PREVALENCE OF VANCOMYCIN AND HIGH LEVEL AMINOGLYCOSIDE RESISTANT ENTEROCOCCI AMONG HIGH-RISK PATIENTS

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Abstract. Enterococci have been recognized as clinically important pathogens in high-risk populations of hospitalized patients. The role of enterococci in nosocomial infections is being recognized with increasing frequency. The main source of these infections is usually fecal carriage of the microorganisms. In this study, gastrointestinal colonization with vancomycin resistant enterococci (VRE) and high-level aminoglycoside resistant enterococci among 316 high-risk hospitalized patients were investigated. One hundred and ninety-eight enterococci strains were isolated from stool specimens. All strains were identified to species level and 90 of the isolates were identified as *Enterococcus faecalis* (45%), 85 as *E. faecium* (21.5%), 14 as *E. avium* (7%), 7 as *E. raffinosus* (3.5%), 1 as *E. durans* (0.5%) and 1 as *E. hirae* (0.5%). Eleven of 198 strains were found to be moderately sensitive to vancomycin (MIC: 8-16 μg/ml) by the agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations, and the rest of these strains were found to be sensitive (MIC≤4 μg/ml). Twenty-eight strains showed high-level resistance to streptomycin (2,000 μg/ml) and 26 strains were found to have high-level resistance to gentamicin (500 μg/ml). Twelve of these strains had high-level resistance to both aminoglycosides. By the disk diffusion tests, 53 of 198 strains were found to be resistant to erythromycin, 51 to penicillin, 37 to ampicillin, 18 to ciprofloxacin, 14 to norfloxacin and 3 to nitrofurantoin. No beta-lactamase production was detected in 198 studied strains.

INTRODUCTION

Enterococci were originally classified as enteric Gram-positive cocci and later included in the genus *Streptococcus*. In the 1930s, with establishment of the Lancefield serological typing system, enterococci were classified as group D streptococci, and were differentiated from the nonenterococcal group D streptococci such as *Streptococcus bovis*, by distinctive biochemical characteristics. It was further recommended that the term "enterococcus" should be used specifically for streptococci that grow at both 10º and 45ºC, at pH 9.6, and in 6.5% NaCl and survive at 60ºC for 30 minutes. These organisms were also noted to hydrolyze esculin in the presence of bile. In the 1980s, based on genetic differences, enterococci were removed from the genus *Streptococcus* and placed in their own genus, *Enterococcus*. Enterococci are normal inhabitants of the gastrointestinal tract of humans and animals. Although a dozen enterococcus species have been identified, only two are responsible for the majority of human infections. These organisms are *Enterococcus faecalis* and *E. faecium* (Johnson, 1998; Centinkaya *et al*, 2000). The emergence of enterococci as significant pathogens is worrying because these organisms are inherently resistant to cephalosporins and aminoglycosides. The limited choice of efficient therapy in serious enterococcal infections is complicated by emergence of ampicillin resistance, high-level aminoglycoside resistance and glycopeptide resistance (Harthug *et al*, 2000).

Over the past 20 years, in the United States, there has been a remarkable rise in antimicrobial resistance among enterococci, especially *E. faecium*. Vancomycin-resistant enterococcus (VRE) isolates were first reported in the UK in

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1987, and subsequent reports of infection have come predominantly from Europe and North America. Outbreaks of VRE infection and long-term stool carriage associated with a clonal strain of *E. faecalis* or *E. faecium* in hospitalized patients have been reported (Johnson, 1998; Hsueh *et al*, 1999; Centinkaya *et al*, 2000; Harthug *et al*, 2000).

In hospitals, the emergence and spread of resistant pathogens can be limited by improving management procedures, such as isolating carriers or infected patients to prevent cross-colonization, and by implementing antibiotics policies to reduce the selection of resistant bacteria (Bertrand *et al*, 2000). The main source of resistant enterococci is usually fecal carriage of the microorganisms.

The aims of this study were to determine the intestinal colonization by resistant enterococci; the identification and resistance characterization of these microorganisms.

MATERIALS AND METHODS

Within 6 months, a total of 501 stool specimens from 316 inpatients at medical and surgical units of Istanbul University Medical Hospital were collected in Stuart transport media. All patients had a history of high level and long-term antibiotic therapy. Hospitalized patients included bone marrow transplant recipients, solid organ transplant recipients, cancer and cardiovascular patients.

