CLINICAL MICROBIOLOGY REVIEWS, Jan. 1990, p. 46–65
0893-8512/90/010046-20$02.00/0
Vol. 3, No. 1

The Life and Times of the Enterococcus

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INTRODUCTION

The name "entérocoque" was first used by Thiercelin in a paper from France published in 1899 (206); the name was proposed to emphasize the intestinal origin of this new gram-positive diplococcus. In the same year, MacCallum and Hastings reported a case of endocarditis caused by an organism they called *Micrococcus zymogenes*; later papers suggest that this organism was actually a hemolytic enterococcus (191). The name *Streptococcus faecalis* (*faecalis*, relating to feces) was first coined in 1906 by Andrewes and Horder, who isolated this organism from a patient with endocarditis and considered that this streptococcus was "so characteristic of the human intestine that the term 'streptococcus faecalis' may justly be applied to it" (2).

Over the next decade or so, various authors studied and wrote about isolates that they also referred to as *S. faecalis*, but in 1919 Orla-Jensen used different terminology when he described strains of *S. glycerinaceus* and *S. faecium* (163). For a number of years, these names were largely ignored and the organisms were considered to be the same as *S. faecalis* (191). Differences of opinion regarding nomenclature existed not only for *S. faecalis*, but also for the common name, enterococcus. In an excellent review in 1937, Sherman (191) emphasized that the term enterococcus had been used to mean different things ranging from the broad definition of any fecal streptococcus to a restricted definition of organisms that appeared to be identical to *S. faecalis*. Sherman proposed a classification scheme which separated strepto-

cocci into four divisions: pyogenic, viridans, lactic, and enterococcus. The latter term was used for organisms that (for the most part) grew at 10 and 45°C, in 6.5% NaCl, and at pH 9.6 and which survived 60°C for 30 min; the ability to split esculin was also noted (191). Many of these characteristics became widely used to distinguish between enterococci and nonenterococcal streptococci, such as *S. bovis*, and some are still used today to help identify enterococci. Sherman's classification scheme also correlated with the serological scheme originated by Lancefield in the early 1930s. In that system, the enterococci reacted with group D antisera, while the pyogenic streptococci reacted with group A, B, C, E, F, or G and the viridans streptococci were nongroupable; *S. bovis*, classified by Sherman as a viridans streptococcus, was later shown to react with group D antiserum. Within the enterococci, Sherman described what were at that time the generally recognized enterococcal species, *S. faecalis*, *S. zymogenes*, *S. liquefaciens*, and *S. durans*. However, because of the similarities of the first three of these, he suggested that the more appropriate terminology would be as follows: *S. faecalis* (hemolysis negative, proteolysis negative), *S. faecalis* var. *liquefaciens* (hemolysis negative, proteolysis positive), *S. faecalis* var. *hemolyticus* (hemolysis positive, proteolysis negative), *S. faecalis* var. *zymogenes* (hemolysis positive, proteolysis positive), and *S. durans*. More recent work has shown that hemolysis is plasmid mediated and can be transferred to nonhemolytic strains, confirming the inappropriateness of using this characteristic to distinguish species (102).

Although the species that Orla-Jensen in 1919 had named *S. glycerinaceus* and *S. faecium* were considered by Sherman to be the same as *S. faecalis* (191), a number of studies in the 1940s and 1950s showed that organisms referred to as *S. faecium* had biochemical characteristics that distinguished them from *S. faecalis*. Such differences included inhibition by potassium tellurite, fermentation reactions, and failure to reduce tetrazolium to formazan (8, 49, 93). Although *S. faecium* was not officially recognized as a separate species in the 1957 *Bergey's Manual of Determinative Bacteriology* (19), the species status of these organisms was nonetheless widely accepted and was incorporated into official nomenclature by the mid-1960s (8, 19, 23, 49, 117). During this period, *S. durans* was sometimes listed as a separate species and sometimes referred to as a variant of *S. faecium* (19, 23, 49).

In addition to the familiar organisms mentioned above, a number of other enterococci have been isolated from human, animal, food, and plant sources. Motile enterococci were noted as early as 1935, and some that resembled *S. faecium* became known as *S. faecium* subsp. *mobilis* (23, 88, 101, 117, 169). Some motile enterococci were noted in the 1950s to produce a yellow pigment, and in 1968 the name *S. faecium* var. *casseliflavus* (for yellow colored) was suggested (88, 147). An enterococcus isolated from Gouda cheese was referred to in 1955 as "malodoratus" because of its bad smell (43). Some strains of enterococci were found to react not only with Lancefield's group D antiserum but often with group Q as well. Because these organisms resembled enterococci from chickens, the name "*Streptococcus avium*" was proposed in 1967 (160); this name was recognized in the 1974 *Bergey's Manual* (50).

In the past decade, the use of nucleic acid relatedness has clarified and expanded the classification scheme for enterococci. Farrow et al. in 1983 presented biochemical and DNA hybridization data that indicated that *S. faecalis*, *S. faecium*, *S. casseliflavus*, *S. avium*, *S. durans*, and *S. faecalis* var. *malodoratus* were all distinct; that "*S. faecium* var. *mobilis*" was the same as *S. casseliflavus*; that *S. faecalis* and its former subspecies *liquefaciens* and *zymogenes* were indeed one species; and that some group D enterococci from chickens, designated *S. gallinarum*, were distinct from *S. avium* (20, 70). Schleifer and Kilpper-Bälz in 1984 used DNA-DNA and DNA-rRNA hybridization to show that *S. faecalis* and *S. faecium* were so distantly related to streptococci, including *S. bovis*, that they should be transferred to another genus; it was similarly proposed that the group N lactic streptococci should be transferred to a new genus, *Lactococcus* (187). The proposal to transfer enterococci to a new genus named *Enterococcus* had been previously suggested, and it was this genus name that was proposed by Schleifer and Kilpper-Bälz (187). Shortly thereafter, Collins, Jones, and Farrow, working with Kilpper-Bälz and Schleifer, used similar methodology to show that strains called *S. avium*, *S. casseliflavus*, *S. durans*, *S. faecalis* subsp. *malodoratus*, and *S. gallinarum* were sufficiently closely related to other members of the genus *Enterococcus* to be transferred to this genus but sufficiently distinct to be considered separate species (43). The names proposed were *Enterococcus avium*, *E. casseliflavus*, *E. durans*, *E. malodoratus*, and *E. gallinarum* (43). These two important papers were published during the preparation of the 1984 *Bergey's Manual* (186), and the genus *Enterococcus* was not incorporated into the official nomenclature; however, this work was cited in *Bergey's Manual* and considered to support fully the creation of a new genus to encompass the enterococcal group of organisms (186). Nucleic acid studies have also been used to define other species within the proposed genus, including *Enterococcus hirae* (*hirae*, meaning of the intestine or gut), which includes some organisms previously classified as *S. durans*, and *E. mundtii*, which includes some atypical nonmotile pigmented strains (42, 69, 110). Three other new species, *E. raffinosus*, *E. solitarius*, and *E. pseudoavium*, have also been proposed (66), and some enterococci still have not been identified to species. The following is a list of the new and proposed enterococcal species (42, 43, 69, 110, 187; Collins et al., in preparation): *E. faecalis*, *E. faecium*, *E. durans*, *E. avium*, *E. casseliflavus*, *E. malodoratus*, *E. gallinarum*, *E. hirae*, *E. mundtii*, *E. raffinosus*, *E. solitarius*, and *E. pseudoavium*.

IDENTIFICATION AND TYPING

Presumptive Identification

The genus *Enterococcus* consists of gram-positive, facultatively anaerobic organisms that are ovoid in shape and may appear on smear in short chains, in pairs, or as single cells. Like streptococci, these organisms do not have cytochrome enzymes and are thus catalase negative, although some strains do produce pseudocatalase (65, 187). Most react with group D antisera and some react also with group Q antisera. Hydrolysis of L-pyrrolidonyl-β-naphthylamide (PYR) is a characteristic feature that is seen also with group A streptococci but not other streptococci. Most strains in the newly defined *Enterococcus* genus possess the characteristics summarized by Sherman in 1937 such as the ability to grow in 6.5% NaCl and at pH 9.6, to grow at 10 and usually 45°C, and, for the most part, to survive at 60°C for 30 min, although *E. avium* grows poorly if at all at 10°C (65, 187, 191).

In clinical laboratories, enterococci have been presumptively identified for years by their appearance on smear and

TABLE 1. Tests used to differentiate selected gram-positive organisms[a]

Test	% Positive					
	Enterococci	Lactococci	Aerococci	Pediococci	Leuconostocs	Lactobacilli
Gas from glucose	<1	0	0	0	100	50
Vancomycin resistance[b]	<1	0	0	100	100	90
Reaction with streptococcal group D antiserum	80	0	0	95	35	25
Bile-esculin positive	99	75	60	100	90	50
PYRase positive[c]	100	69	100	0	0	7
Growth						
In 6.5% NaCl broth	100	56	100	35	60	40
At 45°C	99	25	0	83	0	60
At 10°C	85	100	0	4	75	100

[a] Adapted from Facklam et al. (67).
[b] Although still very rare, acquired resistance to vancomycin has now been described (see text).
[c] Hydrolysis of PYR.

culture plus the demonstration of their ability to hydrolyze esculin in the presence of bile and to grow in the presence of 6.5% NaCl (62, 63). However, because some enterococci may require up to 48 h of incubation for the correct reaction to occur (17) and because it is often important clinically to known quickly whether an isolate is likely to be an *Enterococcus* or a *Streptococcus* sp., more rapid screening procedures have been sought. One such system is a 2-h test that uses 0.2% esculin in a buffered 5% NaCl solution which was reported to be positive for all of 239 enterococci after 2 h (170). Although the test itself is rapid, the heavy inoculum used (McFarland standard no. 3 in 0.5 ml) would likely require 24 h of growth of the organism following its isolation. Several other screening systems use the ability of enterococci to hydrolyze PYR. Bosley et al. found that all 78 enterococci (including 32 *E. faecalis*, 28 *E. faecium*, 13 *E. avium*, and 5 *E. durans*) were positive at 4 h in PYR broth (17); the inoculum consisted of a loopful of colonies from an overnight growth. Since group A streptococci also hydrolyze PYR, this system included serologic testing with group D antisera (17). A number of different PYR preparations have been used, including one which yields results in 10 min (222). PYR has also been used to identify successfully 24 of 25 enterococci from real or simulated positive blood cultures; this method required using bacterial pellets following centrifugation. Group D serologic testing was not reliable in this system, but excellent results were obtained with antisera for group A streptococci, the only streptococci that are PYR positive. Thus, a combination of Gram stain, positive PYR test, and negative group A serologic test was proposed as a useful screening test (86).

