DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI (VRE) ISOLATED FROM URINARY TRACT INFECTIONS (UTI) IN TEHRAN, IRAN

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ABSTRACT
This report describes the frequency of Enterococci phenotypic and genotypic susceptibility patterns of VRE (Vancomycin Resistant Enterococci) from three hospitals in Tehran, Iran. One hundred and twenty enterococcal urine cultures were isolated from patients with urinary tract infection (UTI). After identification of enterococcal species by biochemical tests, glycopeptide susceptibility of each isolate was assessed by disk agar diffusion method according to NCCLS guideline. Glycopeptide minimum inhibitory concentration (MIC) for each VRE isolate was determined by the agar dilution method and the vanA gene was detected by PCR. Seven percent (8/120) of the isolates were VRE, including E. faecalis 38% (3/8), E. faecium 25% (2/8), E. mundtii 25% (2/8), and E. raffinosus 12% (1/8). All 8 isolates resistant to vancomycin showed vancomycin MIC of >512µg/ml, and teicoplanin MIC’s ranging from 8->64µg/ml, and they all possessed the vanA gene. Six (75%) of VRE were isolated from a referral tertiary care hospital, i.e. Ahari Children Medical Center (ACMC). Almost 90% of Enterococci were E. faecalis (57%) and E. faecium (30%). The remaining 13% were identified as E. mundtii (6%), E. avium (3%), E. durans (1%), E. hirae (2%), and E. raffinosus (1%). The diverse VRE species combined with high rate of VRE isolation in Iran, as well as isolation of E. raffinosus and E. mundtii in the Middle East (ME) region for the first time, suggests a rapid spread of resistance among Enterococci along with an emerging shift in VRE distribution in Iran.

Key words: Vancomycin Resistant Enterococci (VRE), Urinary Tract Infection, Disk Agar Diffusion Method.

INTRODUCTION
Enterococcal species have emerged as important pathogens in Iran as well as throughout the world. Enterococci are rated as the second leading cause of urinary tract infections (UTI) and comprise about 10% of nosocomial UTI (1,2,3). Furthermore, the emergence of vancomycin resistant Enterococci (VRE) in Iran has presented serious challenges for hospital infection control practitioners as well as clinicians treating patients with enterococcal infections in Iranian hospitals (4). Infections caused by VRE in Iran, like many other countries, have been associated with high morbidity and mortality rates especially in immuno-compromised patients (5, 6). 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 (4, 7).

Despite the sporadic reports of VRE isolation from Iranian medical centers, morbidity and mortality caused by enterococcal infections in Iran, is on the rise (8). This is primarily because appropriate antimicrobial therapy of enterococcal infections has become progressively more difficult for Iranian physicians due to the lack of adequate information regarding the prevalence of VRE and levels of their glycopeptide resistance. In this study the frequency of enterococcal species and VRE isolation in Tehran and VRE glycopeptide susceptibility levels in three different health care settings were investigated.

MATERIALS AND METHODS

Patient specimens and bacterial strains
One hundred and twenty enterococcal isolates were recovered from urine specimens of patients with urinary tract infections (UTI) from three hospitals in Tehran, namely, Ahari Children Medical Center (ACMC), which is a referral tertiary care center and a Tehran University of Medical Sciences teaching hospital, Mehrad Hospital and Pars Hospital. The last two hospitals are tertiary care and secondary care facilities, respectively. Only one Enterococcal isolate was analyzed from each patient. Enterococcal genus identification was performed based on the following microbiological tests: Gram reaction, catalase reaction, presence of pyrolysidon arylamidase (PYR), growth on bile-aesculin agar and 6.5% NaCl media. A previously published scheme (9, 10) was used in this study to identify the enterococcal species. This scheme utilized a motility test, arginine
decarboxylation in Moeller decarboxylase media, pyruvate utilization, and fermentation of carbohydrates (Arabinose, Raffinose, Mannitol, Ribose).

