In vitro antimicrobial susceptibility in clinical isolates of Enterococcus species

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Abstract

Objective. To describe the antimicrobial activity of several antimicrobial agents against 97 clinical significant isolates of Enterococcus spp. **Material and Methods**. During a 2-year prospective study at Instituto Nacional de Pediatria (National Institute of Pediatrics) in Mexico City. Ninety seven strains of Enterococcus spp. (60 E. faecalis and 37 E. faecium) were tested against 11 antibiotics. Susceptibility tests were performed with agar, according to the standards of the sNational Committee for Clinical Laboratory Standards (NCCLS). Isolates were screened for high-level resistance (HLR) to β-lactams, aminoglycosides, glycopeptides and other antibiotics, as well as for vancomycin-phenotypes. Differences between proportions were evaluated with χ^2 of Fisher exact fest. **Results**. Overall resistance rates to the antibiotics tested were: 17/97 (17.5%) to penicillin, ampicillin, amoxicillin-clavulanate and imipenem. There was neither HLR nor β-lactamase production; 74/97 (48.4%) were resistant to erythromycin; 60% to ciprofloxacin; 31/97 (32%) to gentamicin, and 55/97 (56.7%) to streptomycin. Seven strains were vancomycin-resistant enterococci (VRE), all of them identified as E. faecium; 5/7 with Van A and 2/7 with Van B phenotypes. All the isolates were susceptible to linezolid. The difference in susceptibility among species was significant. **Conclusions**. Mutidrug-resistant enterococci is a real problem and continuous surveillance is necessary. The microbiology laboratory is the first line of defense against

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Resumen

Objectivo. Describir la actividad antimicrobiana de varios antibióticos, contra 97 cepas de Enterococcus spp., consideradas como aislamientos clínicamente significativos. **Material y métodos**. En un estudio prospectivo de dos años, (enero de 1998 a diciembre de 1999) hecho en el Instituto Nacional de Pediatría en la Ciudad de México, se procesaron 97 cepas de Enterococcus (60 de Enterococcus faecalis y 37 de Enterococcus faecium, contra 11 antibióticos. La prueba de susceptibilidad se elaboró con agar, de acuerdo con los estándares del Comité Nacional para el Laboratorio Clínico (NCCLS). Todos los aislamientos fueron probados para determinar la resistencia elevada en contra de β-lactámicos, aminoglucósidos y glicopéptidos. Asimismo, se determinó el fenotipo de resistencia hacia la vancomicina. Se evaluaron diferencias de proporciones con χ^2 o prueba exacta de Fisher. **Resultados**. La resistencia en general hacia los antibióticos probados fue 17/97 (17.5%) a penicilina, ampicilina, amoxicilina-clavulanato e imipenem. No se encontró resistencia elevada ni presencia de producción de β-lactamasas; 74/97 (48.4%) fueron resistentes a eritromicina, 60% resistentes a ciprofloxacina, 31/97 (32%) resistentes a gentamicina y 55/97 (56.7%) resistentes a estreptomicina. Siete cepas fueron resistentes a vancomicina, todas ellas E. faecium; 5/7 con el fenotipo A y 2/7 con el fenotipo B. Todas las cepas aisladas fueron susceptibles al linezolid. La diferencia en la susceptibilidad antimicrobiana entre las especies fue significativa. **Conclusiones**. La resistencia anti-

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the spread of multiantibiotic-resistan enterococci in the hospital environment .All the strains recovered should be tested for susceptibility to ampicillin, streptomycin, gentamicin and glycopeptides.

Key words: drug resistance, microbial; enterobacteriaceae infections; Mexico

microbiana múltiple de Enterococcus spp. es un problema real y es necesaria su vigilancia. El laboratorio de microbiología es la primera línea de defensa en contra de la diseminación de enterococos con resistencia múltiple en el ambiente hospitalario. Todas las cepas aisladas deberían ser probadas en contra de ampicilina, estreptomicina, gentamicina y glicopéptidos.

Palabras clave: resistencia microbiana a las drogas; infecciones por enterobacteriaceae; México

Enterococci are normal inhabitants of the gastroin testinal tract and part of the normal intestinal flora. They are not particularly pathogenic organisms in humans. Despite their lack of pathogenicity, enterococci have emerged as significant nosocomial pathogens.[1-9] Enterococci are also commonly recovered from infections of the abdomen, the pelvis, the biliary tract and wounds. Polymicrobial flora is common in these sites. Enterococci cause infections of other sites less frequently, for example, in bone, joints and the meninges.[3,4,10-13]

Progress in medical technology, such as the use of various intravascular access devices, magnified the impact of organisms of relatively low virulence, such as enterococci.[13] Of critical importance is the intensive use of broad-spectrum antibiotics in hospitals, which fosters a selective pressure favoring the growth of intrinsically drug-resistant commensal organisms like enterococci.[6,14-18]

Resistance to a number of antimicrobial drugs is characteristic of the genus Enterococcus, although some species are more intrinsically resistant than others.

