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MINIREVIEW

β-Lactamase-Producing Enterococci

BARBARA E. MURRAY

Center for Infectious Diseases and Departments of Internal Medicine and of Microbiology and Molecular Genetics, The University of Texas Medical School at Houston, Houston, Texas 77030

Enterococci have a long and well-established role in causing human disease, beginning with a report of what was probably an enterococcus isolated from the blood of a patient with endocarditis around the turn of this century (16). In recent years, enterococci have become recognized as a common cause of nosocomial infections and were recently cited as being the second most common pathogens isolated from hospitalized patients (31). The increasing importance of enterococci in nosocomial infections may be at least partially related to the many inherent (i.e., naturally occurring) and acquired resistances found in these organisms; these multiple resistances presumably provide a selective advantage, allowing enterococci to survive many different antimicrobial regimens. Inherent resistance to cephalosporins, semisynthetic penicillinase-resistant penicillins, low levels of aminoglycosides, and clindamycin is typically seen. Relative to streptococci, enterococci also have decreased susceptibility to penicillin, ampicillin, and ureidopenicillins, with *Enterococcus faecalis*, the most commonly isolated species, being less resistant to these compounds than *Enterococcus faecium* and *Enterococcus raffinosus* (2, 6, 7). Enterococci have also acquired resistance to a number of antimicrobial agents, including the tetracyclines, erythromycin, chloramphenicol, trimethoprim, high levels of clindamycin, high levels of aminoglycosides, high levels of penicillins (via penicillinase and nonpenicillinase mechanisms), and, most recently, vancomycin (16). All of these resistances add to the clinical dilemma posed by enterococci because they decrease the number of available therapeutic options and because they increase the number of agents whose use may provide a positive selective pressure for resistant organisms. This review described the emergence, characterization, and spread of β-lactamase, an enzyme common to other organisms for decades but found only recently in enterococci.

IS β-LACTAMASE PRODUCTION A NEW PROPERTY IN ENTEROCOCCI?

Staphylococcus aureus, which has the same β-lactamase as that now found in *E. faecalis*, was originally found to produce this enzyme in the early to mid-1940s. The first published account of a β-lactamase in enterococci was in 1983, when this laboratory reported that a strain of *E. faecalis* isolated in Houston, Tex., in 1981 produced β-lactamase (19). The recognition that the first isolate, called HH22, produced β-lactamase was really a serendipitous observation since, as will be discussed further below, this trait was not and typically is not detected by routine susceptibility test methods unless a higher-than-normal inoculum is used. HH22 was collected as part of a survey of high-level gentamicin resistance in enterococci and was being studied by time-kill synergy curves to verify that its high-level gentamicin resistance correlated with lack of synergism between penicillin and gentamicin. During these experiments it was noted that at the inoculum usually used in enterococcal synergy experiments (10^7 CFU/ml), HH22 was not inhibited by penicillin but that it was inhibited at an inoculum of 10^5 CFU/ml. Such an inoculum effect is typical of β-lactamase-producing (Bla^+) organisms and led to further studies which confirmed that HH22 could hydrolyze nitrocefin and could inactivate penicillin G (19). The next report of a Bla^+ enterococcus was in 1987 and described an organism isolated in Philadelphia, Pa., in 1983 (11). Since then, a large number of Bla^+ isolates of *E. faecalis* have been reported from at least 11 cities in four countries, including several small clusters and two large outbreaks (8, 13, 21, 27); a Bla^+ isolate of *E. faecium* has also recently been reported (3). Since all of these Bla^+ organisms were isolated since 1981, it seems likely that β-lactamase production is a recently acquired trait in members of the genus *Enterococcus*. As was mentioned above, however, one problem with this conclusion is the fact that at routinely used inocula, Bla^+ enterococci often are not any more resistant to penicillin or ampicillin than other enterococci, and until β-lactamase production was first reported, it is doubtful that laboratories were testing enterococci with a specific β-lactamase test. On the other hand, the conclusion regarding recent emergence is supported by largely unpublished results from a number of investigators who began to specifically test for β-lactamase after 1983 but who either have failed to find Bla^+ isolates or tested for some years before finally finding such strains (27). Additional indirect evidence supporting the conclusion that β-lactamase has been relatively recently acquired by enterococci is that it is almost always associated with high-level resistance to gentamicin (12, 20, 21, 26). Evidence for the recent acquisition of high-level resistance to gentamicin by enterococci is strong, since some laboratories had tested for this property in earlier years without finding it and have documented its appearance in their hospitals only over the past decade or so (5, 14, 38).

