysis

Data were statistically analyzed using chi-square and Fisher’s exact tests while difference significance (p < 0.05) was determined using SPSS software (version 22).

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Primer name</th>
<th>Sequence(5'-3')</th>
<th>Size(bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddl</td>
<td>ddl E.faecium-F</td>
<td>TTAGGAGCAACGACAGATTGACG</td>
<td>658</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>ddl E.faecium-R</td>
<td>TATGACAGCCGCTTCGATCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vanA</td>
<td>vanA-R</td>
<td>CATGAAATAGAATAAAGTGGTAATA</td>
<td>1030</td>
<td>[17]</td>
</tr>
<tr>
<td>vanB</td>
<td>vanB-F</td>
<td>CCCCTTAAACGCTTAATACGATCACC</td>
<td>433</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>vanB-R</td>
<td>GCCGCATCTCCTGCAAAAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>esp</td>
<td>esp-F</td>
<td>AGATTGCACTTGGATTCG</td>
<td>510</td>
<td>[21,22]</td>
</tr>
<tr>
<td></td>
<td>esp-R</td>
<td>AATTGATTTGATCAGTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hyl</td>
<td>hyl-F</td>
<td>ACCAGAGAGCTGCAGCGAAAATG</td>
<td>276</td>
<td>[21,22]</td>
</tr>
<tr>
<td></td>
<td>hyl-R</td>
<td>GACTGACCTCCAGTTTCAAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Primers used in this study.
3. Results

Among the 600 samples collected from different sources in ICU wards from three hospitals, 57 (42%) E. faecalis and 79 (58%) E. faecium isolates were recovered. The results of PCR assay for the ddl faecalis and ddl faecalis genes confirmed the phenotypic identification of the E. faecium and E. faecalis isolates, respectively. Among all samples from different sources, 51 (8.5%) VRE were found. Furthermore, the results showed a high rate of vancomycin resistance 64.5% (n = 51) in the E. faecium isolates. Out of 100 clinical samples received from hospitalized patients, 13 VREfm strains were isolated. The most common specimen source was clinical samples received from hospitalized patients, 13 VREfm strains were isolated. Among all samples from different sources, 64.5% (n = 51) in the E. faecium isolates. Out of 130 rectal swab samples and 50 hands of stuff samples, 20 VREfm isolates were recovered. The results of PCR assay for the vanA and vanB genes from VRE faecium isolates.

3.1. Antibiotic resistance patterns

VREfm isolates were tested for susceptibility to eight antimicrobial agents by disk diffusion method. All (100%) VREfm isolates showed resistance to ampicillin, teicoplanin, ciprofloxacin, and erythromycin, followed by gentamicin (87%), chloramphenicol (55%), nitrofurantoin (29%), and all the isolates were susceptible to linezolid. All VREfm isolates showed high MICs to vancomycin (64 to >256 μg/ml) and teicoplanin (16–256 μg/ml). Similarly, all of them carried vanA gene (Fig. 2).

3.2. Molecular typing

Overall, the genetic relationship among 44 VREfm isolates was analyzed by PFGE and pulsotype groups were detected according to a similarity cut off value ≥ 85%. Analysis of PFGE results revealed 31 different PFGE patterns including 10 (32%) common types (CT) comprised of 23 isolates with a predominant pulsotype (CT3) including four isolates. Twenty-one (68%) isolates showed unique typing pattern. Out of 10 examined common types, eight of them (80%) consisted of isolates from different sources whereas two of them (CT5 and CT7) had strains isolated from the same sources (rectal swab). In CT3, as a predominant pulsotype, there were four isolates from all of the four studied sources. Other common types were recovered from different sources including CT1 and CT9 from clinical and environmental, CT2 from environmental, and hands of HCW, CT4 and CT8 from rectal swab and hands of HCW, and CT10 from rectal swabs and environmental sources. Of the 10 examined CT in three hospitals, in three of them (30%) (CT3, CT5 and CT9), we found strains that were common between H2 and H3 hospitals. In addition, in four CTs (40%) (CT1, CT4, CT6, and CT7) we recovered strains that were common between different wards of the same hospital and in three CTs (30%) (CT3, CT5, and CT9), we found strains that were common in a same ward in a hospital. On the other hand, in four clones (40%), we found strains that had been isolated at different times. The information including the isolation site, date of isolation, MIC value, the frequency of virulence genes of VREfm isolates and PFGE types are shown in Fig. 3.

