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ANTIBIOTIC SUSCEPTIBILITIES OF ENTEROCOCCI ISOLATED FROM TURKISH CHILDREN*

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SUMMARY: Akan Ö, Kanra G, Ecevit Z, Ceyhan M, Seçmeer G, Berkman E. (Infectious Disease Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine. Ankara, Turkey). Antibiotic susceptibilities of enterococci isolated from Turkish children. Turk J Pediatr 1997; 39: 13-17.

To evaluate the antibiotic resistance rates of enterococci isolated at Hacettepe Children's Hospital, in vitro antibiotic susceptibility tests were performed in 77 enterococci (32 hospital, 45 nonhospital strains) isolated from various clinical specimens in 1994. Microbroth dilution tests against ampicillin, vancomycin, gentamicin and streptomycin were performed according to the NCCLS standards. High-level resistance to aminoglycosides was investigated. Ampicillin resistance rates were 21.9 percent and 2.2 percent for hospital and nonhospital strains, respectively ($p < 0.01$). The same rates were 46.9 and 13.3 percent for gentamicin ($p < 0.01$), and 15.6 and 13.3 percent for streptomycin ($p = 0.25$). No resistance was detected against vancomycin. Six strains (7.8%) showed high-level resistance to both aminoglycosides studied. Special attention should be paid to enterococci isolated from hospitalized patients. Appropriate antibiotic use in serious infections can only be achieved by choosing an appropriate regimen according to antibiotic susceptibility tests. Periodic evaluation of the antibiotic susceptibility patterns of enterococci is necessary for the empirical treatment of infections due to these microorganisms. *Key words: enterococci, antibiotic susceptibility, high-level aminoglycoside resistance.*

For years enterococci have been considered as harmless inhabitants of the gastrointestinal tracts of humans and animals. Despite their low virulence, recent studies have documented that enterococci are increasing as significant nosocomial pathogens[1]. Enterococci do not possess the common virulence factors found in many other bacteria. Their intrinsic and acquired resistance against various antimicrobials explain the pathogenic potential of these microorganisms. Because of the diverse antimicrobial resistance mechanisms, successful treatment of enterococcal infections with current antimicrobial agents is becoming difficult, especially in serious infections[2]. This study was performed to show the antibiotic resistance rates of enterococci isolated in Hacettepe University Children's Hospital in order to give information to clinicians about the antibiotic therapy of enterococcal infections until susceptibility results are available.

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Material and Methods

Seventy-seven enterococci isolated in 1954 from urine (49), wound (23) and blood (5) specimens were evaluated in this study. All isolates were identified by colony morphology, hemolytic properties on sheep blood agar plates, Gram stain, negative catalase reactions, bile-esculin hydrolysis and growth in 6.5 percent NaCl. Streptococcal group antigens were also detected using group D antisera (Slidex Strepto-Kit, bio-Merieux-France). Biochemical reactions (mannitol, lactose, sorbitol fermentations) were used for differentiation of the species, and some of the strains were identified using API 20 Strep kit (bio-Merieux-France)[3].

The antibiotic susceptibility tests using ampicillin, vancomycin, streptomycin and gentamicin powders were performed using the microbroth dilution technique according to NCCLS standards[4]. High-level resistance against aminoglycosides (MIC's > 500 mcg/ml and > 2000 mcg/ml for gentamicin and streptomicin, respectively) was detected. Statistical analysis was performed using Fisher's exact test.

Results

Forty-five of the 77 total strains were isolated from outpatient clinics, and 32 were hospital isolates. Seventy-three of them were identified as E.fecalis, three E. faecium and one was E. durans. The antibiotic resistance rates for the hospital and nonhospital isolates are shown in Table I. The hospital isolates were more resistant to ampicillin and gentamicin ($p < 0.01$). Four of the seven ampicillin-resistant, and 11 of the 15 high-level gentamicin-resistant hospital strains were from pediatric surgical wards. All of the patients had histories of broad-spectrum antibiotic usage. MIC50 values for ampicillin and vancomycin were 0.50 mcg/ml, while MIC90 values were 2 mcg/ml and 8 mcg/ml, respectively. Four strains were resistant to all antibiotics studied except vancomycin. Six (7.8%) strains showed high-level resistance to both aminoglycosides studied.

