Evaluation of Antimicrobial Susceptibility Patterns of Enterococci Isolated from Patients in Tehran University of Medical Sciences Teaching Hospitals

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Received: 15 Jan. 2008; Received in revised form: 9 May 2008; Accepted: 30 Jul. 2008

Abstract- This study investigated the antibacterial resistance among enterococci isolated in Tehran hospitals. A total of 277 *Enterococcus faecalis*, 123 *Enterococcus faecium* and 13 isolates of other enterococcal strains were collected from 1 March 2002 to 15 April 2004 from three teaching hospitals of Tehran University of Medical Sciences. The minimum inhibitory concentrations (MIC) of tested antibiotics were determined by agar dilution method. Susceptible and resistant isolates were defined according to the species-related MIC breakpoints of the Clinical and Laboratory Standards Institute (CLSI) guidelines. Sixty- three percent of isolates were resistant to rifampicin (MIC_{90} 64 µg/ml), 44% to ciprofloxacin (MIC_{90} 16≤ µg/ml), 43% to erythromycin (MIC_{90} 512 µg/ml), 32% to cefazolin (MIC_{90} 256≤ µg/ml), 25% to penicillin (MIC_{90} 32 µg/ml), 21% to ampicillin (MIC_{90} 128≤ µg/ml), 8% to vancomycin (MIC_{90} ≤ 8 µg/ml), and 8% to teicoplanin (MIC_{90} 16≤ µg/ml). All of the vancomycin-resistant strains carried the vanA phenotype and genotype. High level resistance to gentamicin and streptomycin were found in 52% and 83% of the isolates, respectively. The results indicated that a significant percentage of isolates are resistance to different antibiotics, pointing out the need for control strategies to avoid dissemination of resistant isolates and for continuous surveillance for the detection of emerging resistance traits.

Acta Medica Iranica 2009; 47(4): 325-328.

Key words: Antimicrobial susceptibility patterns, enterococci, vancomycin resistant enterococci

Introduction

Enterococci are part of the normal flora of gastrointestinal and genital tract and anterior urethra of humans, and for years have been considered no significant pathogen but recent studies have shown that enterococci have emerged as nosocomial pathogens in Iran as throughout the world (1-4).

More than a dozen species of enterococci are currently recognized but 85-95% of enterococcal infections are caused by *Enterococcus faecalis* and 5-10% caused by *Enterococcus faecium* (1, 2). *Enterococcus* species are intrinsically resistant to many antimicrobial agents including β-lactams and low level resistance to aminoglycosides (1, 5, 6).

They have a great capacity to acquire resistance to other antimicrobial agents including high-level resistance to aminoglycosides and glycopeptides (1, 5). Of great concern is the emergence of resistance to vancomycin, especially in *E. faecium*. Vancomycin resistant enterococci (VRE) have been reported from many countries (2, 5, 7).

Increasing resistance to antibiotics among enterococcal isolates reduce the choices of antibiotics available to treat infections caused by them (5). Despite the sporadic reports of VRE isolation from Iranian medical centers (3, 4) the prevalence of VRE in Iranian hospitals was unknown justifying a need to investigate enterococci isolated from clinical samples in Iran for resistance to vancomycin and other antibiotics. As the first step to generation of data concerning the prevalence of antibiotic resistance in general and resistance to vancomycin in particular, in Iran, enterococci isolated from the urinary tract samples at three teaching hospitals in Tehran were studied for possible resistance to vancomycin, and other antibiotics.

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Patients and Methods

Bacterial strains

A total of 423 isolates of enterococci were collected from urine specimen from different patients from three Tehran University of Medical Sciences teaching hospitals from 1 March 2002 to 15 April 2004.

Enterococci were identified by gram staining, colony morphology, catalase reaction, growth on bile esculin agar and in 6.5% NaCl broth, and presence of pyrrolidonyl arylamidase. Species-level identification was performed by formation of acid in mannitol, sorbitol, sucrose, arabinose, raffinose, pyruvate and sorbose broth, pigmentation, motility, growth on tellurite agar, and arginine hydrolysis.

Antimicrobial susceptibility tests

The minimum inhibitory concentrations (MIC) were determined by agar dilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (9). The antimicrobial agents tested were erythromycin, gentamicin, streptomycin and vancomycin (Sigma, Germany), ampicillin, ciprofloxacin and teicoplanin (ADATAB, Mast, England) penicillin and rifampin (Merck, Germany). *E. faecalis* ATCC 29212 was used as control.