The role of enterococci as a cause of infections has become increasingly important, not only because of their documented pathogenic potential, but also because of the increasing antimicrobial resistance of some strains, especially resistance to vancomycin (VRE).[14] Increasing use of parenteral third-generation cephalosporins and vancomycin for the treatment of intravascular device-related infections might have a role in developing enterococcal resistance.[6] Observations of vancomycin-resistant strains have revealed the presence of several different phenotypes of glycopeptide resistance.[17]

A number of newly-acquired mechanisms of resistance have emerged or become more frequent in Enterococcus species during the past decade, including high-level aminoglycoside resistance, beta-lactamase production, high-level ampicillin resistance, and vancomycin resistance. In United States hospitals, enterococci have become the second most common nosocomial pathogen overall, according to Nationwide Surveillance data.[10,19] In our study, 97 isolates from pediatric patients with Enterococcus species considered as clinically significant strains, were tested against several antimicrobials, to determine the in vitro activity of each agent as well as the phenotype in those with VRE.

Material and Methods

From January 1998 to December 1999, a 2-year prospective study was carried out at Instituto Nacional de Pediatria (National Institute of Pediatrics), a teaching and referral third-level hospital in Mexico City. Only serious infections were included in the study: endocarditis (n=4); primary bacteremia (unknown source) (n=23); catheter- related bacteremia (24); empyema (4); urosepsis (9); meningitis and / or ventriculitis (11); intrabdominal infection (3); and deep surgical wound infection (abscess) (19).

Clinical definition. Clinical significant bacteremia or infection due to Enterococcus spp., was defined by isolation of either species from ≥ 2 blood cultures or from a single blood culture, if there was a clinically apparent and / or culture-positive source of infection.

Bacterial strains. A total of 97 isolates were collected, 60 of them were Enterococcus faecalis and 37 were Enterococcus faecium. All of them were stored in double-strength skim milk (Difco,Labs. Detroit, Mich.) at -70^0 C.

Enterococcal isolates were identified using dried-overnight gram-positive combination panels in the MicroScan WalkAway 96 Instrument (Dade MicroScan, Inc., West Sacramento, CA). Species identification was confirmed by conventional microbiological testing.[20,21]

Prior to testing for susceptibility, isolates were thawed and subcultured twice to ensure purity and viability. Antimicrobials were supplied from the manufacturers as laboratory powders of known potency; stock solutions were prepared as recommended by the manufacturers. Antimicrobial used were: Penicillin G potassium, ampicillin and amoxicillin-clavulanate, imipenem, erythromycin, streptomycin, gentamicin, ciprofloxacin, teicoplanin, vancomycin and linezolid.

Antimicrobial susceptibility testing. The minimal inhibitory concentration (MIC) was determined in duplicate by the broth microdilution method in Mueller-Hinton broth (Difco, Mexico City, Mexico) supplemented with 10 mg of $MgCl_2$/l and 20 mg of $CaCl_2$/l., with a final inoculum of 1.5 X 10^5 CFU/ml, as recommended by the National Committee for Clinical Laboratory standards (NCCLS).[22] All plates were incubated at 35^0 C for 24 h in ambient air before determination of Minimal Inhibitory Concentration (MIC) values. The plates were visually read. NCCLS breakpoints were used to interpret MIC data.[22] Appropriate quality control was performed by use of Enterococcus faecalis ATCC-29212 (vancomycin susceptible). Linezolid is an investigational drug. NCCLS considered strains with a MIC ≤ 2 µg/ml as susceptible, those with a MIC=4 µg/ml as intermediate, and those with a MIC ≥ 8 µg/ml as resistant.[23]

Screening for beta-lactamase production was done using Cefinase disk methodology (a chromogenic substrate nitrocefin, Cefinase, BBL, Microbiology Systems, Cockeysville, MD).

High-level aminoglycoside resistance (HLAR). All the strains with a MIC ≥ 64 µg/ml to gentamicin and streptomycin were used to screen for HLAR. Those strains suspected to be HLAR were confirmed by broth tube dilution using brain-heart infusion broth with 500 and 1000 µg/ml concentrations of gentamicin, as well as with 1000 and 2000 µg/ml concentrations of streptomycin.

Phenotypes. The Van A phenotype include enterococci resistant to high levels of vancomycin (MIC ≥ 64/ml) and teicoplanin (MIC ≥ 8 µg/ml). This resistance is vancomycin- and/or teicoplanin- inducible.[18] Van B organisms are resistant to a range of vancomycin concentrations, from 4 to ≥ 1024 µg/ml; they typically retain their susceptibility to teicoplanin. This resistance is also inducible by vancomycin but not by teicoplanin.[18] Differences between proportions were evaluated with the χ^2 or Fisher exact test (as appropriate).