Table I: Antibiotic Resistance Rates of Hospital and Nonhospital Strains

	Hospital Strains n: 32		Nonhospital Strains n: 45		
	n	R%	n	R%	p value
Antibiotic					
Ampicillin	7	21.9	1	2.2	$p < 0.01$
Vancomycin	0	0	0	0	
Gentamicin*	15	46.9	6	13.3	$p < 0.01$
Streptomycin*	5	15.6	6	13.3	$p = 0.25$

* Highly resistant strains.
n: number of cases.
R: Antibiotic resistance rate.

Discussion

Enterococci cause mostly urinary tract and intraabdominal or pelvic wound infections[5]. They can also cause bacteremia with high mortality[6]. Enterococcal infections of all types have been thought to be acquired from patients' own flora; however, in recent years the spread of these microorganisms among hospitalized patients has been observed. Hospital personnel and nurses can carry these bacteria to their patients[1,5]. In the USA, enterococci are reported to be the second-most common cause of nosocomial infections. They are also capable of causing a variety of community-acquired infections. E.fecalis is the most common isolate (85-90%), followed by E. faecium (5-10%)[1,7]. In our series, urinary tract specimens were most common, followed by wound and blood isolates. E. fecalis was the most common isolate.

In addition to their intrinsic resistance to a wide variety of antimicrobials, such as antistaphylococcal penicillins and most cephalosporins, increasing resistance of enterococci to the limited number of antibiotics used in therapy was become a worldwide problem[2]. Prolonged hospital stays, hospitalization in surgical wards and prior treatment with antimicrobials have been found as predisposing factors for acquisition of resistant strains[5,8]. Our resistance rate was higher in hospital isolates, and the majority of strains were from surgical wards.

Ampicillin is the preferred β-lactam for the treatment of nonserious infections such as urinary tract infections; however, some strains, especially E. faecium isolates, are resistant because of the changes in penicillin-binding proteins. PBP5 is the responsible protein[9]. Since 1983 β-lactamase-producing strains have been reported[10]. Infections caused by these strains can be treated with combinations of β-lactamase inhibitors or with vancomycin[2]. Our ampicillin-resistant strains were mostly E. faecium. Our resistance rate (2.2%) of outpatient isolates was not high, but resistance among hospital isolates (21.9%) was remarkable. A similar resistance rate (22%) was detected in 100 pediatric urine specimens studied in İstanbul[11]. No β-lactamase-producing strains have been found in Turkey yet[11,12]. β-lactamase production in our strains was not evaulated. In serious infections the combination of an aminoglycoside with penicillin/ampicillin or a glycopeptide is used to obtain a synergistic bactericidal effect[13]. However, strains that are highly resistant to aminoglycosides are no longer susceptible to the combination therapy[1]. High-level resistance to streptomycin was first reported in the early 1970's, and at the end of the same decade high-level gentamicin resistance was described[8,14]. Ribosomal resistance and aminoglycoside-modifying enzymes coding on self-transferable plasmids cause high-level aminoglycoside resistance[2,15]. Enterococci with high-level gentamicin resistance are increasing in prevalence and are generally resistant to all aminoglycosides with the exception of streptomycin[14]. Our gentamicin resistance was higher than

in previous reports from Turkey[11, 12]. It may be because no distinction was made between hospital and non-hospital strains in those studies. The fact that high-level streptomycin resistance was less common among our isolates may be due to limited use of this agent in our hospital.

Clinically important infections by enterococci resistant to glycopeptides were first described in France in 1986[16], and have been reported from various parts of the world in both adult and pediatric populations[2, 17]. The alarming point about the spreading potential of resistance is that vancomycin-resistant genes could be transferred among enterococci and from enterococci to staphylococcus species[2]. Although vancomycin-resistant enterococci have not yet been isolated in Turkey, with the wide use of such antimicrobials it may become a problem.

Clinical isolates of enterococci, especially isolated from hospitalized patients, should be screened for ampicillin, vancomycin and high-level aminoglycoside resistance. should be emphasized, especially in surgical wards. Suitable therapy for empirical treatment can only be chosen on the basis of susceptibility studies.

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