Detection of *van* determinants

Vancomycin-resistance genes (*van*A and *van*B) were targeted by multiplex PCR using previously designed primers (10). PCR reactions were performed in a 25 µL volume comprising: 1X PCR buffer, 2.5 mM $MgCl_2$, 0.3µg/ml of each primer, 2.5 U *Taq* DNA polymerase, 0.2 mM dNTP Mix, and 5µL of DNA template (10 µg/ml). The PCR conditions included a pre-denaturation step at 94ºC for 5 min, followed by 30 cycles of 45 sec at 94ºC, 45 sec at 54ºC and 45 sec at 72ºC. A final extension step was performed at 72ºC 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. *E. faecalis* E206 (*van*A positive) and *E. faecium* (*van*B positive), (kindly provided by Dr. Edet Udo) were used as control strains.

Results

Of the 423 enterococci isolates, 277 (66%) were *E. faecalis*, 123 (29%) *E. faecium* and 23 (5 %) other enterococcal species including *E. hirae*, *E. durans* and *E. avium.*

The resistance rates and MIC of *E. faecalis* and *E. faecium* and the overall resistance rates of all *Enterococcus* spp are shown in Table 1.

Table 1. MIC distribution of Enterococcal isolates

Organism (No.)	Antibiotic	Number of isolates with indicated MIC (µg/mL)											
		≤1	**2**	**4**	**8**	**≤**	**32**	**64**	**128**	**256**	**512**	**1024≤**	**%R**
	Ampicillin	-	-	**229**	0	21	2	11	10	4	-	-	15
	Cefazolin	-	-	92	**76**	25	17	31	13	23	-	-	30
	Ciprofloxacin	-	**173**	50	25	29	-	-	-	-	-	-	37
E. faecalis	Erythromycin	**148**	10	12	5	9	0	12	5	20	40	16	39
(277)	Penicillin	-	56	**165**	2	32	16	6	0	-	-	-	19
	Rifampicin	117	0	15	**33**	25	44	22	21	-	-	-	58
	Vancomycin	-	**205**	10	56	0	0	0	3	1	2	-	2
	Teicoplanin	-	**248**	11	7	5	3	1	2	-	-	-	2
	Ampicillin	-	-	**70**	0	3	4	7	23	16	-	-	43
	Cefazolin	-	-	38	19	**7**	6	10	17	26	-	-	48
	Ciprofloxacin	-	52	**26**	31	14	-	-	-	-	-	-	58
E. faecium	Erythromycin	34	6	7	3	5	4	1	**12**	12	20	19	58.8
(123)	Penicillin	-	36	**40**	3	12	25	6	1	-	-	-	40.2
	Rifampicin	32	0	5	16	**18**	22	14	16	-	-	-	74
	Vancomycin	-	**72**	7	12	4	0	0	9	6	10	3	23
	Teicoplanin	-	**84**	2	9	0	2	7	12	7	-	-	23
	Ampicillin			**307**	0	24	6	20	36	20	-	-	21
	Cefazolin	-	-	138	**103**	38	25	28	32	49	-	-	32
	Ciprofloxacin		**230**	81	58	44	-	-	-	-	-	-	44
All Enterococci	Erythromycin	199	**18**	20	9	15	2	4	13	33	62	38	43
(413)	Penicillin	-	95	**207**	7	47	44	12	1	-	-	-	25
	Rifampicin	154	0	22	**50**	44	67	38	38	-	-	-	63
	Vancomycin	-	**272**	25	78	4	0	0	12	7	12	3	8
	Teicoplanin	-	**352**	0	20	7	5	8	14	7	-	-	8

MIC_{50}, bold, MIC_{90},underlined

Table 2. The aminoglycoside MIC distribution of enterococcal isolates

Organism (No.)	Antibiotic	Number of isolates with indicated MIC (µg/mL)						
		≤125	250	500	1000	2000	4000≤	%R
E. faecalis (277)	Gentamicin	17	20	**115**	33	62	30	45
	Streptomycin	6	7	9	12	20	**223**	83
E. faecium (123)	Gentamicin	6	7	26	19	**31**	34	68
	Streptomycin	0	2	3	3	4	**111**	90
All Enterococci (413)	Gentamicin	25	28	146	**55**	94	65	52
	Streptomycin	9	9	13	16	25	**341**	83