3.3. Prevalence of virulence genes in VRE faecium strains

In terms of the gene encoding for potential virulence factors, 59% and 34% of the VREfm strains were positive for esp and hyl, respectively. The distribution of these virulence genes among the VRE isolates from different sources is shown in Figs. 4 and 5. A higher percentage of esp gene was found in clinical isolates (90%) compared to rectal swab isolates (41%), although the percentage of esp gene in VRE isolated from different sources showed a borderline significant trend (p = 0.09). Furthermore, the incidence of the hyl gene among various sources had not a significant difference (p = 0.98). The esp gene was found in 78% of the VRE isolates of the common types and 38% of the isolates from single types suggesting a significant difference (P < 0.05) between them. Furthermore, no significant difference in hyl gene prevalence was found between VRE isolates of common types and single types (43% vs. 23%; p > 0.05).

4. Discussion

In the present study, we detected the frequency of E. faecium in clinical and environmental samples. Furthermore, the frequency of colonization of VRE faecium in hospitalized patients in ICU wards of hospitals in the southwest of Iran was reported for the first time. According to the results, the frequency of E. faecium was higher than E. faecalis isolates; however, E. faecalis is known as the main cause of enterococcal infections. In recent years, an increase in...
*E. faecium* in nosocomial infections can be seen in hospitals, probably due to the emergence of VRE strains in hospitals environments, especially ICU wards [23,24]. The ratio of prevalence of *E. faecium* to *E. faecalis* was 1.3:1 (58% versus 42%). This result is statistically significant, as it is higher than the results of other studies conducted in Iran and other countries [14,25–27].

A high level of resistance (64.5%) to vancomycin was detected in *E. faecium* isolates; this rate of VRE in ICUs from Ahvaz is higher than VRE rate reported from ICUs of Iran and other countries [28–31]. The emergence of *E. faecium* strains with a high level of resistance to vancomycin, ampicillin, gentamicin, and ciprofloxacin as the main classes of antibiotics against enterococcal infections is a
warning in ICUs. In other similar studies, linezolid was identified as the most effective antibiotic against VRE strains [14,32]. Any resistance to linezolid could be because of cost and limitations in using this antibiotic in Iran. Intestinal colonization with VRE strains is the main risk factor for VRE infection [33]. The prevalence of VRE rectal colonization in our study was 15.5%. In another research conducted to review 26 studies on VRE colonization in different countries, it was found that the VRE acquisition rates vary widely from 1.1% to 29.4% [31]. According to reports of Shadel et al., the prevalence of VRE was 309 (17%) of 1872 patients [34]. The prevalence of VRE rectal colonization in the study of Nateghian was 25% in ICU ward of pediatric center in Tehran, Iran [35].

One of the remarkable findings was the isolation of ≥25% VRE strains in the medical equipment, and environment of ICUs. Evidence has confirmed the importance of the environment as a reservoir of E. faecium spreading. The contamination of the hospital environment and the ability of enterococci to survive outside the human body for a long time are regarded as factors influential in the occurrence of cross-contaminations either through the patient or ICU environment [36,37].