For selective isolation of VRE and all enterococci from feces, specimens were inoculated onto two Slanetz and Bartley agar (Oxoid) plates, with and without vancomycin, within 24 hours. The vancomycin supplemented medium was prepared weekly and stored at 4ºC. Suspicious colonies of Gram-positive cocci on each medium were presumptively identified as enterococcus by negative catalase, positive pyrrolidonyl arylamidase (PYR) tests; by the ability to hydrolyze esculin and to grow in the presence of 6.5% NaCl. Identification to species level was performed by the conventional tests scheme proposed by Facklam and Collins or by API Rapid ID Strep (Bio Merieux) if necessary.

Enterococci were tested for their susceptibility to eight antimicrobial agents of erythromycin, penicillin, ampicillin, ciprofloxacin, norfloxacin, nitrofurantoin, vancomycin and teicoplanin. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method. An inoculum of 10^4 CFU/spot from a log-phase culture was delivered by a multi-point inoculator onto Mueller-Hinton agar plates containing appropriate antibiotic concentrations. Interpretative criteria for susceptibility status were those of the National Committee for Clinical Laboratory Standards (NCCLS). High-level resistance to gentamicin (500 mg/ml) and streptomycin (2,000 mg/ml) was investigated by the growth on agar containing specific concentrations of these antibiotics, and beta-lactamase production by nitrocefin tests (Oxoid), (Sykes, 1978; Swenson *et al*, 1994; Suppola *et al*, 1996; NCCLS 1997a,b). The *Enterococcus faecalis* ATCC (American-Type Culture Collection) 29212 was used as a control.

RESULTS

A total of 501 stool specimens from 316 inpatients were investigated and 198 enterococcus strains were isolated from 167 hospitalized patients.

Six different species were identified, of which *E. faecalis* was the most prevalent. Out of 198 enterococcus strains, 90 (45%) were identified as *E. faecalis*, 85 (43%) as *E. faecium*, 14 (7%) as *E. avium*, 7 (3.5%) as *E. raffinosus*, 1 (0.5%) as *E. durans* and 1 (0.5%) as *E. hirae.*

Eleven of 198 strains were found to be moderately sensitive to vancomycin (MIC:8 μg/ml for 5 strains and 16 μg/ml for 6 strains) and the rest of these strains were found to be completely sensitive (MIC≤4 μg/ml). One of the vancomycin-moderately sensitive strains was *E. faecalis* and the remaining 10 strains were *E. faecium.*

Table 1 shows the results of disk diffusion tests for the enterococcus strains. Resistance to erythromycin was the commonest and it was found in 53 (27%) strains; whereas resistance to penicillin was observed in 51 (26%) isolates, to ampicillin in 37 (19%), to ciprofloxacin in 18 (9%), to norfloxacin in 14 (7%) and to nitrofurantoin in only 3 (1.5%) strains. Resistance to van-

Table 1
Results of disk diffusion tests for enterococcus strains.

Antibiotics	E	P	AM	CIP	NOR	FM	V	TP
Resistant	53(27%)	51(26%)	37(19%)	18(9%)	14(7%)	3(1.5%)	-(0%)	-(0%)

E: erythromycin, P: penicillin; AM: ampicillin, CIP: ciprofloxacin, NOR: norfloxacin, FM: nitrofurantoin, V: vancomycin, TP: teicoplanin

Table 2
High-level resistance determined by agar dilution tests in enterococcus strains.

		Resistant to		
Strains	n	Streptomycin (2,000 μg/ml) n (%)	Gentamicin (500 μg/ml) n (%)	Streptomycin+Gentamicin (2,000 μg/ml+500 μg/ml) n (%)
E. faecium	85	13(15)	14(16)	8(9)
E. faecalis	90	15(17)	12(13)	4(4)

comycin and teicoplanin was not detected.