MIC_{50}, bold , MIC_{90},underlined

The results show that 106 isolates including 53 *E. faecium*, 43 ~~of~~ *E. faecalis*, and 12 isolates out of other enterococcal species were resistant to ampicillin with an MIC of 16–256µg/ml. Resistance to rifampicin (63%), ciprofloxacin (44%), erythromycin (43%), cefazolin (32%) and penicillin (25%) were prevalent but only 34 (8%) isolates were resistant to vancomycin and teicoplanin. All VRE isolates carried the *van*A gene and their MIC values for vancomycin were 128-1024 µg/mL.

High-level resistance (HLR) to the aminoglycosides gentamicin (MIC >500 µg/mL) and streptomycin (MIC >2000 µg/Ml) were detected in 52% and 83 % of enterococcal isolates. HLR to the aminoglycosides was more common in *E. faecium* than in *E. faecalis* (Table 2).

Discussion

Despite the fact that enterococci have been considered to have a relatively low virulence, in the past few years these organisms, among all nosocomial pathogens, have emerged as a significant concern. VRE may cause a range of infections associated with high mortality especially in VRE bacteremia (11, 12). VRE are often concomitantly resistant to multiple antimicrobial classes. Increasing HLR to penicillin, ampicillin, and aminoglycosides has been documented in recent years, particularly in strains of vancomycin-resistant *E. faecium* (13). This study investigated the prevalence of antibiotic resistance in enterococci isolated from urine samples at three teaching hospitals in Tehran.

The enterococcal isolates possess an intrinsically relative resistance to penicillin and ampicillin (1). Furthermore, *E. faecium* is less susceptible to β-lactam agents than *E. faecalis* because their penicillin-binding proteins (PBPs) have lower affinities for these antibiotics and some strains have plasmid-encoded β-lactamase (14,15). In our study, the 15% resistance rate to ampicillin in *E. faecalis* isolates was higher than the 1-12% resistance rates reported in Lebanon, Kuwait, Turkey, and Brazil (15-18). However, the 43% resistance rate to ampicillin in *E. faecium* isolates was lower than the 47–100% rates reported from Kuwait and Turkey (16, 17).

Of the 413 isolates 63, 44 and 43 % were resistant to rifampicin, ciprofloxacin, and erythromycin respectively which were higher than the levels reported for these antibiotics among enterococci isolated in ~~the~~ Brazil and India and similar to Kuwait hospitals (5, 18, 19).

HLR to streptomycin and gentamicin due to aminoglycoside modifying enzymes (AME) is possibly one of the fastest spreading phenotypes of resistance among enterococci (20). In the present study, high-level gentamicin resistance was 52%, and high-level streptomycin resistance was 83%. These results are similar to those from Kuwait and another report from Iran (21, 22) and higher than Turkey (17). However, other studies indicated variable percentages of high-level aminoglycoside resistance (7, 23).

The 8% rate of VRE prevalence in the present study is in agreement with reports of VRE prevalence (7%) in Tehran (3, 4). In addition, presence of alarmingly high rate of vancomycin resistance in Iran is in sharp contrast with studies from other countries in the Middle East, where low incidence (0-1%) of VRE has been reported (15, 16). Despite the recent isolation of an enterococcal strain with a single *van*B genotype from a Tehran hospital (24), the finding that all VREs isolated in this investigation had *van*A genotype illustrates that *van*A genotype is the predominant type of enterococcal vancomycin resistance in Iran, as reported in other countries (2 ,7, 16). In conclusion, multidrug resistant enterococcal strains, in particular *E. faecalis* and *E. faecium*, cause serious problems in the treatment of patients with enterococcal infections due to inappropriate use of antibiotics. The emergence of resistance to major antibiotic classes such as β-lactams and aminoglycosides emphasizes the necessity for use of new drugs.