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, Steinhelm, GM). Teicoplanin susceptibility testing was performed by the disk diffusion method on Mueller Hinton agar (DIFCO, Detroit, Michigan, USA) containing 30 µg/mL teicoplanin (MAST Grp. Ltd, Merseyside, UK). Glycopeptide minimum inhibitory concentrations (MIC) were determined by the agar dilution method on BHI agar according to National Committee of Clinical Laboratory Standards guidelines (11). All susceptibility test results were assessed after 24 h incubation at 35°C. An enterococcal strain susceptible to vancomycin (ATCC 29212) was used as a negative control and resistant Enterococcus strains [E. faecalis E206 (vanA positive) and E. faecium E2781 (vanB positive), courtesy of Dr. Edet Udo] were used as the positive control for this study. The MIC break point value considered for resistant isolates using vancomycin and teicoplanin was at ≥ 32 µg/mL. For vancomycin, isolates with MIC of ≤ 4 µg/mL and for teicoplanin MIC of ≤ 8 µg/mL were considered susceptible. All figures including frequency and antimicrobial susceptibility results were rounded down if they were <0.5, and were presented as whole numbers if they were ≥0.5.

Enterococcal DNA extraction
Total DNA was extracted from Enterococci as previously described (12). Briefly, enterococcal strains were grown overnight at 35°C on BHI agar. Two or three colonies of each culture were scraped from the surface of the agar plates and resuspended in 200 µL of sterile distilled water. The cell suspension was heated for 15 min at 100°C and then centrifuged at 12,000 g for 10 min. The DNA in the supernatant fluid was used as a template for vanA gene amplification by polymerase chain reaction (PCR).

Detection of vanA gene by PCR
Genes encoding the vancomycin-resistance determinants vanA and vanB were investigated by PCR using specific primers (vanA-1 5’- GGG AAA ACG ACA ATT GC-3’, vanA-2 5’-GTA CAA TGC GGC CTT TA-3’ and vanB-1 5’-ATG GGA AGC CGA TAG TG-3’, vanB-2 5’-GAT TTC GTT CCT CGA CC-3’)(13). PCR reactions were performed in a 50 µL volume consisting of: 1X PCR buffer, 3.5 mM MgCl2, 0.2 mM dNTP Mix and 3 µL of DNA template (10 µg/µL). The PCR conditions consisted of a pre-denaturation step at 94°C for 5 min, followed by 30 cycles of 45 sec at 94°C; 45 sec at 54°C and 45 sec at 72°C. A final extension step was performed at 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1.5 % agarose gel. DNA bands were visualized by staining with ethidium bromide and photographed under UV illumination.

RESULTS
In the present study, of 120 Enterococcal isolates 7% (8/120) were resistant to vancomycin. Various VRE species were isolated, including E. faecalis 38% (3/8), E. faecium 25% (2/8), E. munditii 25% (2/8), and E. raffinosis 13% (1/8). Table 1 depicts the distribution of VRE species according to individual health institution, where each VRE was isolated. In addition to isolation of the majority (75%) of VRE from ACMC, the isolates from this hospital showed the highest diversity (Table 1). Surprisingly, in contrast to the isolated VRE from ACMC, there was no diversity in the few VRE strains isolated from Mehrad hospital and all were identified as E. faecium.

Interestingly, although 50% of VRE isolates from ACMC were identified as E. faecalis; no E. faecium were among VRE isolates recovered from this hospital. Furthermore, all Enterococcal isolates from Pars hospital were sensitive to vancomycin and no VRE were recovered from this hospital. In addition, all isolates of E. faecalis isolate in Mehrad and Pars hospitals were susceptible to vancomycin.

Figure 1. A representative agarose gel electrophoretic analysis of PCR products of vanA gene from two Vancomycin Resistant Enterococci (VRE), showing the 732 bp amplicon (lane 3 and 9). Positive and negative control samples are shown in lanes 1 and 4, respectively. (M = Molecular weight markers).

