MÆDICA - a Journal of Clinical Medicine

ORIGINAL PAPERS

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

Babak POURAKBARI[a]; Mojtaba Kamali AGHDAM[b]; Shima MAHMOUDI[a]; Mohammad Taghi Haghi ASHTIANI[c]; Farah SABOUNI[b]; Zahra MOVAHEDI[d]; Amir Esmael ALYARI[b]; Reihane Hosseinpour SADEGHI[a]; Setareh MAMISHI[a,b]

[a]Pediatrics Infectious Diseases Research Center, Tehran University of Medical Sciences, Tehran, Iran
[b]Department of Pediatric Infectious Disease, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[c]Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[d]Department of Pediatric Infectious Disease, School of Medicine, Qom University of Medical Sciences, Qom, Iran

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

Address for correspondence:
Setareh Mamishi, Department of Pediatric Infectious Diseases, Children Medical Center Hospital School of Medicine, Tehran University of Medical Sciences, No.62, Gharib St., Keshavarz Blvd., Tehran, Iran. Tel: +98- 21- 6642- 8996. Fax: +98- 21- 6642- 8996
E-mail: smamishi@sina.tums.ac.ir

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, ceftriaxone, cefotaxime, cefixime, 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).

Antibiotic	Resistance	
	N	%
Meropenem	63	69.2
Imipenem	51	56
Gentamicin	67	73.6
Amikacin	54	59.3
Ampicillin	48	52.7
Ampicillin/sulbactam	48	52.7
Chloramphenicol	51	56
Vancomycin	19	20.8
Teicoplanin	14	15.3
Ceftriaxone	85	93.4
Cefotaxime	84	92.3
Cefixime	87	95.6
Nitrofurantoin	12	13.1
Trimethoprim/sulfamethoxazole	79	86.8
Erythromycin	89	97.8
Clindamycin	77	84.6
Linezolide	0	0

TABLE 1. Antimicrobial resistance of *E. faecalis* strains isolated from urinary tract infections.

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.

Table2 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

ward (n=3), CICU (n=3), urology ward (n=3) and NICU (n=2).

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 ≥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 phenotypically VanB (12). In the study of Malani et al. from Michigan hospitals during a 10 year period, only 2% of *E. faecalis* isolates were vancomycine 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.

N	Sex	Age (month)	Period of hospitalization	Time of isolation	Ward	Vancomycin MICs
1	F	14	40	12	gastroenterology	1024
2	F	6	35	18	gastroenterology	>1024
3	F	11	1	1	gastroenterology	>1024
4	F	72	24	16	gastroenterology	256
5	M	0.7	48	23	NICU	1024
6	M	0.5	42	23	NICU	1024
7	M	1	6	2	CICU	>1024
8	F	24	31	13	CICU	>1024
9	F	24	31	12	CICU	>1024
10	F	63	29	14	urology	>1024
11	M	5	37	30	urology	512
12	F	33	28	21	urology	128
13	M	19	34	8	infectious	>1024
14	F	7	53	29	infectious	>1024
15	F	77	5	3	infectious	>1024

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

References

1. **Oprea SF, Zaidi N, Donabedian SM, et al. –** Molecular and clinical epidemiology of vancomycin-resistant Enterococcus faecalis. *J Antimicrob Chemother* 2004; 53:626-630
2. **Pournaras S, Tsakris A, Palepou MF, et al. –** Pheromone responses and high-level aminoglycoside resistance of conjugative plasmids of Enterococcus faecalis from Greece. *J Antimicrob Chemother* 2000; 46:1013-1016
3. **Sood S, Malhotra M, Das BK, et al. –** Enterococcal infections & antimicrobial resistance. *Indian J Med Res* 2008; 128:111-121
4. **Mato R, Almeida F, Pires R, et al. –** Assessment of high-level gentamicin and glycopeptide-resistant Enterococcus faecalis and E. faecium clonal structure in a Portuguese hospital over a 3-year period. *Eur J Clin Microbiol Infect Dis* 2009; 28:855-859
5. **Torres C, Escobar S, Portillo A, et al. –** Detection of clonally related vanB2-containing Enterococcus faecium strains in two Spanish hospitals. *J Med Microbiol* 2006; 55(Pt 9):1237-1243
6. **Leclercq R –** Epidemiological and resistance issues in multidrug-resistant staphylococci and enterococci. *Clin Microbiol Infect* 2009; 15:224-231
7. **C Winn-Junior W, Allen S, Janda W, et al. –** Koneman,s color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006; 700-4
8. **National committee for clinical laboratory standard –** Performance standards for antimicrobial susceptibility testing. Sixteen informational supplement 2006; M100-S16 (M7-A7)
9. **Kariyama R, Mitsuhata R, Chow JW, et al. –** Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. *J Clin Microbiol* 2000; 38:3092-3095
10. **Arias CA, Murray BE –** Emergence and management of drug-resistant enterococcal infections. *Expert Rev Anti Infect Ther* 2008; 6:637-655
11. **Edmond MB, Wallace SE, McClish DK, et al. –** Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis* 1999; 29:239-244
12. **Chiew YF, Ling ML –** Two cases of vancomycin-resistant enterococci from Singapore. *J Infect* 1998; 36:133-134
13. **Emaneini M, Aligholi M, Aminshahi M –** Characterization of glycopeptides, aminoglycosides and macrolide resistance among Enterococcus faecalis and Enterococcus faecium isolates from hospitals in Tehran. *Pol J Microbiol* 2008; 57:173-178
14. **Lopez M, Rezusta A, Seral C, et al. –** Detection and characterization of a ST6 clone of vanB2-Enterococcus faecalis from three different hospitals in Spain. *Eur J Clin Microbiol Infect Dis* 2012; 31:257-60
15. **Malani PN, Thal L, Donabedian SM, et al. –** Molecular analysis of vancomycin-resistant Enterococcus faecalis from Michigan hospitals during a 10 year period. *J Antimicrob Chemother* 2002; 49:841-843
16. **Melo-Cristino J –** Antimicrobial resistance in staphylococci and enterococci in 10 Portuguese hospitals in 1996 and 1997. POSGAR. Portuguese Study Group of Antimicrobial Resistance. *Microb Drug Resist* 1998; 4:319-324
17. **Werner G, Coque TM, Hammerum AM, et al. –** Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill* 2008; 13(47) pii: 19046
18. **Wisplinghoff H, Bischoff T, Tallent SM, et al. –** Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; 39:309-317
19. **Edmond MB, Ober JF, Weinbaum DL, et al. –** Vancomycin-resistant Enterococcus faecium bacteremia: risk factors for infection. *Clin Infect Dis* 1995; 20:1126-1133