References

1. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev 2000; 13(4): 686-707.
2. Schouten MA, Hoogkamp-Korstanje JA, Meis JF, Voss A; European VRE Study Group. Prevalence of vancomycin-resistant enterococci in Europe. Eur J Clin Microbiol Infect Dis 2000; 19(11): 816-22.
3. Fatholahzadeh F, Hashemi FB, Emaneini M, Aligholi M, Nakhjavani FA, Kazemi B. Detection of Vancomycin resistant Enterococci (VRE) isolated from Urinary Tract Infections (UTI) in Tehran, Iran. Daru 2006; 14(3): 141-5.
4. Feizabadi MM, Asadi S, Aliahmadi A, Parvin M, Parastan R, Shayegh M, et al. Drug resistant patterns of enterococci recovered from patients in Tehran during 2000-2003. Int J Antimicrob Agents 2004; 24(5): 521-2.
5. Udo EE, Al-Sweih N, Phillips OA, Chugh TD. Species prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals. J Med Microbiol 2003; 52(Pt 2): 163-8.
6. Murray BE. The life and times of the Enterococcus. Clin Microbiol Rev 1990; 3(1): 46-65.
7. Reinert RR, Conrads G, Schlaeger JJ, Werner G, Witte W, Lütticken R,et al. Survey of antibiotic resistance among enterococci in North Rhine-Westphalia, Germany. J Clin Microbiol 1999; 37(5): 1638-41.
8. Forbes BA, Sahm DF, Weisfeld A. Bailey and Scott's Diagnostic Microbiology. 10th ed. St. Louis, Missouri; Mosby; 1998.
9. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A4. National Committee for Clinical Laboratory Standards (NCCLS): Wayne, PA, USA, 2004.
10. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol 1995; 33(1): 24-7.
11. Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis 2001; 32 Suppl 2: S133-45.
12. Edmond MB, Ober JF, Dawson JD, Weinbaum DL, Wenzel RP. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis 1996; 23(6): 1234-9.
13. Jones RN, Sader HS, Erwin ME, Anderson SC. Emerging multiply resistant enterococci among clinical isolates. I. Prevalence data from 97 medical center surveillance study in the United States. Enterococcus Study Group. Diagn Microbiol Infect Dis 1995; 21(2): 85-93.
14. French GL. Enterococci and vancomycin resistance. Clin Infect Dis 1998; 27 Suppl 1: S75-83.
15. Zouain MG, Araj GF. Antimicrobial resistance of enterococci in Lebanon. Int J Antimicrob Agents 2001; 17(3): 209-13.
16. Udo EE, Al-Sweih N, John P, Chugh TD. Antibiotic resistance of enterococci isolated at a teaching hospital in Kuwait. Diagn Microbiol Infect Dis 2002; 43(3): 233-8.
17. Kaçmaz B, Aksoy A. Antimicrobial resistance of enterococci in Turkey. Int J Antimicrob Agents 2005; 25(6): 535-8.
18. Titze-de-Almeida R, Rollo Filho M, Nogueira CA, Rodrigues IP, Eudes Filho J, Nascimento RS, et al. Molecular epidemiology and antimicrobial susceptibility of Enterococci recovered from Brazilian intensive care units. Braz J Infect Dis 2004; 8(3): 197-205.
19. Chaudhary U, Shamma M, Yadav A. Antimicrobial susceptibility patterns of common and unusual enterococcus species isolated from clinical specimens. J Infect Dis Antimicrob Agents 2007; 24: 55-62.
20. Hryniewicz W, Zareba T, Kawalec M. Susceptibility patterns of Enterococcus spp. isolated in Poland during 1996. Int J Antimicrob Agents 1998; 10(4): 303-7.
21. Udo EE, Al-Sweih N, John P, Jacob LE, Mohanakrishnan S. Characterization of high-level aminoglycoside-resistant enterococci in Kuwait hospitals. Microb Drug Resist 2004; 10(2): 139-45.
22. Feizabadi MM, Maleknejad P, Asgharzadeh A, Asadi S, Shokrzadeh L, Sayadi S. Prevalence of aminoglycoside-modifying enzymes genes among isolates of Enterococcus faecalis and Enterococcus faecium in Iran. Microb Drug Resist 2006; 12(4): 265-8.
23. del Campo R, Tenorio C, Rubio C, Castillo J, Torres C, Gómez-Lus R. Aminoglycoside-modifying enzymes in high-level streptomycin and gentamicin resistant Enterococcus spp. in Spain. Int J Antimicrob Agents 2000; 15(3): 221-6.
24. Emaneini M, Hashemi FB, Aligholi M, Fatholahzadeh B, Kazemi B, Sadeghi F. Detection of vanB genotype enterococci in Iran. Int J Antimicrob Agents 2005; 26(1): 98-9.