Table 2 shows that in agar dilution tests, a total of 13 (15%) *E. faecium* isolates and 15 (17%) *E. faecalis* strains were highly resistant to streptomycin (MIC≥2,000 μg/ml). Out of 85 *E. faecium* isolates, 14 (16%) strains and out of 90 *E. faecalis* isolates, 12 (13%) strains were highly resistant to gentamicin (MIC≥500 μg/ml). High-level resistances to streptomycin and gentamicin were found in 8 (9%) strains of *E. faecium* and 4 (4%) strains of *E. faecalis*. No beta-lactamase production was detected in 198 strains.

DISCUSSION

Enterococci have emerged as increasingly important nosocomial and community-acquired pathogens. Although they are generally considered to be of low pathogenic potential, it is now recognized that these organisms can cause serious invasive infections, including endocarditis, bacteremia, urinary tract and pelvic infections.

The role of enterococci as a cause of infection has become increasingly important, not only because of their documented pathogenic potential but also because of increasing antimicrobial resistance (especially resistance to glycopeptides) in some strains (Reid *et al*, 2001). Over the last decade, a major focus of interest has been the emergence and spread of vancomycin resistant enterococci (VRE). The UK has the dubious distinction of being the first country in the world to experience a nosocomial outbreak involving such organisms. At about the same time, there were also case reports of VRE infection or colonization from France, and over the next few years, VRE were reported from many other countries (Swenson *et al*, 1994; Suppola *et al*, 1996; Johnson, 1998).

Numerous studies have been undertaken to determine risk factors for infection or colonization with VRE. The results indicate a multiplicity of factors including prior treatment with antibiotics, particularly vancomycin and/or third-generation cephalosporins; proglonged hospitalization; severe underlying disease (*eg* cancer, renal failure or diabetes), hospitalization; in renal, oncology, hematology, surgical or intensive-care units and invasive procedures. The intestinal tract is the most important source for spread of enterococcus, including VRE (Centinkaya *et al*, 2000; Johnson, 2000; Reid *et al*, 2001). Arabshahi and

co-workers reported fecal carriage rates for VRE of 2.07% for hospitalized patients in Iran in 1998. Gordts *et al* detected VRE in 3.5% of hospitalized patients in Belgium in 1995. In the UK, Jordens *et al* in 1994 reported fecal VRE carriage rates of 2%. The prevalence of gastrointestinal colonization with VRE among the US adult population ranges from 0% to 75% among various high-risk groups, varying by state, institution, and patient population. In the Netherlands, 135 stool samples were screened from leukemic or bone marrow transplant children; none were colonized with VRE (Trabulsi *et al*, 1998). Few studies have addressed the epidemiology of VRE among hospitalized patients in Istanbul. In our study we did not find VRE, but eleven of the 198 strains isolated from stool of inpatients were found to be moderately sensitive to vancomycin (MIC:8-16 μg/ml). An enterococcus, for which the MIC of vancomycin is ≤ 4 μg/ml, is categorized as susceptible to vancomycin. One of the vancomycin moderately susceptible strains was *E. faecalis* and the remaining 10 strains were *E. faecium.* All vancomycin moderately sensitive strains were isolated in both media, with or without vancomycin. These results were interpreted as the addition of vancomycin to isolation medium would not increase the isolation rate of vancomycin-moderately sensitive or vancomycin-resistant strains. It was also observed that vancomycin usage in the Istanbul University Hospital did not result in the intestinal colonization of vancomycin-resistant enterococci.

Some studies suggested that stool surveillance does not seem reliable in predicting VRE recovery from clinical specimens, and VRE was not isolated consistently from rectal swabs from individuals previously known to be colonized (Trabulsi *et al*, 1998). We believe that the surveillance of stool for VRE is important in hospitals in order to estimate the rate of carriage, especially among high-risk groups.

For the first time, VRE was reported in Turkey in 1999. This VRE was isolated from a fluid sample of an 11 month-old boy who was subjected to empirical treatment with vancomycin and amikacin combination for bronco-pulmonary infection (Vural *et al*, 1999).

In the present study, of 198 enterococcus isolates, 175 (88%) were identified as *E. faecalis* and *E. faecium*. Our observation was comparable and up to a certain degree in accordance with some other studies (Jorden *et al*, 1994; Gordts *et al*, 1995; Trabulsi *et al*, 1998). Eskiturk *et al* reported 91.5% *E. faecalis* and *E. faecium* in fecal samples of hospitalized patients in 1997. Some investigations reveal that about 90-95% of clinical enterococcal isolates are *E. faecalis* and *E. faecium* and less frequently by other species (Johnson, 1998; Bertrand *et al*, 2000; Centinkaya *et al*, 2000).