Although the above screening tests appeared to be sufficient in the past to identify enterococci presumptively, it is now recognized that other less commonly encountered gram-positive cocci can also give a positive reaction in some of these tests (65–67). For example, some cultures of *Lactococcus*, *Aerococcus*, *Pediococcus*, and *Leuconostoc* spp. are bile-esculin positive or can grow in 6.5% salt or both. Strains of *Pediococcus* and *Leuconostoc* spp. can be group D positive, and some lactococci and aerococci are PYR positive; lactococci and aerococci, however, should not react with group D antisera. Leuconostocs show no zone of inhibition around a 30-μg vancomycin disk, are PYR negative, and produce gas from glucose, whereas enterococci are PYR positive and do not produce gas from glucose; also, although vancomycin resistance has now been reported, the vast majority show a zone of inhibition around vancomycin disks. A battery of tests, including gas from glucose, vancomycin susceptibility testing, group D antiserum reaction, bile-esculin, PYR, and growth in 6.5% NaCl and at 45 and 10°C, may be necessary (Table 1). For further details, the reader is referred to recent papers by Facklam and Collins (66) and Facklam et al. (67).

Species Identification

In many instances, it may not be necessary to identify enterococci to species. For example, with urinary tract and wound isolates, presumptive identification to the genus level together with determination of susceptibility to selected antimicrobial agents such as penicillin, ampicillin, and vancomycin is generally sufficient. However, with certain infections, especially endocarditis, differentiation between *E. faecalis* and *E. faecium* (the two most commonly encountered enterococcal species) can be helpful because of the naturally occurring differences in the susceptibility of these species (see below). Currently, it is not known whether the less commonly encountered enterococcal species differ importantly in their susceptibility, but now that criteria are available for identifying them, perhaps more laboratories will look for these species and accurate numbers regarding their incidence and susceptibility can be determined (66).

Another reason for species identification of enterococci is that it may be useful for epidemiologic surveillance within hospitals. Although enterococci were not previously thought of as nosocomially spread pathogens, recent studies have confirmed this route of transmission (126, 232); an increase in the number of isolates of *E. faecium* or especially an increase in the rarely encountered species would be an epidemiologic clue to the presence of an outbreak (46, 216).

Between 80 and 90% of clinical enterococcal isolates in recent studies have been *E. faecalis* (114, 129, 172, 193), with *E. faecium* a distant second; therefore, this is the differentiation that should be most commonly made. In addition to the low degree of DNA relatedness between these species, a number of biochemical differences facilitate their differentiation. *E. faecalis*, for example, has the Lys-Ala_{2-3} type of peptidoglycan and has demethylmenaquinones, whereas *E. faecium* contains the Lys–D-Asp type of peptidoglycan and has neither menaquinones nor ubiquinones (187). Differences that can be readily detected in clinical laboratories include the ability of most *E. faecalis*, but generally not *E. faecium*, to grow on medium containing 0.04% tellurite and to reduce tetrazolium to formazan; however, many of the other enterococcal species resemble *E. faecium* in these tests, and *E. mundtii* are often like *E. faecalis*. Other differences are that most *E. faecalis* but not *E. faecium* produce acid from D-tagatose, sorbitol, and

glycerol, whereas most *E. faecium* but not *E. faecalis* produce acid from melibiose and L-arabinose (43, 65, 66, 187). Several biochemical reactions can be suggestive of the other enterococcal species (42, 43, 66, 69). *E. casseliflavus*, for example, is motile and produces yellow pigment; *E. mundtii* produces yellow pigment and is not motile; *E. gallinarum* is motile but does not produce yellow pigment; and the other enterococcal species neither are motile nor produce yellow pigment (42, 43, 187). The papers describing these new species used a combination of conventional tests and dehydrated miniaturized tests (42, 43, 69, 187, 229), the results of which usually, but not always, agree (78).

Clinical laboratories that identify enterococci to species generally use a commercially available system, such as the API 20S (Analytab Products, Plainview, N.Y.), the GPI (gram-positive identification) system (Vitek Systems, Inc., Hazelwood, Mo.), the Rapid Strep System (also available from API), or the RapID STR system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.). Most of these test systems provide an identification in approximately 4 h, although the inoculum size usually requires that the organism be incubated for 24 h after its isolation. A number of studies of these rapid systems showed that the majority of *E. faecalis*, *E. faecium*, *E. avium*, and *E. durans* are correctly identified (3–5, 64, 68, 105, 229). However, these systems were developed prior to the recent taxonomy changes and some identifications may be in error, especially for species other than *E. faecalis*; these systems need to be reevaluated with these new species definitions.

It is not clear at present how aggressively the routine clinical microbiology laboratory should pursue the identification of organisms that are presumptively identified as enterococci and have a consistent antibiogram, but for a detailed analysis of the biochemical reactions typifying these organisms, the reader is referred to Facklam and Collins's recent identification scheme of the 12 enterococcal species (66).

Typing Schemes

Until recently, it has been difficult to do epidemiologic studies of transmission of enterococcal strains because the organism is present in most healthy individuals and, within a given species, there have not been good mechanisms for distinguishing one isolate from another. Easily determined phenotypic characteristics, such as biochemical reactions and antibiotic resistance patterns, do not usually show enough variation to be useful, except for unusual variant strains such as asaccharolytic *E. faecalis*. Typing based on total plasmid content has been used with a wide variety of other organisms and is most useful when multiple plasmids are present. Zervos et al. used total plasmid content plus the rather uncommon, at that time, marker of high-level resistance (HLR) to gentamicin to distinguish strains (232). Luginbuhl et al. used a combination of plasmid typing and physiologic tests to investigate an outbreak in neonates (126). Both of the studies strongly support the concept of nosocomial transmission of enterococci based on finding strains with the same total plasmid content in different patients. Total plasmid content analysis is applicable because enterococci do not have species-specific plasmids or plasmid patterns; that is, unrelated strains have different plasmid contents.

Phage and bacteriocin typing have been applied to enterococci at various times over the past two decades (33, 109, 115, 166, 167, 212). A recent publication reported a typing scheme, using both phage and enterococcines, with over 900 enterococci from two hospitals (114). A large percentage (79%) could be typed into one of 25 phage types, although 61% belonged to a single phage type. Seventy-nine percent could also be placed into one of six enterococcine groups which consisted of 85 enterococcinotypes; half belonged to one group. When phage typing was combined with enterococcine typing, 87% of strains could be typed. Among these strains, 187 types were obtained by combining species, phage type, and enterococcinotype (114).

At the current time, it is not known whether the standard for epidemiologic typing of enterococci should be plasmid typing, phage with or without bacteriocin typing, or some other technique, such as chromosomal digestion patterns, which may be applied to enterococci in the future. It will be of interest to compare these various techniques in epidemiologic studies of the same strains.

HABITAT

Enterococci are found in the feces of most healthy adults; in several recent studies from Japan, Federal Republic of Germany, and Scandinavia, enterococci were found in 97% of 71 individuals studied (14, 15, 55, 59, 134, 159, 190). When enterococci from feces have been identified to species, many studies report that *E. faecalis* is more common and is found in higher numbers than *E. faecium* (14, 59, 114, 159, 190). Studies from some locations, however, have reported that *E. faecium* is found more often than *E. faecalis* (134). Noble examined feces from 6- to 7-day-old infants and from inpatient and outpatient adults (159). He found *E. faecalis* in 48% of 21 infants and *E. faecium* and *E. avium* in none. Among adults, *E. faecalis* was found in 8 of 10 inpatients and 14 of 29 outpatients (56% overall); in most of these, 10^5 to 10^7 CFU/g of stool were found. *E. faecium* was found in 15 of 39 adults (38.5%), with average counts of 10^4 to 10^5 CFU/g. *E. avium* was found in 3 of 39 adults, and *E. durans* was not found in any (159). Benno et al. compared the fecal microflora of nine rural Japanese with eight urban Canadians and found that 100% of both groups had *E. faecalis*; these organisms were present at about 6.5 $\log_{10}$ CFU/g (14). *E. faecium* was found in two individuals in each location; *E. durans* was found in one Japanese and in no Canadian individuals (14). Two other recent studies from the Federal Republic of Germany found *E. faecalis* in 21 of 22 persons before a course of a fluoroquinolone and in 22 of 22 approximately 1 week after therapy was stopped; *E. faecium* was found in 9 of 22 (59, 190).

Enterococci are less commonly found at other sites such as in vaginal (17% in one study [12]) and oral (116, 196) specimens, and results are sometimes quite variable. In a study of the dental plaque of healthy students, academic staff, healthy toothache patients, and hemodialysis patients and staff, enterococci were found in approximately the same percentages in the various groups (10% overall), although patients and staff in one particular hospital had carriage rates of 60%; almost all isolates were *E. faecalis* (196). In another study, higher rates of enterococcal carriage were found among long-term hemodialysis patients and cardiac patients than among their staff and acute dental patients; in this study, *E. faecium* outnumbered *E. faecalis* isolates (31).

CLINICAL INFECTIONS

Historical Perspective

Enterococci have been recognized as being potentially pathogenic for humans since the turn of the century. What

was probably the earliest review of the literature on human diseases associated with enterococci appeared in 1912, and several reviews were published in the 1920s (reviewed by Sherman [191]). Even though some of these early isolates were probably not enterococci as they are now defined, many of the clinical syndromes described years ago continue to be seen today.

In Thiercelin's original description of entérocoque in 1899, he found these organisms in patients with enteritis, appendicitis, and meningitis (206), none of which are commonly associated with enterococci today. Also in 1899, as was mentioned earlier, an organism isolated from a patient with endocarditis was called "*Micrococcus zymogenes*," but was later thought to be *S. faecalis* var. *zymogenes* (191). The organism Andrewes and Horder called *Streptococcus faecalis* in 1906 was also isolated from a patient with endocarditis (2); Hicks in 1912 (191) and a number of subsequent workers confirmed the association of enterococci with endocarditis (61, 191). The role of enterococci in urinary tract infections (UTIs) was first reported by Andrewes and Horder in 1906 and was extended in subsequent decades by other investigators (2, 61, 191). Early authors also noted an association of enterococci with puerperal sepsis and with purulent abdominal infections following damage to the intestinal tract (61, 191). In World War I, a number of authors reported enterococcal wound infections, although it was not established whether this organism was colonizing or truly causing infection (61). Other enterococcal infections that were reported in the early years include osteomyelitis, cholecystitis, and dental infection (61). Some of these reports may not have been describing organisms that were truly enterococci or the cultures may have contained other organisms that were missed or were disregarded. One of the more obvious examples is the role that was postulated for enterococci in enteritis or food poisoning or both. This apparent misconception probably arose because of the common occurrence of enterococci in the intestinal tract and thus in fecally contaminated foods. On the other hand, a recent article implicated a strain of *E. hirae*, which probably came from the stool of a human with diarrhea, as a cause of diarrhea in suckling rats (60).