Consistent with other studies, the urinary tract was found as the most important source of VRE infections [38,39]. About 77% of VREs were isolated from the urine samples of the patients. Furthermore, isolation of about 8% of VREfm from blood cultures indicated the risk of enterococcal septicemia in the patients of ICU wards. The results of PFGE typing showed heterogeneity among VRE strains isolated from various sources. Thus, out of 44 isolates studied, 21 isolates were placed in a single type, and 23 isolates were in common type, suggesting the diverse populations of VRE strains in ICU. It is notable that, in long-term, these strains can transfer the resistance genes and virulence factors to different strains of enterococcus and other bacteria, such as MRSA. The results are in line with the findings of other researchers in Iran [14,28] but in contrast to studies conducted in USA and Europe which reported clonal spread of VREfm strain in hospitals [40,41].

In addition, the diversity of pulsotypes reflects the variety of resources for these strains. Overall, 30% of CTs were common between H2 and H3 hospitals. Since the studied hospitals were major hospital and patients admitted to ICU wards were continuously exchanged between them, the pulsotype patterns may indicate the probability of inter-hospital transmission. Furthermore, our results showed the inter-ward and inter-hospital circulation of VRE isolates. In this study, there were isolates with intestine colonization origin in 70% of CTs, which can highlight the role of VRE colonized people in terms of dissemination of infection in high risk wards. Since VRE colonized patients are not screened in Iranian hospitals at admission, it seems necessary to implement the guidelines in this regard. Several studies showed that surfaces around patients such as pillows, door handle, tables, and medical devices used for patients are contaminated to a large extent with VRE, and in most cases, this contamination is involved in bacterial cross transmission among hospitalized patients in ICU wards, which occurs mainly through the hands of health care workers [12]. In this study, 25% of all isolates of VREfm was isolated from environmental sources, which is higher than that in another study [42]. The most common specimen sources were a pillow, nurse station, and the surface of the ventilator.

The presence of VREfm strains from environmental sources among 40% of CTs shows the importance of paying more attention to disinfection of surfaces and medical supplies to prevent the spread of VRE in hospitals. On the other hand, analysis of the date of bacteria isolation from each common type showed that a number of strains in a CT were isolated at different times, indicating the presence of resistant strains which have been probably circulating in a relatively long period of time in the hospital and highlighting the need for an urgent control of infection to stop circulating the VRE strains. Among the virulence genes, esp plays an important role in the attachment of bacteria to the urinary tract and formation of biofilm [15]. In our study, 59% of VRE isolates carried esp gene, which is consistent with the incidence found by other researchers [43]. The hyl gene encodes hyaluronidase and facilitates colonization and invasion of bacteria [21]. In this study, the hyl gene was found in 34% of VRE isolates, which is consistent with studies reporting a hyl rate of 31.3% and 27% [11,21]. In addition, the percentage of esp gene in VRE isolated from different sources showed a borderline significant trend (p = 0.09); however, if more samples were obtained from various sources, this difference could have been significant. Further, the higher percentage of esp gene was found in clinical isolates (90%) compared to intestine-colonizing isolates (41%), confirming the results of another investigator who revealed a higher incidence of this gene among clinical isolates compared with colonizing isolates [21]. Besides, the incidence of the hyl gene among various sources had not a significant difference (p = 0.98), which is in line with the results of another investigator [44]. In this study, we showed a higher rate of esp gene in common types isolates that reflect the importance of this gene as a marker of the nosocomial VREfm lineage. The pattern of virulence genes esp and hyl in some isolates varies in a common type, and this can be justified with acquisition or loss of pathogenicity island on which these genes are located.

In conclusion, the results suggested the high prevalence of diverse VRE with threatening antibiotic resistance patterns in clinical and environmental sections in the ICU wards of Ahvaz hospitals. The spreading ability of VRE and genetic linkage between clinical and environmental isolates should be of great concern, which may result in the longer stay and higher cost of hospitalization and thus high rate mortality. Hence, more attention should be paid to surveillance programs in hospitals, specific standards for admission and management of patients in ICU wards, providing effective therapy of infections due to VREs and appropriate training to healthcare workers in dealing with ICU patients.

Conflict of interest

The authors declare that they have no conflict of interest.

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