High Frequency of Vancomycin-Resistant Enterococcus Faecalis in an Iranian Referral Children Medical Hospital

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ABSTRACT
Background: Enterococci have emerged in recent years as important nosocomial pathogens. Although most enterococcal human infections are caused by Enterococcus faecalis, studies on vancomycin resistance are usually limited to Enterococcus faecium isolates and a little is known about E. faecalis. Therefore we undertook this study to obtain information about the prevalence of vancomycin-resistant E. faecalis (VREF) and genes responsible for resistance.

Material and methods: Ninety-one E. faecalis isolates of different patients admitted at Children’s Medical Center from August 2009 to June 2010 were included in this cross-sectional study. Antimicrobial testing was performed by Kirby-Bauer disk diffusion method according to Clinical Laboratories Standards Institute (CLSI).

Results: Among all isolates, 15 (16%) were identified as VR E. faecalis. PCR analysis revealed that all VREF isolates were positive for the vanA gene.

Conclusion: The present study reports the highest range of VREF in Iran. The increased frequency of VREF, as seen with rapid rise in the number of VanA isolates should be considered in infection control practices.

Keywords: vancomycin resistant, E. faecalis, Iran

Article received in the 10th March 2012. Article accepted on the 21st August 2012.
INTRODUCTION

Enterococci consider as important nosocomial pathogens and have the capacity to develop and transfer antimicrobial resistance (1,2). Historically, Enterococcus faecalis has been the predominant pathogen among enterococci and the ratio of infections due to this species to those due to all other enterococcus spp. is approximately 10:1 (3). Although most enterococcal human infections are caused by E. faecalis, vancomycin resistance is more frequently related to the Enterococcus faecium (4,5). Even though several glycopeptide resistance mechanisms such as acquiring vanA and vanB genes have been described (6), studies on vancomycin -resistant enterococci (VRE) are usually limited to E. faecium isolates and vancomycin -resistant E. faecalis (VREF) isolates have been sporadically recovered. Therefore we undertook this study to obtain information about the prevalence of VREF and genes responsible for resistance in the referral Children’s Medical Center (CMC), Tehran, Iran.

MATERIAL AND METHODS

Ninety-one E. faecalis isolates of different patients admitted at Children’s Medical Center (CMC) from August 2009 to June 2010 were included in this cross-sectional study. The CMC is a referral tertiary teaching hospital affiliated to Tehran University of Medical Sciences admitting patients from all regions of Iran. Clinical information on E. faecalis patient isolates was collected from medical records. Information included age, sex, length of hospital stay, time and ward of strain isolation and microbiological data. All isolates were identified using standard Microbiology methods (7). Antimicrobial testing was performed by Kirby-Bauer disk diffusion method to detect resistance to gentamicin, amikacin, ceftiazidone, cefotaxime, celexime, nitrofurantoin, trimethoprim/sulfamethoxazole, erythromycin, clindamycin, vancomycin, teicoplanin, linezolide, imipenem, meropenem and chloramphenicol according to Clinical Laboratories Standards Institute (CLSI). In addition, susceptibility of isolates to vancomycin was determined by a microdilution assay using standards recommendations (8).

DNA Extraction

DNA was extracted from VR E. faecalis isolates using QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer’s instruction.

Polymerase chain reaction (PCR) amplification of resistant genes

The vanA and vanB genes were detected by PCR as described by Kariyama et al (9). E. faecium BM4147 (vanA-positive) and E. faecalis V583 (vanB-positive) were used as positive controls.

RESULTS

In this study, 91 E. faecalis were recovered from children aged <1 month to 12 years old. All of the isolates were recovered from urinary tract infections. Average length of hospital stay in all patients was 24 days. Twenty-one of the patients were hospitalized in urology ward, whereas the others were distributed in infectious ward (n=14), surgical ward (n=12), gastroenterology ward (n=11), nephrology ward (n=11), NICU (n=7), CICU (n=7), oncology (n=4) and PICU (n=4). Resistance rates of the strains to antibiotics are shown in Table 1. Most of the isolates were resistant to most expanded-spectrum cephalosporins. The highest resistance was seen in erythromycin (97.8%) followed by trimethoprim/sulfamethoxazole (86.8%) and clindamycin (84.6%). High-level resistance to gentamicin was expressed by 73.6% of the strains. Among all isolates, 15 (16%) were identified as VREF.

Table 2 illustrated the vancomycin MIC of all VREF isolates, patient details and duration of hospital stay, ward and date of isolation. Four patients were hospitalized in gastroenterology ward, the others were distributed in infectious wards.
VREF isolates were susceptible to linezolide (100%), nitrofurantion (93.4%), chloramphenicol (53.4%), and clindamycin (26.7%) whereas they were resistant to other antibiotics. For all VREF isolates, vancomycin MICs was $\geq$128 mg/L. PCR analysis revealed that all VREF isolates were positive for the vanA gene.

**DISCUSSION**

In our study the emergence of high resistance to the most common anti-enterococcal antibiotics can make a real challenge for treatment of these infections (10). In this study, high-level resistance to aminoglycosides, which is one of the traditionally most useful anti-enterococcal antibiotics were found among E. faecalis. VRE have a broad geographical distribution but majority of them belong to E. faecium (2). A report from the United States hospitals indicate that up to 50% of E. faecium and 3% of E. faecalis are resistant to vancomycin (11). In contrast with E. faecium, there is a little information on the epidemiology of VREF. Finding of alarmingly high rate of VREF (16%) in Iran is in sharp contrast with studies from other countries (12-18). In 1995, two VREF isolates were obtained from urinary specimens in Tan Tock Seng Hospital in Singapore and both strains were pheno-typically VanB (12). In the study of Malani et al. from Michigan hospitals during a 10 year period, only 2% of E. faecalis isolates were vancomycin resistance (15). In the recent study in Spain, the frequency of VREF with acquired resistance in the three hospitals was very low (range 0.2-1.1%) and all of them harbored the vancomycin resistance vanB gene (14). The large VRE surveillance study in Portugal revealed rates of 1% VREF among isolates causing urinary tract and invasive infections (16). Rates of VREF in Germany remain very low (<1%) (17). In Italy, the frequency of VREF has increased but has remained below 5% (from <1% in 2002 to 4% in 2006) (17).

Another surveillance study conducted in the United States hospitals from 1995 through to 2002 showed that 2% of E. faecalis isolates were vancomycin-resistant (18). In previous report from Iran in 2008, VREF was found in 4% (8/210) of isolates from different hospitals in Tehran (13).

In this study an increasing number of VREF isolates, as well as an increase in recovery of vanA were obtained. Emergence of vanA gene is of concern since this gene confers high-level resistance to glycopeptides. As compared with vanB, the vanA is known to have increased transferability, which may explain the rapid increase in the number of VanA isolates. In addition, this type of gene implicated in the transfer of vancomycin resistance from E. faecalis to S. aureus (1) and colonized or infected individuals can be at risk of developing severe infections when cancer, transplantation or surgery suppress normal host defenses (19).

In conclusion, the present study reports the highest range of VREF in Iran. The increased frequency of VREF, as seen with rapid rise in the number of VanA isolates should be considered in infection control practices.

**TABLE 2.** Patient details, date and ward of isolation, and vancomycin MICs of VREF isolates.

<table>
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<tr>
<th>N</th>
<th>Sex</th>
<th>Age (month)</th>
<th>Period of hospitalization</th>
<th>Time of isolation</th>
<th>Ward</th>
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