Endocarditis

Enterococci cause an estimated 5 to 15% of bacterial endocarditis. As with other enterococcal infections, most isolates are *E. faecalis* (81, 141, 185, 226); however, other species can also cause this disease. Among isolates sent to the Centers for Disease Control, endocarditis was the diagnosis given for patients from whom *E. avium*, *E. casseliflavus*, *E. durans*, *E. gallinarum*, and *E. raffinosus*, as well as *E. faecalis* and *E. faecium*, were isolated (66). The average age of patients in one recent study of enterococcal endocarditis was 65 years; this is somewhat older than the age in studies of several decades ago, perhaps reflecting a generally aging population (111, 131, 226). Enterococcal endocarditis occasionally occurs in children and rarely in infants (128, 203). Males predominate and, at least in the earlier studies, have been older than the women with this disease. Mandell et al., reporting patients from 1945 to 1968, found an average age of 59 years in the 22 men and 37 years in the 16 women they studied (131). During this time period, Koenig and Kaye also reported a mean age of 56 years in men and 35 years in women (111). The disease may present as an acute or subacute illness. Common risk factors for enterococcal endocarditis included genitourinary and biliary portals (111, 131, 226). In Mandell's study, 50% of the men had preceding genitourinary instrumentation or UTI; 38% of the women had a preceding genitourinary source including abortion or instrumentation (131). Underlying heart disease is often present but is not a prerequisite for the development of this disease (111, 129, 131, 172, 174, 226). Enterococci can also cause endocarditis in drug addicts. In this population, they are estimated to cause approximately 5 to 10% of cases, but one study in Cleveland, Ohio, in 1970 to 1974 reported that 11 of 20 cases of endocarditis (55%) were caused by enterococci (172). Unlike staphylococcal endocarditis in addicts, the valves involved by enterococci are usually aortic and mitral. These patients usually have no history of antecedent valvular disease and often present with congestive heart failure (172).

Enterococcal Bacteremia

Enterococcal bacteremia is much more common than enterococcal endocarditis. In two studies, which reviewed all blood cultures positive for enterococci, 2 of 114 (1.7%) and 2 of 79 (2.5%) patients were thought to have endocarditis (80, 221). Higher percentages have also been reported, but the cases were selected differently. For example, Shlaes noted in his review that there were 13 cases with pure cultures of enterococci from the blood in the last 4 years of the study and 6 patients (32%) with endocarditis; this study included only patients with two or more positive blood cultures and did not include those with polymicrobial bacteremia, a common accompaniment to enterococcal bacteremia (193). Malone et al. studied 55 patients with enterococcemia and found endocarditis in 5 patients (9%); however, this study included only patients with two or more positive blood cultures or one positive blood culture plus isolation of the organism from normally sterile sites or from surgical or autopsy specimens (130). Using a similar definition, Maki and Agger found 13 cases of endocarditis among 153 cases of enterococcemia (8%) (129). Twelve of their cases of endocarditis occurred among the 33 patients with community-acquired enterococcal bacteremias (36%) and only one occurred among the 120 patients with nosocomial bacteremia (0.8%). This review also emphasized that there had been a steady increase in the number of patients with enterococcemia since 1975, even though there was little change in the number of admissions; this increase was due entirely to an increase in nosocomial bacteremias (129).

The source of enterococcal bacteremia without endocarditis is often the urinary tract: 23% of patients in the study by Shlaes et al. (193), 19% in the study by Garrison et al. (80), and 24% in the study by Malone et al. (130). In Maki and Agger's report, 77% had either a urinary or an intravascular catheter (129). Intraabdominal, biliary, pelvic, and wound sources are also common (80, 122, 129, 130, 193). Other sources include burn wounds, peripartum mothers and infants, bone, and intravascular catheters (45, 80, 129, 130, 193). Polymicrobial bacteremia, either simultaneous or in separate but temporally related blood cultures, is very common, ranging from 24% in a study requiring two or more positive enterococcal blood cultures for inclusion to 45% in a study which included all positive enterococcal blood cultures (80, 129, 193).

Mortality of enterococcal bacteremia has generally been high, most probably because of the underlying complicating factors. In the study by Shlaes et al., it was 34% and was significantly associated with burns, hospital-acquired infections, and serious underlying illness but not with the sex of

the patient or with the presence of polymicrobial bacteremia (193). Nine of 14 diabetics, 6 of 10 patients with malignancy or granulocytopenia, 7 of 8 with renal failure, and 3 of 5 alcoholics died (193). In the study by Malone et al., the mortality was 44%; this study did not assess the same factors assessed in the previous study but found that male sex and a rapidly or ultimately fatal underlying disease were significantly associated with increased mortality (130). In Maki and Agger's study, 46% died (30% of those with endocarditis and 48% of those without). Age over 56 years, hospital acquisition, polymicrobial bacteremia, fatal underlying disease, prior antibiotic therapy, intraabdominal origin, and multiple sites of local infection were each associated with a significant increase in case fatality (129). It is not known whether the high mortality in patients with enterococcal bacteremia is due to the enterococcus per se or to the underlying condition which, in turn, predisposes to the development of the enterococcal bacteremia. It is also not known how enterococcemia can be prevented or better treated. Maki and Agger recommend using empiric anti-enterococcal therapy (pending culture results) for intraabdominal or pelvic infection, postgastrointestinal or genitourinary surgery wound infection, burn wound infection, urosepsis, or line sepsis when enterococci have been cultured from a site of local infection, the patient has valvular heart disease, the infection is nosocomial, or the patient is immunosuppressed (129). Although data are lacking that show improved efficacy of this approach, these suggestions seem reasonable.

UTI

UTIs are commonly caused by enterococci, particularly among hospitalized patients; enterococcal prostatitis and perinephric abscess have also been reported (54, 89, 92, 146). Among young healthy women who have not undergone instrumentation, do not have recurrent infections, and do not have structural abnormalities, enterococci cause $<5\%$ of UTIs (199). In persons who have been instrumented, received antibiotics, have structural abnormalities, and/or have recurrent UTIs, the rate of urinary colonization and infection by enterococci rises (92, 215). A dramatic example illustrating some of these features was the study by Gross et al. from a Veterans Administration Hospital in 1972 (89). In that hospital, whose patients were mainly elderly men, enterococci caused 21% of UTIs (defined as a urine culture containing $>10^5$ CFU/ml) and was the leading cause of that infection; *Escherichia coli* was found in 15%. This contrasted with 36% *Escherichia coli* and 4% enterococci in UTIs at a general hospital reported in 1973 (18, 89). Although most of the patients in the Veterans Administration study were asymptomatic and the positive urine cultures may have represented colonization as opposed to actual infection, it is important to note that 30 of 34 had undergone instrumentation. These authors also found that, of nine patients with enterococcal bacteremia, four had positive urine cultures with $>10^5$ enterococci per ml and three had positive urine cultures with $>10^5$ enterococci per ml (89). In a study from the United Kingdom over the years 1980 to 1986, the percentage of urine cultures containing $>10^5$ CFU of enterococci per ml increased from 11 to 20% for specimens from catheterized patients but showed little change (6.1 to 7.8%) for midstream urine specimens from noncatheterized patients (124). The U.K. national survey of infections reported that enterococci caused 7.2% of UTIs in hospitalized patients in 1980 (136). The Centers for Disease Control's National Nosocomial Surveillance Study lists enterococci as the third most common cause of nosocomial UTIs, causing 14.7% in the 1984 report (36). As will be discussed further below, the hospital setting is complex and a number of factors may contribute to acquisition of enterococcal urinary infection, including frequent instrumentation, prior therapy with antibiotics that select for resistant organisms, debilitated patients, and transmission of resistant organisms.

Neonatal Infections

Although group B streptococci and *Escherichia coli* are the most common causes of neonatal infections, it has been well documented that enterococci can also cause infection in this population. In a retrospective study at Cincinnati Children's Hospital, nine cases of neonatal enterococcal sepsis or meningitis or both were found between 1970 and 1976 (24). Most of these infants had not been instrumented and were of normal to near-normal birth weight; one infant was 25 days old and was admitted from home (24). Siegel and McCracken reported the incidence rates (cases per 1,000 live births) of bacterial diseases among 30,059 live births at Parkland Memorial Hospital in Dallas, Tex., from 1974 to 1977 (194). Group B streptococci were the most common organisms found with a rate of 3.2; the rate of "group D streptococci" was 1.2, and for *Escherichia coli*, it was 0.9. Among the group D streptococci, the incidence rates for enterococci ranged from 0.6 to 2.0 over the 4-year period as compared with the rates for *Escherichia coli*, which ranged from 0.4 to 1.4. Early-onset septicemia was the usual presentation for neonatal group D streptococcal disease (194).

An outbreak of *E. faecium* occurred in infants in the Neonatal Intensive Care Unit at the Medical College of Virginia Hospitals in 1982 (46). The children had many of the features commonly associated with nosocomial enterococcal bacteremia: all had severe underlying disease, nasogastric tubes, and multiple intravascular devices, were premature, and had received total parenteral nutrition. Cultures of blood and cerebrospinal fluid (CSF) were positive in four patients, blood only was positive in two patients, and CSF only was positive in one patient. The lack of recovery of *E. faecium* from neonates over the previous year plus certain biochemical characteristics of these isolates led these authors to conclude that these *E. faecium* represented the same strain and that the outbreak was most likely due to spread by hospital personnel (46).

Another outbreak of neonatal enterococcal sepsis was recently reported by Luginbuhl et al. from Denver, Colo. (126). During the 6-month outbreak period, enterococci caused 8 of 19 (42%) episodes of neonatal bacteremia versus only 8 of 159 episodes (5%) during the rest of the 5-year period reviewed (126). All *E. faecalis*-infected infants were premature and of low birth weight and often had bowel resection and a longstanding central line in place (126). Nosocomial transmission has also been documented among a large number of infants at Boston Children's Hospital, Boston, Mass.; beta-lactamase production, an unusual feature, proved to be an important epidemiologic marker (E. Rhinehart, C. Wennersten, E. Gross, G. Eliopoulos, R. Moellering, N. Smith, and D. Goldmann, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1073, 1988).

