

SPECIES IDENTIFICATION AND INVESTIGATION OF VANCOMYCIN AND HIGH-LEVEL AMINOGLYCOSIDE AMONG CLINICAL ISOLATES OF ENTEROCOCCAL STRAINS

S.R. MOADDAB,* Ph.D., AND A. RAFI,** Ph.D.

From the *Department of Clinical Microbiology, Istanbul University, Istanbul, Turkey, and the **Department of Pathobiology, Tabriz University of Medical Sciences, Tabriz, I.R. Iran.

ABSTRACT

A study was performed to determine the species and antimicrobial susceptibility of 100 enterococcus strains from various clinical specimens as etiological agents at the Clinical Microbiology Laboratory, Istanbul University, Medical Faculty Hospital.

Out of 100 enterococcal isolates, 86 were identified as *E. faecalis*, 11 as *E. faecium*, 1 as *E. avium* and 2 as *E. raffinosus*.

By the disk diffusion tests 36 of 100 strains were found to be resistant to erythromycin, 32 to penicillin, 30 to ampicillin, 7 to ciprofloxacin, 6 to norfloxacin and 5 to nitrofurantoin. No resistance was observed to vancomycin or teicoplanin.

Twenty-six strains showed high-level resistance to streptomycin (2000 µg/mL) and 13 strains exhibited high-level resistance to gentamicin (500 µg/mL). Ten of these strains had high-level resistance to both aminoglycosides. None of the strains produced beta-lactamase.

MJIRI, Vol. 16, No. 3, 165-168, 2002.

INTRODUCTION

Enterococci are normal inhabitants of the gastrointestinal tract of humans and animals. Urinary tract infections are frequently caused by these bacteria. Intra-abdominal or pelvic wound infections, and bacteremia with or without endocarditis are among other common enterococcal infections. Despite their innate low virulence, these organisms have emerged as major nosocomial pathogens.¹

Enterococcus ranks as the second most common pathogen isolated in nosocomial infections, and the third most common cause of nosocomial bacteremia in the USA. Enterococci are intrinsically resistant or tolerant to many antibiotics including the penicillinase-resistant anti-staphylococcal penicillins and most cephalosporins.^{1,2}

An inherent property of enterococci is their low-level resistance to aminoglycosides, and studies have shown that enterococci highly-resistant to aminoglycosides are

becoming increasingly common. When a cell wall inhibitor such as a beta-lactam antibiotic or vancomycin is used together with an aminoglycoside, the cell wall inhibitor enhances the uptake of the aminoglycoside by changing the permeability of the cell wall and thus enhances the effect of the aminoglycoside.^{2,3} The emergence of high-level aminoglycoside resistance in enterococcal strains and the increasing reports of vancomycin resistant enterococci create great difficulty to cure serious infections.^{1,4,5}

Thus the aims of this study were to determine the species distribution of enterococci, to determine their susceptibility to vancomycin and some other antibiotics, beta-lactamase production and high-level resistance to aminoglycosides.

MATERIAL AND METHODS

A total of 100 strains of enterococci isolated from various

Enterococcal Resistance to Antibiotics

clinical specimens of patients in a four month period (September to December 1999) at the Clinical Microbiology Laboratory, Istanbul University, Medical Faculty Hospital were identified by morphology negative catalase positive PYR (N, N-dimethylaminocinnamaldehyde) tests, and by the ability to hydrolyse esculin and to grow in the presence of 6.5% NaCl.⁶ Identification of these 100 strains to the species level was carried out according to Facklam and Collins' criteria and by API Rapid ID Strep (Bio Merieux) when necessary. Antibiotic susceptibility tests were performed and evaluated according to NCCLS (National Committee for Clinical Laboratory Standards) proposals. Five mL of Mueller-Hinton broth was inoculated to 4-5 colonies in tryptic soy agar incubated at 37°C for 3-4 hours, and the turbidity was adjusted to MacFarland No. 0.5 tube. The surface of Mueller Hinton agar plates were spread with this suspension, antibiotic disks were applied and the diameters of inhibition zones were recorded after a 24 hour incubation at 37°C. For agar dilution tests a drop containing 10¹⁴ CFU (colony-forming unit) of a strain was placed on Mueller-Hinton agar containing appropriate concentrations of vancomycin. In agar dilution tests of aminoglycosides, 10⁶ CFU/drop bacterial suspension and brain-heart infusion agar were used. Any growth within 24 hours at 35°C was evaluated as a sign of resistance to the indicated concentration of antibiotic.^{7,8} Beta-lactamase production was investigated by positive nitrocefin test (nitrocefin; Oxoid Ltd).⁹ *Enterococcus faecalis* ATCC (American-Type Culture Collection) 29212 was used as control.