The results of teicoplanin and vancomycin MIC levels among various VRE isolates which were examined in this study are shown in Table 2. Overall, the vancomycin MIC levels among isolates of VRE were quite high (i.e. >512 µg/mL) indicating high level of resistance among the ACMC and Mehrad enterococcal isolates. Although, teicoplanin MIC levels of E. faecalis, E. munditii and E. raffinosis isolates ranged from 8 µg/mL to 32 µg/mL, the isolates of E. faecium displayed the highest teicoplanin resistance levels (i.e. ≥64 µg/mL). Importantly, 100% (8/8) of examined VRE species were identified as having the vanA genotype suggesting no diversity in susceptibility genotype among VRE isolates (Figure 1).
As shown in Table 3, the frequency of enterococcal species isolation varied among the hospitals examined in this study. Regarding the overall distribution of the enterococcal isolates, by and large, the majority of Enterococci were identified as *E. faecalis* and *E. faecium* accounting for 87% of isolates. Other species (*E. mundtii*, *E. avium*, *E. hirae*, *E. durans* and *E. raffinosus*) recovered from the patients with UTI from the hospitals in this study, accounted for the remaining 13% of all Enterococcal isolates. Other enterococcal species consisted of *E. mundtii* (6%); *E. avium* (3%); *E. hirae* (2%); *E. durans* (1%) and *E. raffinosus* (1%). Again, the highest diversity of enterococcal isolates was shown from ACMC, followed by Pars and Mehrad hospitals. Although Pars hospital provides mostly primary care for patients, this hospital showed a higher diversity of enterococcal isolates than Mehrad hospital. Moreover, despite the absence of any VRE isolates from Pars hospital, low prevalence enterococcal isolates such as *E. avium*, *E. hirae*, and *E. durans* comprised 26% of all *Enterococci* recovered from this hospital.

### DISCUSSION

The spread of antimicrobial resistance among Enterococcal 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 common cause of bacteremia and the second most frequent cause of UTI. (1, 2). Comprehensive data concerning the endemic prevalence and susceptibility patterns of *Enterococci* in various health institutions is also necessary to prevent spread of antimicrobial resistance in Iran. This investigation indicates a severe problem of antimicrobial resistance among *Enterococci* in some hospitals in Tehran. The 7% rate of VRE prevalence in the present study is in agreement with earlier reports of high VRE prevalence (7%) in Tehran (4). In addition, finding of alarmingly high rate of vancomycin resistance in Iran is in sharp contrast with...
Vancomycin resistant enterococci from urinary tract infections

Vancomycin-resistant enterococci have emerged as a major healthcare problem worldwide. In Iran, the prevalence of vancomycin-resistant enterococci (VRE) has been reported to be low, at 0.1%. However, studies from other countries in the Middle East (ME) have reported higher rates of VRE, with an incidence of 1-2% (1-3). This discrepancy highlights the importance of surveillance and monitoring of VRE in the ME region.

In Iran, VRE have been isolated in various hospitals, including a tertiary hospital in Tehran. The first report of VRE in Iran was from a hospital in Tehran, indicating a change in the VRE species circulating in the hospital population. This change might be due to the increased clinical importance of enterococcal species, other than Enterococcus faecalis and Enterococcus faecium, in the ME region.

The wide spectrum of diversity among VRE species in Iran calls for physicians' vigilance in rapid identification of glycopeptide antibiotic resistance. Additionally, the high level of resistance among VRE species in Iran calls for physicians' vigilance in rapid identification of glycopeptide antibiotic resistance during the treatment of life-threatening enterococcal infections in Iran. Finally, our results highlight the need for a national Iranian surveillance program, which continuously monitors the changes in bacterial resistance nationwide and will help to set national priorities for local intervention efforts in Iran. Evidence gathered in such programs may help to confirm findings of this investigation with comprehensive endemic surveillance data from all regions of Iran. Reliable bacterial susceptibility surveillance data from Iran and other countries in the ME will further strengthen the reliability of ongoing global surveillance programs in the developed countries, and thus, will enhance attempts to control the spread of VRE worldwide.

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REFERENCES