Results

A total of 97 clinical isolates of Enterococcus spp. (60 E. faecalis and 37 E. faecium) were collected, identified, and analyzed over a 24-month study period.

Table I shows the in vitro activity of antimicrobial agents that were tested according to different species.

β-lactam resistance. 5/60 (8.3%) E. faecalis and 27/37 (73.0%) E. faecium were resistant (overall 32/97; 33%) to penicillin; 2/60 (3.3%) E. faecalis and 15/37 (40.5%) E. faecium were resistant (overall 17/97, 17.5%) to am-

Table I

IN VITRO ACTIVITY OF SEVERAL ANTIMICROBIAL AGENTS AGAINST 97 ISOLATES OF ENTEROCOCCUS FAECALIS AND ENTEROCOCCUS FAECIUM. NATIONAL INSTITUTE OF PEDIATRICS, MEXICO CITY, 1998-1999

	E. faecalis *(60)*			E. faecium *(37)*		
Antimicrobials	*MIC range*	*MIC_{50}/MIC_{90}*	*%*	*MIC range*	*MIC_{50}/MIC_{90}*	*%*
Penicillin	1->16	4/>16	91.6	1->16	4/>16	27.0
Ampicillin	<0.25->16	1/4	96.6	0.05->16	2/16	59.5
Amoxicillin/clav	<0.25->16	1/4	96.6	0.5->16	2/>16	59.4
Imipenem	0.5->8	2/4	–	≤0.25->8	>8/>8	–
Erytromycin	<0.25->8	>8/>8	–	0.5->32	>4/>8	–
Streptomycin	<0.5->128	0.5/>128	46.6	<0.5->128	0.5/>128	37.8
Gentamicin	≤0.5->128	0.5/>128	75.0	<0.5->128	0.5/>128	56.7
Ciprofloxacin	<0.5->2	0.5/>2	33.3	0.5->2	1/2	27.0
Teicoplanin	≤0.25->16	0.25/0.5	100	0.5->16	0.25/>16	86.4
Vancomycin	0.5-8	0.5/1	100	0.5-16	0.5/>16	81.0
Linezolid	≤0.25-2	0.5/1	100	<0.25->2	0.5/≥4	100

MICs in µg/ml, %=Percent susceptible determined using NCCLS interpretative criteria; (–) no interpretative criteria published by the NCCLS

MIC: minimal inhibitory concentration

picillin and amoxicillin-clavulanate; 15/97 (15.4%) –all of them E. faecium– were resistant to imipenem. Resistance between species against β-lactams was significant. There was neither high-level penicillin resistance nor β-lactamase production among the clinical strains tested.

High-level aminoglycoside resistance. Fifthteen of sixty (25.0%) E. faecalis and 16/37 (43.3%) E. faecium were resistant (overall 31/97; 32%) to gentamicin; 32/60 (53.4 %) E. faecalis and 23/37 (62.1%) E. faecium were resistant (overall 55/97; 56.7%) to streptomycin. Resistance to aminoglycosides between species was significant.

Vancomicin-resistant enterococci. Seven strains were resistant to vancomycin, all of them E. faecium; 5 of 7 strains were also resistant to teicoplanin. All E. faecalis strains were susceptible to vancomycin and teicoplanin.

Phenotypes. Five of seven VRE isolates exhibited the Van A phenotype, and 2/7 exhibited the phenotype Van B.

Other antimicrobials. Erythromycin inhibited more than 50% of all strains at or below their respective susceptible breakpoint concentrations. More than 60% of the strains tested were resistant to ciprofloxacin. Notably, 100% of all the isolates tested were inhibited by ≤4 µg/ml of linezolid.

Discussion

Enterococci are not generally regarded as highly virulent bacterial pathogens, however, resistance to many antimicrobial drugs complicates the treatment of enterococcal infections. Acquired resistance to high concentrations of ampicillin, aminoglycoside, and glycopeptide antibiotics, specifically vancomycin, has exacerbated this problem.[6,8,13,14,18,24,25]

In the last decade enterococci have become recognized as leading causes of nosocomial bacteremia, surgical wound infections, and urinary tract infections.

Two types of enterococci cause infections: a) those originating from patients´native flora, which are unlikely to possess resistance beyond that intrinsic to the genus, and to be spread between patients from bed to bed, and b) isolates that possess multiple antibiotic resistance traits and are capable of nosocomial transmission. The therapeutic challenge of multiple-drug resistance enterococci has brought their role as important nosocomial pathogens into sharper focus.

Although E. faecium strains are resistant to ampicillin, aminoglycosides, and glycopeptides more than E. faecalis strains, the relative proportion of infections caused by these species has not dramatically changed in recent years.[14]

Different patterns of resistance have been informed from many countries.[18] That information is scarce in Mexico, particularly in pediatric patients.[26,27] In this study the activity of several antimicrobial agents against 97 clinical isolates is reported.