RESULTS

A total of 100 enterococcus strains were isolated from various clinical specimens of patients within four months.

Seventy of these 100 strains were isolated from urine specimens, 18 from pus, 8 from blood cultures, 2 from vaginal swabs and 2 from cerebrospinal fluid (CSF).

Of 100 enterococcus strains, 86 were identified as *E. faecalis*, 11 as *E. faecium*, 1 as *E. avium*, and 2 *E. raffinosus*.

Table I shows the results of disk diffusion test for enterococcus strains. A total of 36 enterococcus isolates were resistant to erythromycin. The number (and percentage) of resistant strains varied from 0-32 for other antibiotics.

Beta-lactamase production was not seen in any of the strains. Vancomycin and teicoplanin resistance were not detected. The vancomycin MICs for all strains were <2 µg/mL.

Table II shows that in agar dilution tests, a total of 5 (45%) *E. faecium* isolates and 21 (25%) *E. faecalis* strains were highly resistant to streptomycin (MIC>2000 µg/mL). Out of 11 *E. faecium* isolates, 6 strains (55%) and out of 86 *E. faecalis* isolates, 7 strains (8%) were highly resistant to gentamicin (MIC>500 µg/mL).

High-level resistance to streptomycin and gentamicin was found in 5 strains (45%) of *E. faecium* and 4 strains (5%) of *E. faecalis*. Results suggested that high-level resistance was more common in *E. faecium* strains when compared with *E. faecalis* ($p<0.001$).

DISCUSSION

Enterococci are normal inhabitants of the gastrointestinal tract of humans and animals. They can cause different infections, for example urinary tract and intra-abdominal wound infections, and can also be responsible

Table I. Results of disk diffusion tests for 100 enterococcus strains.

Antibiotics	E	P	AM	CIP	NOR	FM	V	TP
Resistant (No.)	36(36%)	32 (32%)	30 (30%)	7 (7%)	6 (6%)	5 (5%)	0 (0%)	0 (0%)

Abbreviations used in the Table: E: Erythromycin, P: Penicillin, AM: Ampicillin, CIP: Ciprofloxacin, NOR: Norfloxacin, FM: Nitrofurantoin, V: Vancomycin, TP: Teicoplanin

Table II. High-level resistance determined by agar dilution tests in 100 enterococcal strains.

Species	Strains n	Resistant to:		
		Streptomycin (2000 µg/mL) n (%)	Gentamicin (500 µg/mL) n (%)	Streptomycin+Gentamicin (2000 µg/mL+500 µg/mL) n (%)
<i>E. faecium</i>	11	5 (45%)	6 (55%)	5 (45%)
<i>E. faecalis</i>	86	21 (25%)	7 (8%)	4 (5%)

for severe life-threatening diseases such as bacteremia and endocarditis. Despite their innate low virulence, enterococci have emerged as major nosocomial pathogens.¹ These bacteria are intrinsically resistant to several antibiotics and possess the ability of acquired resistance through the exchange of genetic material, thus they have become more resistant to multiple antibiotics.¹⁰

As some studies show, the most common enterococcal infection is urinary tract infection¹¹ and this fits in very well with the present study considering that 70 out of 100 of our isolates had been isolated from urine specimens.

Investigations reveal that about 85-90% of clinical enterococcal isolates are *E. faecalis* strains, which are followed by *E. faecium* (5-10%) and less frequently by other species.^{12,13}

In this study, of 100 enterococcus isolates, 86 were identified as *E. faecalis*, 11 as *E. faecium*, 1 as *E. avium* and 2 as *E. raffinosus*. Our observation concurs with other studies, with 80-90% of clinical isolates being *E. faecalis*, and *E. faecium* accounting for most of the remainder.