Central Nervous System Infections

In addition to causing neonatal meningitis, enterococci can also cause central nervous system infections in older

children and adults. Most cases seem to be related to an underlying disorder. In a 1961 review, 12 of 294 cases of meningitis appeared to be caused by enterococci; many of these patients were said to have had a long-term primary illness, invasive procedures of the central nervous system, or prior antibiotic therapy or all three (56).

In case reports reviewed by Bayer et al., enterococcal meningitis was reported in one patient with meningeal leukemia who was receiving intrathecal chemotherapy, in two children with spina bifida, in one with meningomyelocoele, in one patient following a basilar skull fracture, and in one patient (who had recurrent enterococcal meningitis) with a communication between a mediastinal cyst and the spinal subarachnoid space (11). Of the three patients in this paper who were studied by the authors, one had meningomyelocoele and one was recovering from intracranial surgery for repair of an arteriovenous malformation; the third patient had no known central nervous system abnormality but had obstruction of the urinary tract due to prostatic cancer and an enterococcal UTI. All three of these patients had CSF leukocyte counts of $<50/mm^3$, and most of the patients reviewed had CSF leukocyte counts of $<200/mm^3$ (11).

Ryan et al. reported a patient with enterococcal meningitis that was atypical for two reasons (178). First, the patient's only compromising condition was that he was receiving prednisone (30 mg) every other day for chronic obstructive lung disease; second, his CSF leukocyte count was $4,000/mm^3$ on admission, decreased to $311/mm^3$ on chloramphenicol, and then increased to $7,600/mm^3$ (with a CSF glucose of 27 and a repeat positive CSF culture) when he relapsed, still on chloramphenicol (178). Enterococci have also been reported as a cause of central nervous system shunt infections, particularly those that terminate in the peritoneum (2 of 19 ventriculoperitoneal infections) or ureters (2 of 14 ventriculoureteral infections and 1 of 9 lumboureteral infections) (188).

Intraabdominal and Pelvic Infections

Although enterococci are part of the normal intestinal flora of most persons and are found in about 17% of routine vaginal cultures, the need to empirically or prophylactically treat for the enterococcus in intraabdominal and pelvic infections remains somewhat controversial (12, 52). The data from animal experiments indicate that enterococci do not cause sepsis when injected intraperitoneally alone but can act synergistically with other organisms or substances to cause abscess formation (162, 214, 220, 223a). Moreover, antibiotic regimens that have little or no in vitro activity against enterococci, but which have activity against anaerobes and *Escherichia coli*, have been successfully used in most patients with intraabdominal and pelvic infections (10, 32, 71, 94, 168, 201, 225). The discrepancy between in vitro and clinical results is also seen in the rat model of enterococcal intraabdominal abscess in which combinations of clindamycin or metronidazole plus gentamicin have some benefit and are bacteriostatic in vivo against enterococci (223a).

Despite the difficulty in establishing pure enterococcal infections, it is clear that enterococci can cause and contribute to abdominal and pelvic abscess and sepsis. For example, enterococci have been reported as a cause of spontaneous peritonitis in both cirrhotics and nephrotics and can cause peritonitis in patients on chronic ambulatory peritoneal dialysis (48, 87, 123, 219); some of these isolates were reported to be pure cultures of enterococci, although it is not always stated that anaerobic cultures were performed. Enterococci have also caused acute salpingitis, peripartum maternal infection (such as endometritis) with bacteremia, and abscess formation following Cesarean section (83, 122, 161, 193). In one study of 144 bacteremias in obstetrical and gynecological patients, 18 (13%) were due to enterococci, placing it second in frequency behind *Escherichia coli*; although the sources were not identified, it is clear that the majority were not from a urinary source (122). Enterococcal abscesses and bacteremia also occur with intraabdominal and biliary infections (53, 80, 129, 193, 231). What is not known is who will develop these infections and who would be likely to benefit from early therapy with anti-enterococcal agents. Dougherty has suggested that it would be reasonable to use such therapy in patients hospitalized long term, with persistent or recurrent intraabdominal sepsis, and possibly those who are immunodepressed, such as transplant and burn patients (52). Although these are the patients who seem most likely to develop enterococcal infections, the efficacy of early therapy has not been evaluated.

Nosocomial Infections and Superinfections

In most instances of nosocomial enterococcal disease, it is not known whether the organism comes from the flora the patient was admitted with or whether the organism was acquired following hospitalization. Although some early studies did not suggest transmission within the hospital, more recent work, including studies of outbreaks among neonates, has demonstrated person-to-person spread. Regardless of the source, it has become clear in recent years that enterococci are an important cause of hospital-acquired infections (98). As mentioned, the 1984 National Nosocomial Infection Surveillance Summary listed the enterococcus as the third most common cause of nosocomial infection; enterococci caused approximately 10% of all such infections, including 14.7% of UTIs and 7% of bacteremias (36). In support of this was Morrison and Wenzel's study which showed that the rate of enterococcal UTIs increased over a 10-year period from 12.3 to 32.2 per 10,000 patient discharges; the percentage of hospital-acquired UTIs caused by enterococci increased from 6 to 16% (146). In Maki and Agger's study, the number of nosocomial enterococcemias increased approximately threefold in 1982 to 1983 versus 1970 to 1973, while the number of community-acquired cases was relatively constant (129).

Whether the increase in enterococcal disease seen in various studies is due to an increase in the use of antimicrobial agents to which enterococci are resistant; to an increase in the use of multiple vascular access lines, urinary catheters, and other invasive devices; to an increase in the number of seriously ill and debilitated patients; or to a combination of these factors is difficult to assess, especially since these factors often occur simultaneously in the same patient. However, the importance of these factors has been emphasized in a number of reports. In Morrison and Wenzel's study showing a 2.5-fold increase in enterococcal UTIs over a 10-year period, they found that cephalosporin use had increased from 64,000 to 114,000 g/year (146). Zervos et al. found that patients colonized with gentamicin-resistant enterococci had more likely received a cephalosporin or an aminoglycoside within the prior 3 months than patients without gentamicin-resistant enterococci (232). In four other studies, 42 to 78% of patients with enterococcal bacteremia had received antimicrobial agents prior to onset of the enterococcal infection (9, 89a, 129, 130).

The use of antimicrobial agents lacking enterococcal activity has been implicated as an important factor in the development of enterococcal superinfection (16, 46, 74, 98, 137, 176, 207, 230). Moellering reviewed 2,107 patients treated with moxalactam and found that 2.1% developed an enterococcal superinfection during or shortly after moxalactam therapy (137). This infection occurred in 38 (6.6%) of 572 patients who had a UTI; of note, 28 of these 38 had urinary catheters, which is also considered a compounding factor in the development of enterococcal UTIs. The sources of seven enterococcal bacteremias were wound, peritoneal fluid, the bile duct, the urinary tract, and unknown (137).

Pneumonia, an infection rarely caused by enterococci, and other infections have also been reported in patients treated with moxalactam (16, 137, 207, 230). Enterococcal pneumonia has also been seen following the use of polymyxin aerosol for prevention of *Pseudomonas aeruginosa* pneumonia; in addition to patients with pneumonia, 11 of 292 patients were colonized in the respiratory tract by enterococci (74). Enterococci were found in the urine of 8 of 45 patients (17.8%) treated with aztreonam; 7 of these had a Foley catheter and the eighth had a nephrostomy tube. Three of these patients were thought to be colonized and five were thought to be infected, including one with bacteremia (37).

Zervos et al. reported two patients with enterococcal infection following intravenous ciprofloxacin therapy. Both had long, complicated illnesses typical of enterococcal superinfection. One had suffered a perforated cecum, and *E. faecalis* was present in the initial cultures. Following courses of piperacillin, imipenem, ceftazidime, tobramycin, amphotericin, and, finally, ciprofloxacin plus tobramycin for recurrent *P. aeruginosa* infection, an abdominal abscess and bacteremia due to *E. faecalis* occurred. The other patient had a necrotic left colon resected and was treated with piperacillin, tobramycin, imipenem, and then ciprofloxacin plus tobramycin for *P. aeruginosa*. After about 3 weeks of these various therapies, *E. faecalis* was found in abdominal abscesses along with *P. aeruginosa*, *Candida* spp., and *Proteus vulgaris*. After 5 more days of ciprofloxacin and tobramycin, the patient became bacteremic with *E. faecalis* (231). These patients likely represent persistence of *E. faecalis* following fecal spillage and illustrate the important role enterococci can play in this type of patient, even when anti-enterococcal therapy has been used.

Nosocomial enterococcal infection has also been reported in transplant patients. A recent paper from England reported an unusually high rate of enterococcal bacteremia in liver transplant patients. Although prior antimicrobial therapy is not specifically mentioned, it is highly likely that most received some type of antibiotic (216). In this report, 21 of 104 episodes of bacteremia were due to *E. faecalis* and 23 of 104 were due to *E. faecium*; recurrent bacteremia was seen in 4 patients with each species. The most common source was the biliary tract; enterococcal colonization of T-tubes, which were usually left in for 2 to 3 months, was said to be common (216).

RESISTANCE OF ENTEROCOCCI TO ANTIMICROBIAL AGENTS

Recent attention has focused on enterococci not only because of their increasing role in nosocomial infections, but also because of their remarkable and increasing resistance to antimicrobial agents. These two factors are mutually reinforcing since resistance allows enterococci to survive in an environment in which antimicrobial agents are heavily used; the hospital setting provides the antibiotics which eliminate or suppress susceptible bacteria, thereby providing a selective advantage for resistant organisms, and the hospital also provides the potential for dissemination of resistant enterococci via the usual routes of nosocomial spread.

Antimicrobial resistance can be divided into two general types, that which is an inherent or intrinsic property and that which is acquired. The terms inherent or intrinsic resistance are used here to indicate resistance which is a usual species characteristic present in all or most of the strains of that species (139). The genes for intrinsic resistance, like other species characteristics, appear to reside on the chromosome. Acquired resistance results from either a mutation in the existing DNA or acquisition of new DNA. The various intrinsic (inherent) traits expressed by enterococci include resistance to semisynthetic penicillinase-resistant penicillins, cephalosporins, low levels of aminoglycosides, and low levels of clindamycin. Examples of acquired resistance include resistance to chloramphenicol, erythromycin, high levels of clindamycin, tetracycline, high levels of aminoglycosides, penicillin by means of penicillinase, fluoroquinolones, and vancomycin. Resistance to high levels of penicillin without penicillinase and resistance to fluoroquinolones are not known to be plasmid or transposon mediated and presumably are due to mutation(s).