Considerable resistance of E. faecium isolates to most of the antibiotics tested was demonstrated during the study period.

The results of this study confirm that E. faecalis strains resistant to ampicillin and vancomycin are uncommon; in contrast, E. faecium strains resistant to vancomycin (7/ 37 strains) and ampicillin (15/37 strains), increased alarmingly. This observation is similar to those reported by other authors.[13,14,28-30]

At least for E. feacalis and E. falcium against penicillin, ampicillin and imipenem.

High-level resistance to aminoglycosides is a real problem, this resistance overcomes the synergy of killing combination therapy. Ampicillin and vancomycin are not bactericidal unless combined with an aminoglycoside.[10,12,18] High-level gentamicin resistance is most often associated with high-level resistance to all alternative aminoglycosides

Since enterococcal resistance to gentamicin and streptomycin occurs by different mechanisms, it is important to test susceptibility to both agents. Enterococci with HLR to streptomycin are susceptible to gentamicin. Gentamicin resistance is a good predictor of resistance to other aminoglycosides; also, ampicillin resistance is a predictor of imipenem resistance.[31-33]

Glycopeptide-resistance in Enterococcus spp. (7/ 97 or 7.2%) is higher than that found by Miranda and cols.; 5 /235 (2.12%) in E.faecalis and E.faecium strains.[26] Those isolates confirm the various levels of resistance to vancomycin and teicoplanin.

In this study, five of seven isolates of E. faecium were phenotype Van A and the other 2 were phenotype Van B. It is useful to identify which species are vancomycin-resistant in enterococcal isolates. Identification of Van A organisms has implications for treatment and infection control.[34,35]

Other studies on VRE clinical isolates found that most were Van A phenotype strains of E. faecium; they were associated with outbreaks in special wards with immunocompromised patients on long term antimicrobial regimens, with extended lengths of stay and higher severity of illness scores.[36-38]

Several limitations of the data from this study make firm conclusions problematic. First, all of the microorganisms tested came from a single institution. Second, a relatively small number of E. faecium and E. faecalis were tested; it is possible that these strains mig-

ht represent only a few clones. Third, no species other than E. faecalis and E. faecium were included.

Once vancomycin-resistant enterococci are established in the hospital environment, their frequent resistance to multiple antibiotics make it difficult to avoid further selective pressure in their favor. Enterococcal infections tend to occur in more debilitated or seriously ill hospitalized patients. Mortality in patients with VRE bacteremia may reach 60-70%. [3,14] From 1989 through 1997, the percentage of infections caused by VRE increased from 0.4 to 23.2 % among patients in the intensive care unit (ICU), and from 0.3 to 15.4% among patients not in the ICU.[2]

Because most enterococci are resistant to the bactericidal activity of β-lactam and glycopeptide antibiotics, bactericidal synergy between one of these antibiotics and an aminoglycoside is needed to treat most serious enterococcal infections. The synergistic bactericidal effect between aminoglycosides and β-lactam or glycopeptide antibiotics is lost if there is high-level resistance to either class of drug. The increasing use of parenteral vancomycin for the treatment of intravascular device-related infections might have a role in enterococcal resistance.

Treatment of multidrug-resistant enterococci is under an investigational new drug program for treatment of patients with life-threatening infection due to vancomycin-resistant E. faecium bacteremia. There has been a considerable effort to develop alternative agents; for example, dalfopristin-quinupristin is a streptogramin antibiotic that has been studied in the treatment of infections due to vancomycin-resistant E. faecium. Other investigational agents with activity in vitro against Enterococcus spp. susceptible or resistant to glycopeptides include the oxazolidinones. These are a new class of synthetic antibiotics with good antienterococcal activity and are different from any other class. Mechanisms of resistance that affect antibiotics in current clinical use do not affect the activities of oxazolidinones. Linezolid is one of the investigational agents. [39,40] In this study linezolid showed excellent activity against multiantibiotic- resistant enterococci. Clinical efficacy and safety studies are needed to determine its real utility. Linezolid has recently been approved by the Food and Drug Administration.

The microbiology laboratory is the first line of defense against the spread of multiantibiotic-resistant enterococci in the hospital environment. Cooperation and communication between the laboratory and the infection control program is essential in recognizing enterococci-resistant isolates from colonization and infection. All of the strains recovered should be tested for susceptibility to ampicillin, streptomycin, gentamicin, and glycopeptides.

It will be necessary to study additional E. faecalis and E. faecium strains from different hospitals and, if possible to include less common enterococcal species such as E. gallinarum and E. casseliflavus, which are relatively infrequent causes of human infections but they have intrinsic resistance to low concentrations of vancomycin.

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