As shown in Table I, the most active antibiotics after teicoplanin and vancomycin were nitrofurantoin, norfloxacin and ciprofloxacin with 5%, 6% and 7% of resistance (determined by disk diffusion tests) respectively. A total of 36% of the enterococcal strains were found to be resistant to erythromycin. Penicillin and ampicillin resistance percentages were 32% and 30% respectively. Our results were comparable and up to a certain degree in accordance with some other studies.^{14,15}

High-level aminoglycoside resistance has very important consequences in the therapy of enterococcal bacteremia and endocarditis. Although it may be regarded as important for severe infection isolates,^{14,15} we determined the presence of high-level antibiotic resistance in strains to have an idea about the frequency of this kind of resistance among these isolates in Turkey.

The percentages of high-level resistance to gentamicin (500 µg/mL) and streptomycin (2000 µg/mL) were 55% and 45% respectively for *E. faecium* strains. The percentages of high-level resistance to gentamicin and streptomycin among *E. faecalis* isolates were 8% and 25% respectively. When the combinations of aminoglycoside resistance are taken into consideration, 45% of *E. faecium* and 5% of *E. faecalis* strains were found to be highly-resistant to both streptomycin and gentamicin. Our results showed that high-level resistance among *E. faecium* strains were higher than *E. faecalis* strains ($p < 0.001$).

Two different mechanisms are known for high-level aminoglycoside resistance, the first one is ribosomal resistance and the second one is known as enzymatic modification. Most of the enterococci with high-level resistance to aminoglycoside are known to have the enzymatic

type of resistance.⁴

The high-level resistance of *E. faecalis* from blood isolates was seen for the first time in the USA in 1985, and the prevalence of such strains amounted to 9% in 1985-8 and to 35% in 1989-91. A study revealed 31% high-level gentamicin resistance in *E. faecium* strains and 37% in *E. faecalis* strains and reported 62% high-level streptomycin resistance in *E. faecium* strains and 36% in *E. faecalis* strains.¹⁶

Gokahmetoglu et al. reported an incidence of 71% high-level gentamicin resistance among isolates of *E. faecium* and 22.2% among *E. faecalis* in blood cultures. In that study, high-level streptomycin resistance had been found in 71% of *E. faecium* isolates and 33.3% of *E. faecalis* strains.¹⁷ On the other hand Ma et al. observed only 22.3% high-level gentamicin resistance in *E. faecalis* with none in other species.¹⁸

In serious enterococcal infections, a combination of a beta-lactam and an aminoglycoside is used for the synergistic effects of these antibiotics. If the strain has high-level resistance to aminoglycosides, the synergy between these two groups of antibiotics disappears and vancomycin should be considered. Vancomycin-resistant enterococci (VRE) were first described in 1988, and unfortunately in the last decade enterococci with acquired resistance to vancomycin have been isolated with increasing frequency.^{1,19,20} For this reason, vancomycin resistance was also searched in our strains and no glycopeptide (vancomycin and teicoplanin) resistance was seen in our investigation. The vancomycin MICs for all strains were < 2 µg/mL. However, it is suggested that further studies be done for the early detection of such strains in Turkey.

Although beta-lactamase producing enterococcal isolates have been reported from some other countries, none of our 100 strains gave positive results by nitrocefin test suggestive of no production of beta-lactamase and this was in concurrence with some other investigations.^{12,18,21} Regarding not to isolate any beta-lactamase producing enterococcus strains, it seems that it is not necessary to investigate the production of beta-lactamase among enterococci, at least in Turkey.

REFERENCES

1. Mellmann A, Orth D, Dierich MP, Allerberger F, Klare I, Witte W: Nosocomial cross-transmission as a primary cause of vancomycin-resistant enterococci in Austria. *Journal of Hospital Infection* 44: 281-7, 2000.
2. Ricaurte JC, Boucher HW, Turett GS, Moellering RC, Labombardi VJ, Kislak JW: Chloramphenicol treatment for vancomycin-resistant *Enterococcus faecium* bacteremia. *Clin Microbiol Infect* 7: 17-21, 2001.
3. Landman D, Quale JM: Management of infections due to resistant enterococci: A review of therapeutic options. *J Antimicrob Chemother* 40: 161-70, 1997.