Intrinsic Resistance

Beta-lactams. The resistance or relative resistance of enterococci to beta-lactams is a characteristic feature of these organisms; this appears to be due to low affinity of the penicillin-binding proteins (224). Even strains isolated from primitive populations in the Solomon Islands (who had had little or no exposure to manufactured antibiotics) as well as strains isolated early in the antibiotic era display this property (139).

In vitro testing of *E. faecalis* shows that MICs of penicillin (average, 2 to 8 μg/ml) are at least 10 to 100 times greater than those for most streptococci (158, 165, 209, 210). *E. faecium* is even more resistant and typical MICs of penicillin are often 16 to 32 μg/ml or higher (138, 139). Occasional papers have listed strains of enterococci with penicillin MICs of 25 to >100 μg/ml (13, 46, 82, 209, 224) (see also below). MICs of ampicillin and the ureidopenicillins for *E. faecalis* are usually 1 to 4 μg/ml, about 1 dilution lower than for penicillin. Resistance to the semisynthetic penicillinase-resistant penicillins is more pronounced, with MICs in the range of 8 to 50 μg/ml for nafcillin and usually ≥50 μg/ml for methicillin (72, 153, 158, 209, 210). In addition, Glew and Moellering reported decreased susceptibility of enterococci to nafcillin, oxacillin, and methicillin when tested in 50% serum–50% broth versus broth alone and showed that the presence of 50% serum completely eliminated synergism with oxacillin and eliminated 80% of the synergistic effects seen with nafcillin (84). MICs of carbenicillin and ticarcillin are often 64 μg/ml, comparable to those for *P. aeruginosa* (72, 158). As with the penicillins, imipenem is more active against *E. faecalis* than against *E. faecium* but is not bactericidal (7, 153, 158). Aztreonam lacks activity against gram-positive organisms.

In addition to the high MICs, enterococci are typically "tolerant" to all beta-lactams; that is, they are not killed by concentrations manyfold higher than the MIC. Although in some reports the MBCs as determined by the broth microdilution method have been low, the MBCs of penicillin,

ampicillin, and other penicillins in broth macrodilution systems are typically >100 μg/ml (7, 113, 139).

A notable weakness of cephalosporins is that none of these agents routinely inhibits enterococci sufficiently to warrant its clinical use. MICs of cephalothin range from 6.3 to >100 μg/ml; but MICs of cefoxitin and moxalactam are higher (72, 113, 158, 209, 218). Although the in vitro activity of cephalosporins against enterococci is poor when tested by conventional methods, MICs may be lower in the presence of serum and lysed blood (179). The significance of this observation is not known since enterococcal superinfections occur in patients (and thus in the presence of serum and blood) receiving cephalosporins or cephalosporins plus aminoglycosides (218).

Clindamycin. Another characteristic feature of enterococci is their resistance to clindamycin and lincomycin (107, 209). The MICs for most strains are 12.5 to 100 μg/ml, although some strains also have acquired HLR (MICs, >1,000 μg/ml; see below). Low-level resistance was found by Moellering and Krogstad in 31 of 32 enterococci isolated from Solomon Islanders who had had no previous exposure to clindamycin or lincomycin (139).

Aminoglycosides. Low-level resistance to aminoglycosides is also an inherent property of enterococci. The actual level of resistance varies among the different aminoglycosides. For *E. faecalis*, the average MIC of streptomycin or kanamycin is around 250 μg/ml, whereas gentamicin and tobramycin MICs are 8 to 64 μg/ml (135, 138, 210). HLR also occurs and is discussed below. Low-level aminoglycoside resistance among *E. faecalis* strains appears to be due to low uptake of these agents. If one looks at the uptake of radiolabeled aminoglycosides, it can be shown that, when enterococci are grown in the presence of cell wall synthesis inhibitors, such as penicillin or vancomycin, uptake of the aminoglycoside is markedly enhanced (142, 236). In turn, the presence of an aminoglycoside results in enhanced killing, the well-known synergistic effect of cell wall synthesis inhibitors plus aminoglycosides.

For all *E. faecium* strains reported, MICs of tobramycin, kanamycin, netilmicin, and sisomicin are higher than those for *E. faecalis*; moreover, combinations of penicillin plus these aminoglycosides fail to show synergism against *E. faecium* (138, 145, 223). Strains of this species that have been examined produce low levels of an aminoglycoside 6′-acetyltransferase (6′-AAC) that can barely be detected unless strains are pregrown on one of its substrate aminoglycosides (38, 145). This enzyme and low-level resistance could not be transferred or cured by novobiocin, suggesting that the enzyme may be encoded in the chromosome (145).

TMP/SMX: in vivo resistance? The in vitro and in vivo activities of trimethoprim-sulfamethoxazole (TMP/SMX) against enterococci are controversial. Part of this stems from discrepancies in results of susceptibility testing noted with different media (210). Media containing thymidine allow many bacteria to escape the inhibition of TMP/SMX; thymidine phosphorylase eliminates this escape mechanism by converting thymidine to thymine, which generally cannot be used. Enterococci, however, can use thymine and MICs will be higher in media that are not essentially thymidine- and thymine-free (47). Another potential problem is that enterococci can use exogenous folinic acid, dihydrofolate, and tetrahydrofolate (91). When concentrations of folinic acid equal to those present in urine were added to Mueller-Hinton broth, the mean MICs of TMP/SMX increased 25-fold to 3.3 μg/ml (a concentration still achievable in the urine) (235); smaller increases were seen in another study in Iso-Sensitest agar (91). When enterococcal strains were tested in urine, the mean MIC increased 60-fold; this effect was reversed by methotrexate (235). In addition to the problems with MIC determination, there are also conflicting reports as to whether or not TMP/SMX is bactericidal against enterococci (47, 155). Unless an animal model or clinical data clearly indicate efficacy in vivo, TMP/SMX should not be considered bactericidal. A report of two patients who developed enterococcal bacteremia while or after receiving TMP/SMX for enterococcal UTIs illustrates the potential problems with this combination when used for enterococcal infections (85).

Acquired Resistance

Resistance to antimicrobial agents may be acquired by a mutation in existing DNA or by acquisition of new DNA. In general, acquisition of DNA occurs by transformation (a process not known to occur in nature with enterococci), transduction (a process recently suggested for enterococci), or conjugation. Three different conjugative transfer systems have been reported in enterococci (22, 39, 40). One involves broad-host-range plasmids, some of which (like pAMβ1) can transfer among *E. faecalis*, many species of streptococci, *Staphylococcus aureus*, *Lactobacillus* spp., and *Bacillus subtilis*, among others (22, 39, 183). Transfer of this type of plasmid is largely dependent on forced cell contact on membrane filters and is very inefficient in broth. Broad-host-range plasmids may be the reason that staphylococci and enterococci share so many resistance genes (e.g., gentamicin, erythromycin, and penicillinase) (183). Another type of transfer involves narrow-host-range plasmids (found so far only in *E. faecalis*) that transfer at a high frequency in broth (41). These plasmids respond to clumping-inducing agents, or "pheromones," from recipient cells by producing aggregation substance; this substance causes the characteristic and grossly visible clumping between donors and recipients (40). The third type of conjugative system in enterococci involves conjugative transposons (40). Transfer by this mechanism occurs at a low frequency on filters and in the absence of demonstrable extrachromosomal DNA, although a transient extrachromosomal form may occur during the process. The best-studied conjugative transposon is Tn*916*, which mediates tetracycline resistance (189).

Chloramphenicol, clindamycin, and erythromycin. The first demonstration of transferable resistance among enterococci was made by Raycroft and Zimmerman, who showed that chloramphenicol resistance could be transferred from one strain of *E. faecalis* to another (171). Several studies have reported that 20 to 42% of enterococci are chloramphenicol resistant (1, 6, 22, 209). When studied, this resistance has been mediated by chloramphenicol acetyltransferase (22). Erythromycin resistance plasmids and transposons are also commonly found in enterococci. Although strains from the Solomon Islanders studied by Moellering were all susceptible, strains with high-level erythromycin resistance have shown a steady increase in many locations (6, 139). Erythromycin resistance occurs as part of the macrolide-lincosamide-streptogramin B resistance phenotype; in addition to erythromycin resistance, this determinant also confers HLR to clindamycin (22). The mechanism involves methylation of an adenosine residue in the 23S rRNA. Different erythromycin resistance determinants exist, but an especially common one (*ermB*) is carried by Tn*917* which is widespread in human and animal isolates of many different streptococcal species as well as in enterococci (119); it has recently been found in *Escherichia coli* (21). Tn*917* is also interesting

because its rate of transposition is increased by exposure to low levels of erythromycin (211).

Tetracycline resistance. In various surveys, up to 60 to 80% of enterococci have been resistant to tetracycline (1, 6). Several different genes have been found, including *tetL* (which is contained in the well-studied plasmid pAMα1) and *tetM* (which is on the conjugative transposon Tn*916*) (22, 26, 189). The *tetM* gene has also been found in *Mycoplasma* sp., *Campylobacter* sp., *Neisseria gonorrhoeae*, and *Clostridium* sp., among others (26, 133). Another gene, *tetN*, was originally identified on a plasmid in *S. agalactiae* that was subsequently transferred to and stably maintained in *E. faecalis* (22, 26). *tetO* has also been found in enterococci; it was originally found in *Campylobacter* spp. and shows about 75% homology with *tetM* (118, 197). These various genes confer resistance by two different mechanisms; *tetL* mediates active efflux of tetracycline from cells, the same mechanism commonly found in gram-negative bacilli (133), while *tetM* and *tetN* mediate resistance by a mechanism that protects the ribosomes from inhibition by tetracycline (25). An interesting feature of plasmid pAMα1 (containing *tetL*) is that the resistance genes duplicate or amplify when the host is grown in subinhibitory concentrations of tetracycline. This results in an increase in the size of the plasmid and also results in higher MICs (228).

High-level aminoglycoside resistance and resistance to synergism. As was mentioned above, enterococci typically have intrinsic low-level resistance to aminoglycosides. If this is the only type of aminoglycoside resistance expressed, then the addition of an aminoglycoside to a cell-wall-active agent such as penicillin or vancomycin characteristically results in enhanced killing (except as discussed above for *E. faecium*). This enhanced killing, called synergism, is defined for enterococci as a $\geq$2-$\log_{10}$ increase in killing versus the effect of the cell-wall-active agent alone when the aminoglycoside is used in a subinhibitory concentration (29, 30). Unfortunately, a number of strains of enterococci have acquired HLR (MIC, $\geq$2,000 μg/ml) to an aminoglycoside(s) and, concomitantly, resistance to synergism. A study from the Massachusetts General Hospital, Boston, Mass., showed that, as early as 1973 to 1976, 54% of strains had HLR to streptomycin and 49% had HLR to kanamycin (30). The prevalence of strains highly resistant to various aminoglycosides varies from place to place but is generally highest for streptomycin (112, 144, 152).