Based on our findings, the most active antibiotics after teicoplanin and vancomycin were nitrofurantoin, norfloxacin and ciprofloxacin with 1.5%, 7% and 9% of resistance (determined by disk diffusion tests) respectively. A total of 27% of the enterococcal strains were found to be resistant to erythromycin. Penicillin and ampicillin resistance percentages were 26% and 19% respectively (Table 1).

Although etiological isolates of enterococcus species in some studies show a higher frequency of antibiotic resistance, the results of this investigation indicate the enterococcal isolates exhibiting resistance to variety of antimicrobial agents may also be isolated from fecal samples of hospitalized patients (Pesce *et al*, 1992; Vandamme *et al*, 1996; Eskiturk *et al*, 1997; Arvanitidou *et al*, 2001).

The high percentage of erythromycin-resistant enterococci in our isolates was of particular interest, because macrolides are frequently used in the community for the empirical treatment of infectious disease, as well as for treatment of enterococcal infections, especially when allergy to penicillins is suspected (Arvanitidou *et al*, 2001). The over-use of erythromycin may be responsible for the resistance rates observed in this study. Ampicillin and quinolones are also used frequently in hospitals and thus a considerable percentage of enterococci were found to exhibit ampicillin and quinolone resistance.

Aminoglycosides are frequently used in combination with cell-wall-active antibiotics for severe enterococcal infections. The use of an aminoglycoside and a cell wall synthesis inhibitor in combination for severe enterococcal infections was implemented following studies carried out by Hunter in 1946. The explanation for this

combination's efficacy did not emerge till the 1970s when Moellering *et al* concluded that the cell wall active agent disrupted the bacterial cell wall sufficiently to allow the aminoglycoside to enter and institute its bactericidal effects (Arvanitidou *et al*, 2001, Simjee and Gill, 1997).

Low-level aminoglycoside resistance is an intrinsic characteristic in all enterococcal species. Acquired high-level aminoglycoside resistance may be caused by various aminoglycoside-modifying enzymes and predicts a failure of synergy between cell-wall-active agents and aminoglycoside to which the organism is highly-resistant (Arvanitidou *et al*, 2001; Simjee and Gill 1997). Although high-level aminoglycoside resistance may be regarded as important for severe infections, we determined the high-level resistance in various strains to get an idea of the frequency of this kind of resistance in the enterococcal isolates of fecal samples in hospitalized patients. Based on our results, the percentages of high-level resistance to gentamicin and streptomycin were 16% and 15% for *E. faecium* and 13% and 17% for *E. faecalis*, respectively (Table 2).

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 1985-1988 and to 35% in 1981-1991. A study revealed 31% high-level gentamicin resistance in *E. faecium* and 37% in *E. faecalis* and reported 62% high-level streptomycin resistance in *E. faecium* and in *E. faecalis* (Watanakuankorn, 1992).

Eskiturk *et al* reported an incidence of 11.4% high-level gentamicin resistance among isolates of *E. faecium* and 2.8% among *E. faecalis* in fecal samples in Marmara University Hospitals in Istanbul in 1997. In that study, high-level streptomycin resistance was found in 8.5% of *E. faecium* isolates and in 20% of *E. faecalis* isolates. On the other hand, Ma *et al* in 1998 observed only 22.3% high-level gentamicin resistance in *E. faecalis* with none in other species.

In our study, the frequency of penicillin and ampicillin resistances was high among our isolates, 26% and 19% respectively (Table 1). Betalactamase production was not detected in any isolates, so the resistance was probably due to production of modified penicillin-binding protein (Bertrand *et al*, 2000).

Although none of the fecal samples from our hospitalized patients revealed vancomycin resistant enterococci, we could isolate some strains that showed intermediate resistance to vancomycin, and high-level resistance to aminoglycosides. Administration of antimicrobial agents should be carefully monitored by clinicians to prevent colonization and drug-resistant enterococcal infections. We believe that further surveillance studies are needed for early detection of such strains.

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