Enterococcal Resistance to Antibiotics

4. Murray BE: The life and times of the enterococcus. *Clin Microbiol Rev* 3: 46-65, 1990.
5. Ravizzola G, Cabibbo E, Peroni L, Longo M, Pollara PC, Corulli M, Pizzi R, Dima F, Fallacara C, Turano A: *In vitro* study of the synergy between β -lactam antibiotics and glycopeptides against enterococci. *J Antimicrob Chemother* 39: 461-70, 1997.
6. Facklam RR, Bosley GS, Rhoden D, Franklin AR, Weaver N, Schulman R: Comparative evaluation of the API 20S and automicrobic Gram-positive identification systems for non-beta hemolytic *streptococci* and *aerococci*. *J Clin Microbiol* 21: 535-41, 1985.
7. NCCLS: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved Standard. NCCLS Publications M7-44 and Supplement M100-S7. NCCLS, Wayne, Pa, 1997.
8. NCCLS: Performance standard for antimicrobial disk susceptibility tests, 6th ed. Approved Standard: M2-A6 and Supplement tables M100-S7. NCCLS, Wayne, Pa, 1997.
9. Sykes RB: Methods for detecting beta-lactamases. In: Reeves DS, Phillips I, Williams JD, Wisner R, (eds.), *Laboratory Methods in Antimicrobial Chemotherapy*. Eding burgh: Churchill Livingstone Ltd., pp. 64-9, 1978.
10. Woodford N: Glycopeptide-resistance: a decade of experience. *J Med Microbiol* 47: 849-62, 1998.
11. Moellering RC Jr: Emergence of enterococcus as a significant pathogen. *Clin Infect Dis* 14: 1173-8, 1992.
12. Gray JW, Stewart D, Pedler SJ: Species identification and antibiotic susceptibility testing of enterococci isolated from hospitalized patients. *Antimicrob Agents Chemother* 35: 1943-5, 1991.
13. Guiney M, Urwin G: Frequency and antimicrobial susceptibility of clinical isolates of enterococci. *Eur J Clin Microbiol Infect Dis* 12: 362-6, 1993.
14. Pesce A, Debbia EA, Toni M, Schito GC: Antibiotic resistance of clinical isolates of enterococcus in Italy. *Clin Infect Dis* 15: 490-4, 1992.
15. Vandamme P, Vercauteren E, Lammens C, Pensart N, Leven M, Pot B, Leclercq R, Goossens H: Survey of enterococcal susceptibility patterns in Belgium. *J Clin Microbiol* 34: 2572, 6, 1996.
16. Watanakunakorn C: Rapid increase in the prevalence of high-level aminoglycoside resistance among enterococci isolated from blood cultures during 1989-1991. *J Antimicrob Chemother* 30: 289-93, 1992.
17. Gokahmetoglu S, Sumerkan B, Esel D, Karagoz S: kan kulturelerinden izole edilen enterokok susularinin vankomisin ve yuksek duzey aminoglikozid direnclerinin arastirilmesi. *ANKEM Derg* 13: 57-62, 1999.
18. Ma X, Kudo M, Takahashi A, Tonimoto K, Ike Y: Evidence of nosocomial infection in Japan caused by high-level gentamicin-resistant *Enterococcus faecalis* and identification of the pheromone-responsive conjugative plasmid encoding gentamicin resistance. *J Clin Microbiol* 36: 2460-4, 1998.
19. Wade J, Rolando N, Casewall M: Resistance of *Enterococcus faecium* to vancomycin and gentamicin. *Lancet* 337: 1616, 1999.
20. Venditti M, Tarasi A, Gelfusa V, Nicastrì E, Penni A, Martino P: Antimicrobial susceptibilities of enterococci isolated from hospitalized patients. *Antimicrob Agents Chemother* 37: 1190-2, 1993.
21. Patterson JE, Wanger A, Zscheck KK, Zervos MJ, Murray BE: Molecular epidemiology of β -lactamase-producing enterococci. *Antimicrob Agents Chemother* 34: 302-5, 1990.