Two mechanisms have been described which account for HLR of clinical isolates to streptomycin: ribosomal resistance and enzymatic modification by an adenylyltransferase (57, 112). This enzyme appears to adenylylate the 6-hydroxyl position since it is not active against spectinomycin; unfortunately, even without HLR, spectinomycin is not a bactericidal agent and does not show synergism against enterococci (44, 112, 135). Eliopoulos et al. found streptomycin MICs of 4,000 to 16,000 μg/ml (on dextrose phosphate agar) for strains with streptomycin adenylyltransferase, while MICs were up to 128,000 μg/ml for strains with ribosomal resistance (57). HLR to kanamycin in strains without HLR to gentamicin is due to production of an aminoglycoside 3′-phosphotransferase (44, 112, 135). This enzyme also has activity against amikacin. Although strains do not display HLR to amikacin, Krogstad and Moellering have shown that this enzyme clearly eliminates synergism between penicillin and amikacin and can even cause amikacin to antagonize the effects of penicillin (29, 112).

In 1979, Horodniceanu reported three isolates of *E. faecalis* in France with HLR to gentamicin, kanamycin, and tobramycin, but not to streptomycin (100). In 1983, several reports, including two from my laboratory, documented emergence in the United States of *E. faecalis* with HLR to gentamicin and to all aminoglycosides, including gentamicin and streptomycin. In these studies, which included strains from Houston, Tex., Bangkok, Thailand, and Santiago, Chile, 4.5 to 15% of enterococci had HLR to gentamicin (135, 152). Such strains have now been reported by a number of investigators but perhaps most dramatically by Zervos et al., who found HLR to gentamicin in 55% of clinical isolates of enterococci at the Ann Arbor Veterans Administration Medical Center, Ann Arbor, Mich., in 1985 to 1986 (232). HLR to gentamicin has also been reported in *E. faecium* and in community-acquired *E. faecalis* (58, 154). As with HLR to streptomycin and kanamycin, HLR to gentamicin and other aminoglycosides eliminates synergism between penicillins and these aminoglycosides (46, 100, 135). Various aminoglycoside-modifying enzyme activities have been found in these multiresistant strains, including streptomycin adenylyltransferase, 3′-phosphotransferase, 6′-AAC, and 2″-phosphotransferase (2″-APH) (44, 135, 152). The last two enzyme activities were shown by Ferretti et al. to be due to a single fusion protein with two active regions, one that has 2″-APH activity and one that has 6′-AAC activity (77). The combined activities result in HLR (as well as resistance to synergism) to gentamicin, sisomicin, netilmicin, tobramycin, kanamycin, and amikacin but not streptomycin (44, 135, 154). Many of the strains studied have been able to transfer gentamicin resistance to other strains (100, 135, 152, 233).

Beta-lactamase. Beta-lactamase-producing enterococci have now been reported from five cities: Houston, Tex.; Philadelphia, Pa.; Canonsville, Pa.; West Haven, Conn.; and Boston, Mass., where a cluster of strains was found at Boston Children's Hospital (104, 150, 163a, 164; Rhinehart et al., 28th ICAAC). I have recently been sent from Argentina three enterococci which produce beta-lactamase. In most instances, beta-lactamase has been encoded on a transferable plasmid that also encodes high-level gentamicin resistance; one of these plasmids has been shown to be a pheromone responsive plasmid (148).

The resistance of beta-lactamase-producing strains is not detected by routine disk susceptibility testing (unlike non-beta-lactamase-producing strains with high-level penicillin resistance, discussed below) because of an inoculum effect. When a low inoculum is used (like that used for disk testing), strains appear susceptible, but at a high inoculum (e.g., 10^7 CFU/ml) strains appear resistant (MIC, >500 μg/ml) (149). An inoculum effect is also seen with beta-lactamase-producing staphylococci. It is due to the fact that low numbers of cells do not produce sufficient beta-lactamase to cause resistance. The enterococcal beta-lactamase hydrolyzes penicillin, ampicillin, and piperacillin (and other ureidopenicillins), which correlates with resistance to these compounds (149, 151); there is little or no inactivation of penicillinase-resistant semisynthetic penicillins, cephalosporins, or imipenem. The lack of efficacy of penicillin against beta-lactamase-producing strains has been confirmed in animal model studies (97, 104). Hybridization studies have shown that the beta-lactamase gene is highly homologous to that from *Staphylococcus aureus*, although differences exist in the expression of these enzymes, namely, that the beta-lactamase from enterococci is constitutively produced and cell bound, whereas that from staphylococci is typically inducible and released into the extracellular medium (149, 151, 237). This has been confirmed in my laboratory by DNA sequencing (K. Zscheck and B. E. Murray, 29th ICAAC,

abstr. no. 1121, 1989). Working with Jan Patterson, we have compared the Bla-encoding plasmids from six enterococci and found that, although the restriction endonuclease digestion patterns are different, there is extensive homology between most of these plasmids (149; Patterson et al., 28th ICAAC).

Penicillin resistance without beta-lactamase production. In a 1968 to 1969 survey of 382 enterococcal isolates, penicillin and ampicillin MICs were >100 μg/ml for one isolate and a penicillin MIC of 50 μg/ml was reported for one strain (209); occasional other papers have also reported MICs of penicillin in this range (13, 82, 224). Other examples include the *E. faecium* in a recent neonatal nursery outbreak for which MICs of penicillin were >25 μg/ml (46) and a strain of *E. faecium* reported recently by LeClercq et al. for which the penicillin MIC was 64 μg/ml (120). Recent papers indicate that these highly penicillin-resistant strains are being recognized more often, perhaps because of heightened awareness of penicillin resistance or perhaps because they are becoming more common (28; F. L. Sapico, H. N. Canawati, V. J. Ginunas, and W. Tuddenham, Program Abstr. 28th ICAAC, abstr. no. 1074, 1988; J. M. Boyce, A. A. Medeiros, E. F. Papa, and G. Potter-Bynoe, Program Abstr. 28th ICAAC, abstr. no. 1075, 1988). The MICs of penicillin for these strains may be extreme examples of the "intrinsic resistance" common to *E. faecium* and associated with low-affinity penicillin-binding proteins (224) or may represent acquired resistance.

Vancomycin resistance. The most recent resistance trait to emerge in enterococci is resistance to vancomycin; a number of papers and abstracts have been published since January 1988. Although MICs of vancomycin of ≥50 μg/ml have been noted for occasional enterococcal isolates, such strains apparently were very rare, and it is conceivable that certain of these may have been leuconostocs or other newly recognized, vancomycin-resistant, gram-positive organisms (209, 210). Uttley et al. reported 55 strains of vancomycin-resistant enterococci from 22 patients with end-stage renal failure in England; 48 were *E. faecium* and 7 were *E. faecalis* (213). Sources included blood, intraabdominal fluid, bile, and urine, among others. All were resistant to >64 μg of vancomycin per ml and MICs were >2,000 μg/ml for seven strains. Kaplan et al. reported a patient undergoing hemodialysis who had bacteremia and wound infection with a strain of *E. gallinarum* for which the MIC of vancomycin was 16 μg/ml (106). Lütticken and Kunstmann reported *E. faecium* with a vancomycin MIC of 32 μg/ml (127). Leclercq et al. reported vancomycin-resistant *E. faecium* from the feces of two patients with acute leukemia; the MICs were 512 and 1,024 μg/ml (120). The resistance was shown to be plasmid mediated by novobiocin curing and transformation into *S. sanguis*. In recent publications, vancomycin resistance was reported to be inducible and to be transferable by conjugation or mobilization from *E. faecalis* and *E. faecium* to *E. faecalis* and/or *E. faecium* recipients (121, 158a, 192, 223b). One strain could transfer vancomycin resistance to *S. sanguis*, *Listeria monocytogenes*, and *E. faecalis* but not to restriction-minus *Staphylococcus aureus* or *Bacillus* spp. (121, 192). These represent the first examples among any organisms of transferable vancomycin resistance. It has now been shown that there are two types of resistance to vancomycin. One type, which mediates higher levels of resistance (MIC, ≥256 μg/ml), is associated with an inducible cytoplasmic membrane-associated protein of 39 kilodaltons (192). The other type expresses lower levels of resistance and is associated with an inducible cytoplasmic membrane protein of 39.5 kilodaltons (223b). Although it is postulated that this protein may block vancomycin from its target site, D-Ala-D-Ala, the mechanism is not yet understood. One of the vancomycin resistance genes has been cloned, and a probe from this strain hybridizes only with enterococci with high-level vancomycin resistance (MIC, ≥512 μg/ml) and not with strains for which MICs are 16 to 32 μg/ml, or with vancomycin-resistant leuconostocs or pediococci (R. Le Clercq, V. Coutant, S. Dutka-Malen, J. Duval, and P. Courvalin, Program Abstr. 29th ICAAC, abstr. no. 273, 1989).

MANAGEMENT OF ENTEROCOCCAL INFECTIONS

Therapy

Urinary, soft-tissue, and miscellaneous infections. Enterococcal urinary and soft-tissue infections are typically treated with single-drug therapy, often ampicillin, penicillin, or vancomycin. Ureidopenicillins should also be adequate, although there is no reason to use these agents unless other bacteria are also suspected, such as in mixed abdominal infections. Ticarcillin is only moderately active in vitro against enterococci and has little role in therapy of enterococcal infections. Ciprofloxacin and norfloxacin have been used successfully for enterococcal UTIs, but the organism's susceptibility is marginal for treating systemic infections with this drug and breakthrough has occurred at other sites of enterococcal infections (231). Nitrofurantoin may also be useful for UTIs. Data on the efficacy of other agents such as erythromycin, tetracycline, and chloramphenicol are limited, although erythromycin and chloramphenicol have transiently suppressed symptoms in endocarditis and meningitis, respectively, but are not curative (175, 178). Rifampin is active in vitro but, like other agents, lacks bactericidal activity against enterococci alone or in combination with other agents and, in fact, can be antagonistic to beta-lactams; resistance develops rapidly in vitro when used alone (143). For beta-lactamase-producing strains, imipenem, vancomycin, ampicillin-sulbactam, or amoxicillin-clavulanic acid should adequately replace penicillin or ampicillin. Some vancomycin-resistant strains are also penicillin resistant, and their susceptibility to all possible agents should then be determined.

Enterococcal meningitis is rare and has been treated with various regimens. Although intravenous ampicillin alone has occasionally resulted in cure (11), a more reasonable approach would be to initiate therapy with intravenous ampicillin plus intravenous gentamicin (if the organism is not highly resistant) and to consider switching intravenous gentamicin to intraventricular gentamicin if the patient does not improve and CSF cultures remain positive. Rifampin has been used in enterococcal meningitis, but in one report of successful therapy it was given in conjunction with intraventricular vancomycin after the patient failed to respond to intravenous vancomycin plus gentamicin (178).

Enterococcal bacteremia with a demonstrable extracardiac source generally responds well to the therapy that is directed to the primary site, such as ampicillin for 10 to 14 days. Although some authors may feel that penicillin alone is inappropriate for enterococcal bacteremia (129), most strains are susceptible, with MICs only twice those of ampicillin. Adequate therapy of patients with bacteremia in the face of known valvular disease is not established, but a recent paper by Maki and Akker recommends "a bactericidal regimen for at least four weeks" (129). The same authors also recommend 4 weeks of bactericidal therapy for

patients for whom an extracardiac source cannot be identified, particularly when the enterococcus is present in pure culture and was community acquired (129). Whether or not these lengthy regimens are truly necessary or whether shorter courses or single-drug therapy will suffice is not known.

Endocarditis. Therapy of enterococcal endocarditis has presented a dilemma since the 1940s, when it was noted that a number of patients were not cured by penicillin alone, in contrast to patients infected with "other" streptococci (13, 82, 125). Although perhaps a third of patients have been cured with single-drug therapy, many patients failed such therapy, even when high doses of penicillin were used. This observation, together with the finding that better results were obtained with the use of penicillin plus streptomycin, established the idea that combined therapy is preferred (13, 82, 96, 111, 125, 131, 141, 175, 182, 185, 226). In animal models of enterococcal endocarditis and by time-kill curves, combination therapy has also been shown to be superior to single-drug regimens such as penicillin, ampicillin, imipenem, and vancomycin (7, 35, 95, 184, 200, 204).

For many years, streptomycin was the preferred aminoglycoside for use in enterococcal endocarditis; however, many strains are now highly resistant (MIC, >2,000 μg/ml) to streptomycin and thus are resistant to penicillin-streptomycin synergy (34, 90, 99, 226). Many of these strains lack HLR to gentamicin and can still be treated with combination therapy. Whether or not gentamicin is the agent of choice for strains lacking HLR to streptomycin is somewhat controversial. Disadvantages of streptomycin are that it produces more irreversible ototoxicity and, in most hospitals, serum levels cannot be rapidly obtained. In general, low-dose regimens (such as 3 mg of gentamicin/kg per day) plus high-dose penicillin (20×10^6 U/day) or ampicillin (12 g/day) are preferred (132, 227). The length of therapy is also debated; Wilson et al. (226) suggested that 4 weeks is adequate if patients have been ill for <3 months, although Scheld and Mandell still recommended 6 weeks for most patients (185). For beta-lactamase-producing strains, ampicillin-sulbactam, vancomycin, or imipenem should be used in place of penicillin or ampicillin.

Currently, clinicians are faced with enterococci that are highly resistant to all aminoglycosides. When such strains cause endocarditis, adding an aminoglycoside adds nothing to the effect of the cell-wall-active agent alone but will expose patients to increased toxicity (29, 30, 97, 112, 135). Optimal therapy for these patients is unknown. Based on historical accounts, some patients should be cured by single-drug therapy, but which agent should be used is not known. Some have suggested that ampicillin is inherently more bactericidal than penicillin (13); whether this is just the expected twofold or so higher activity (as is seen in the MICs) or whether the enterococcal killing by ampicillin exceeds this is not clear. Moreover, it is not known whether an MBC, or for that matter any in vitro or animal model criteria, can predict clinical cures. One group reported better success in a rat model of endocarditis by using continuous-infusion ampicillin versus intermittent ampicillin for a strain of enterococcus highly resistant to gentamicin (205). However, that strain also showed a 10^3-CFU/ml decrease from a 10^7-CFU/ml inoculum in time-kill curves, using 5, 10, and 250 μg of ampicillin per ml, and the authors comment that other strains may display more tolerance to ampicillin. Another study showed efficacy of ampicillin alone in animals with endocarditis treated for 21 days via a subcutaneous chamber (208); a single strain was studied. Since some patients and animals with enterococcal endocarditis are also cured by penicillin alone, these results are not surprising. Again, however, care must be taken with generalizations, since failures of ampicillin to cure endocarditis in patients infected with a strain of *E. faecalis* resistant to multiple aminoglycosides have been reported (76, 108). It should also be reiterated that, in the absence of HLR, ampicillin plus an aminoglycoside is superior to ampicillin alone (73, 202).

Other agents also show little promise for reliable, successful, single-drug therapy. Vancomycin alone has been reported to cure some patients with enterococcal endocarditis. In one study, however, the biochemical tests used were insufficient to separate enterococci from some streptococci. To cast further doubt on the bacteriologic identification, all of the enterococci were said to be susceptible to cephalothin and one to clindamycin, unusual properties for enterococci (79). In vitro, vancomycin is usually not bactericidal, and it does no better in animal models than penicillin alone (27, 99, 104, 135, 205, 217). Among other agents that have been evaluated, ciprofloxacin was less effective than procaine penicillin in controlling enterococcal endocarditis (75, 104, 195). Mezlocillin looked better than ampicillin in one study, but higher doses were used (73). Imipenem also gave no better results than other beta-lactams alone (7, 184). Teicoplanin was found to be equivalent to ampicillin in rabbits with enterococcal endocarditis, and the single agents were less active than when combined with gentamicin for strains lacking HLR (202). Rifampin is indifferent or antagonistic to beta-lactams (143). Daptomycin (LY146032), an agent with better in vitro bactericidal activity than beta-lactams and vancomycin, has not had good success in early in vivo trials; this may be related to the lower effect of daptomycin that is observed in 50% human serum in vitro (173). When fosfomycin is combined with daptomycin, there is increased bactericidal activity against enterococci even at low concentrations of daptomycin; this bactericidal activity is not reversed by 50% serum (173).

In summary, although based on little firm data, high-dose ampicillin, perhaps by continuous infusion for a prolonged period of time, seems a reasonable choice for patients with endocarditis whose infecting strain is susceptible to ampicillin but highly resistant to all aminoglycosides. All studies to date indicate that adding an aminoglycoside in this situation adds no increased efficacy.

SUSCEPTIBILITY TESTING

Routine testing for enterococci should include determination of susceptibility to penicillin, ampicillin, and vancomycin; interpretive criteria for ampicillin and penicillin are MICs of 8 (moderately susceptible) and 16 (resistant) μg/ml; for vancomycin, ≤4 μg/ml is considered susceptible and ≥32 μg/ml is considered resistant, with 8 to 16 μg/ml considered intermediate in susceptibility (157). For disk testing, a 16-mm zone diameter is considered resistant with ampicillin 10-μg disks and 17 mm is considered moderately susceptible; for penicillin (10-U disks), a zone of 14 mm is considered resistant and 15 mm is considered moderately susceptible. For vancomycin (30-μg disks), a zone of 9 mm is considered resistant, 10 to 11 mm is intermediate, and 12 mm is considered susceptible (156); however, these criteria have been questioned recently because they failed to classify some strains with MICs of ≥32 μg/ml as resistant (179a, 202a). For urine isolates, fluoroquinolone, erythromycin, tetracycline, and nitrofurantoin susceptibilities may be desired (156). For patients with endocarditis, I would recom-

mend testing for beta-lactamase since the organism may appear susceptible by routine methods; although such isolates are still rare, testing with nitrocefin is a simple method that many laboratories routinely use for other organisms such as gonococci, *Haemophilus influenzae*, and *Staphylococcus aureus*.

For patients with endocarditis and probably other serious infections, such as meningitis, aminoglycoside activity should be determined. A formal MIC determination which shows that the MIC is >2,000 μg/ml is definitive for HLR and resistance to synergism; there are no known exceptions. The converse is not true, however; that is, there are several situations in which strains do not show synergism but do not have HLR to the aminoglycoside used. One example is the lack of synergism between penicillin and amikacin against strains producing the 3′-APH. These strains do not have HLR to amikacin (unless they also produce 6′-AAC–2″-APH) but do have HLR to kanamycin, which should be used to predict resistance to penicillin-amikacin synergism (112). A second example is the failure of penicillin and tobramycin to show synergism against *E. faecium* even if MICs of tobramycin are <1,000 μg/ml; this appears to be a species characteristic (see section on resistance) and should be assumed to be the case for any *E. faecium* unless proven otherwise (38, 138, 145, 223).

An apparently rare example of lack of synergism without HLR was a strain of *E. faecalis* that showed selective resistance to a combination of penicillin plus gentamicin (140). This strain was isolated from the blood of a patient with enterococcal endocarditis who relapsed despite repeated courses of penicillin plus gentamicin. Several isolates from this patient were studied and found to be resistant to the combination of penicillin (ampicillin) plus gentamicin but susceptible to the synergistic combination of penicillin-tobramycin. The strain did not show HLR to gentamicin (MIC of gentamicin, 8 μg/ml). No aminoglycoside-modifying enzymes were found, but the uptake of radiolabeled gentamicin was not enhanced by the addition of penicillin whereas the uptake of radiolabeled tobramycin was enhanced. The patient was subsequently cured by the combination of ampicillin-plus-tobramycin therapy (140).

Another possible, but not proven, exception might be enterococci that are resistant to streptomycin on the basis of production of streptomycin adenylyltransferase; for such strains, MICs of streptomycin are as low as 4,000 μg/ml (57). If a strain is encountered with a MIC of 2,000 μg/ml, it should probably be retested or tested for synergism by time-kill curves. It is also not known what the MICs of gentamicin would be when strains arise that have only the 6′-AAC or 2″-APH activity of the bifunctional enzyme or what would happen in synergy testing. However, since few such strains seem to exist, this does not appear to be a problem at present.

Screening Tests for High-Level Aminoglycoside Resistance

Agar screening. Since most clinical laboratories do not perform full MICs routinely and certainly not synergism studies, screening tests that predict HLR and resistance to synergism have been sought. Moellering et al. first showed that streaking a colony of enterococci to dextrose phosphate agar containing 2,000 μg of aminoglycoside per ml could be used to predict the presence or absence of synergy (144). Other workers have used various media and various inocula. We streaked a heavy unquantitated inoculum onto brain heart infusion agar with 1,000 μg of gentamicin per ml and 2,000 μg of streptomycin per ml and found that all strains that grew, even with only a few colonies, had HLR as confirmed by MIC. For those strains that did not grow, antibiotic MICs were <1,000 μg of gentamicin per ml or <2,000 μg of streptomycin per ml (135, 152; unpublished observations). Sahm and Torres evaluated dextrose phosphate agar, brain heart infusion agar, Mueller-Hinton agar with 5% sheep blood, and Trypticase soy agar with 5% sheep blood with different inocula of *E. faecalis* to screen for HLR (181). Excellent results were obtained with inocula of 10^4 and 10^6 CFU per spot with both gentamicin and streptomycin regardless of the medium used. These authors also obtained good results with tobramycin (for *E. faecalis*) and when kanamycin was used to predict resistance to penicillin-amikacin synergism (181). In our own studies, we did not test for loss of antibiotic activity in the agar plates, but results with plates stored for 6 months at 4 to 8°C were the same as with fresh plates; this observation is consistent with the known marked stability of aminoglycosides.

TABLE 2. Zone of inhibition around antibiotic disks

Antibiotic	Zone diam (mm)[a]		Medium[c]	Reference
	Strains with HLR[b]	Strains without HLR		
Streptomycin (300 μg)	6	≥12	MHA, TSA with SB	177
	6	≥14	MHA with SB	180
	6	≥12	MHA	180
	6–10	≥16	MHA	198
Gentamicin (120 μg)	6	≥16	MHA, TSA with SB	177
	6	≥20	MHA with SB	180
	6–7	≥15	MHA	180
	6	≥17	MHA	198

[a] Disks were 6 mm in diameter.
[b] HLR was defined as a MIC of >2,000 μg/ml, as resistance to synergism, as resistant by one of several other techniques, or a combination of these (177, 180, 198).
[c] MHA, Mueller-Hinton agar; TSA, Trypticase soy agar; SB, 5% sheep blood.

High-content disks. Rosenthal and Freundlich reported the use of high-content disks to identify enterococci with HLR to aminoglycosides (Table 2) (177). Disks containing 120 μg of gentamicin and tobramycin and 300 μg of streptomycin were evaluated on Mueller-Hinton and Trypticase soy agars, both with 5% sheep blood, and the results were compared with MIC determinations on Mueller-Hinton agar. Only 3 of 70 strains had HLR to gentamicin and 14 had HLR to streptomycin; these strains had no zone of inhibition around the high-content disks. All "susceptible" strains (i.e., those lacking HLR) had zones of ≥12 mm for streptomycin and ≥16 for gentamicin; MICs of these agents were ≤125 μg/ml. With tobramycin, strains with MICs of 4,000 μg/ml had no zone of inhibition around the 120-μg tobramycin disk and a strain with a MIC of 2,000 μg/ml had a zone of 8 mm. These authors suggest that, when susceptibility powder is not available, vials of clinical material can be used (177), as has been done in other studies (135, 144, 200).

Sahm and Torres evaluated disks with both 300 and 2,000 μg of streptomycin and 120 and 2,000 μg of other aminoglycosides, using Mueller-Hinton agar as well as Mueller-Hinton agar with 5% sheep blood (Table 2) (180). Twenty *E. faecalis* strains susceptible to synergy with all penicillin-aminoglycoside combinations and 20 *E. faecalis* strains resistant to all combinations were used. With resistant

strains and Mueller-Hinton agar plus blood, the lower-content disks gave zones of 6 mm for streptomycin, gentamicin, and kanamycin and 6 to 10 mm for tobramycin; on Mueller-Hinton agar without blood, the zones were 6 to 7 mm for all four agents. On Mueller-Hinton agar plus blood, synergy-susceptible strains had zones of ≥14 mm for streptomycin and ≥20 mm for gentamicin, tobramycin, and kanamycin; on Mueller-Hinton agar without blood, zones were ≥12 mm for streptomycin, ≥14 mm for gentamicin and tobramycin, and ≥10 mm for kanamycin (Table 2). The results with these disks were easier to evaluate than the results with the higher-content (2,000 μg) disks (180).

Spiegel recently reported using disks with 120 μg of gentamicin and kanamycin and 300 μg of streptomycin on Mueller-Hinton agar (Table 2) (198). The gentamicin disks detected all 35 *E. faecalis* strains that were resistant by other methods; all zones were 6 mm, whereas all 62 strains lacking HLR had zones of ≥17 mm. For streptomycin, 18 of 19 strains resistant by other methods had zones around the streptomycin disk of <10 mm; one strain resistant by other methods had a zone of 10 mm and, on repeat, a zone of 9 mm. Zones for 78 strains not highly resistant to streptomycin by any method were all ≥16 mm. For kanamycin, 49 of 50 strains resistant by another technique had zones of 6 mm. Strains lacking HLR had zones of ≥12 mm (198).

Broth dilution tests. In another screening test, a single concentration of an aminoglycoside in a microdilution well is used. Zervos et al. evaluated 500 μg of gentamicin in Mueller-Hinton broth supplemented with 50 μg of Ca^{2+} and 25 μg of Mg^{2+} per ml; an inoculum of 5×10^5 CFU/ml was used (234). Of 5,797 isolates screened, 508 grew in the single well; gentamicin MICs for 505 were >2,000 μg/ml, and for the other 3, they were 500 to 1,000 μg/ml. Unfortunately, synergy study results were not provided for these three strains. Sahm and Torres evaluated 63 *E. faecalis* strains in a 1-ml volume test with dextrose phosphate broth and Mueller-Hinton broth and an inoculum of 10^5 CFU/ml. Both broths gave good results with streptomycin, gentamicin, and kanamycin, although a few resistant strains were missed, indicating that an aminoglycoside would have been used needlessly; results were fair to poor for tobramycin (181).

Spiegel compared results with the following: (i) an in-house microdilution method with 500 μg of gentamicin per ml, 2,000 μg of streptomycin per ml, Mueller-Hinton broth, and an inoculum of 7.5×10^5 CFU/ml; (ii) the MicroScan microdilution (POS MIC2) panels (MicroScan, W. Sacramento, Calif.), using the same concentrations as above and an inoculum of 10^5 CFU/ml; (iii) an in-house tube macrodilution method (using Mueller-Hinton broth, the same concentrations, and an inoculum of 7.5×10^5 CFU/ml); and (iv) the high-content disks discussed above (198). Resistance was defined as resistance by any method, with confirmation of discrepancies by synergy studies. The MicroScan system detected only 2 of 13 gentamicin-resistant *E. faecalis* strains and 3 more when repeated; only 4 of 15 highly streptomycin-resistant *E. faecalis* strains were detected. The in-house broth microdilution method was applied to more strains and detected all 35 gentamicin-resistant and 16 of 19 streptomycin-resistant *E. faecalis* isolates, which was comparable to the results obtained with these strains with the disk method (see above). The in-house tube macrodilution method detected all 13 gentamicin-resistant *E. faecalis* but missed 8 of 15 streptomycin-resistant *E. faecalis* strains. The reason for the poor results obtained for both aminoglycosides in the MicroScan system is not known, although the inoculum was less than for the in-house system; full-range MICs for discrepant strains were not reported (198). The disk method and the in-house broth microdilution method also detected three of three streptomycin-resistant *E. faecium* strains; none of seven *E. faecium* strains had HLR to gentamicin.

Recommendations for Screening for HLR to Aminoglycosides

(i) The disk method and the single-concentration agar plate method currently appear highly reliable for detecting *E. faecalis* and probably *E. faecium* with HLR to streptomycin and gentamicin. Disks can be made in advance and stored until needed, and plates can probably be stored for at least 6 months. In three separate studies, disks containing 120 μg of gentamicin generated zones of 6 to 7 mm for strains with HLR and ≥15 mm for nonresistant strains (177, 180, 198) (Table 2). For streptomycin, disks containing 300 μg generated zones of 6 to 10 mm for strains with HLR, and for nonresistant strains, zones have been ≥12 mm (177, 180, 198). In the only study reporting strains with a zone of up to 10 mm, all nonresistant strains had zones of ≥16 mm (198).

(ii) In-house broth microdilution systems also appear highly reliable, although a few strains have been missed with streptomycin, 2,000 μg/ml (198, 234). This might result in the use of streptomycin when it was not indicated but is perhaps a less severe error, unless marked toxicity occurred, than failing to use an aminoglycoside when it would result in synergism.

(iii) At the current time, some caution should be used with prepackaged or automated systems that have not been fully evaluated with published data comparing the system with standard methods, such as a full-range MIC determination or the agar plate method. The verification of these systems is particularly important because so many clinical laboratories now depend on prepackaged automated systems for determination of susceptibility. As suggested by one author, "a multicenter study performed under the guidance of the NCCLS [National Committee for Clinical Laboratory Standards] may be appropriate" (198).

(iv) There is usually little need to test aminoglycosides other than streptomycin or gentamicin since these are the agents for which there are the most clinical data. To date, all strains with HLR to gentamicin have also had resistance to synergism and/or HLR to tobramycin, sisomicin, netilmicin, kanamycin, and amikacin by virtue of the 2″-APH–6′-AAC. This enzyme is not active against streptomycin so that gentamicin-resistant strains are not necessarily resistant to streptomycin; in other words, a variable percentage of strains will have HLR to gentamicin while lacking HLR to streptomycin (100, 135, 152, 154). If one considers using amikacin in combination with a cell-wall-active agent, it will be necessary to test for HLR to kanamycin and gentamicin since each causes resistance to synergism with amikacin. If one considers using tobramycin, then screening for HLR to gentamicin and species identification are needed since *E. faecium* are resistant to penicillin-plus-tobramycin synergism (138, 145, 223).

CONCLUSION

The recent attention focused on the enterococcus is likely to continue in the foreseeable future. The ability of enterococci to colonize the gastrointestinal tract, plus the many intrinsic and acquired resistance traits, means that these organisms, which usually seem to have relatively low intrinsic virulence, are given an excellent opportunity to become

secondary invaders. Since nosocomial isolates of enterococci have displayed resistance to essentially every useful antimicrobial agent, it will likely become increasingly difficult to successfully treat and control enterococcal infections. This will be particularly true when the various resistance genes came together in a single strain, an event almost certain to occur at some time in the future.

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