IN VITRO AND ANIMAL MODEL STUDIES OF ENTEROCOCCAL β-LACTAMASE

In vitro hydrolysis studies have shown that the enterococcal β-lactamase, like the ones in staphylococci, has greatest activity against penicillin, ampicillin, and ureidopenicillins; little or no activity against most cephalosporins, penicillinase-resistant semisynthetic penicillins, and imipenem; and intermediate activity against ticarcillin (20). In most staphylococci, β-lactamase production is inducible, whereas to date, β-lactamase production is constitutive in enterococci, i.e., unchanged by preexposure to usual inducers (41). However, even though enzyme production is constitutive,

HH22 (and other enterococci examined) produces much less enzyme than a typical, induced staphylococcus (41), which likely explains the lack of phenotypic resistance of Bla$^+$ enterococci unless a high inoculum is used. An inoculum effect is characteristic of Bla$^+$ organisms, particularly gram-positive bacteria which, lacking an outer membrane, have nothing which impedes the access of the drug to the enzyme and thus must depend on the total amount of enzyme produced by the population of bacterial cells to inactivate the drug. If the total amount of enzyme is insufficient, then the drug can overwhelm the enzyme, inactivate the susceptible target sites, and inhibit growth of the bacteria. A single cell and even a small number of cells may not produce a sufficient amount of enzyme to inactivate the antibiotic and will often appear susceptible; a large number of bacteria collectively produce more enzyme, can inactivate the antibiotic, and thus test resistant. Even Bla$^+$ staphylococci show an inoculum effect and may test susceptible with an inoculum of 10^3 CFU/ml; however, they usually test resistant at the standard clinical laboratory inoculum of 10^5 CFU/ml, unlike Bla$^+$ enterococci, presumably because most staphylococci produce more enzyme. Although all studies of Bla$^+$ enterococci have shown an inoculum effect, the increase in the MIC with a large inoculum has been variable. In our studies of HH22, the MICs of penicillin, ampicillin, or piperacillin were 2 to 4 μg/ml at 10^3 CFU/ml and ≥1,000 μg/ml using 10^7 CFU/ml. Patterson and colleagues using strains from Connecticut and Pittsburgh, Pa., found less of an elevation in the MIC, with MICs of ampicillin or penicillin of 6.25 to 16 μg/ml at 10^7 CFU/ml and of 16 to 500 μg/ml at 10^8 CFU/ml (22, 23, 26). Markowitz et al. examined 40 Bla$^+$ enterococci from a prolonged outbreak and reported MICs of penicillin and of ampicillin of 32 to ≥128 μg/ml at an inoculum of 10^7 CFU/ml (12).

Another difference between Bla$^+$ enterococci and staphylococci is that most strains of staphylococci release their enzyme into the extracellular medium and it can be recovered from cell-free supernatants; with enterococci, we have not found the enzyme in cell-free supernatants (20). When the enterococcal β-lactamase gene was cloned into staphylococci, the enzyme was found in cell-free supernatants, and conversely, when the staphylococcal gene was cloned into enterococci, the β-lactamase remained cell associated (41). When Bla$^+$ enterococci were sonicated, the enzyme was recovered in the very large molecular weight fractions (>100,000) (20); the gene, however, codes for a protein of only ~28,000 kDa (41), raising the possibility that the enzyme remains attached to the cell membrane or is complexed with other components of the outer cell layers such as cell wall peptidoglycan. Since β-lactamases must be transported across the cell membrane in order to exert their effect on beta-lactams, these results suggest that β-lactamase is first transported but either fails to be cleaved from its signal peptide sequence or somehow remains bound to other cell components.

The activity of the enterococcal β-lactamase is reversed in vitro by the β-lactamase inhibitors clavulanate, sulbactam, and tazobactam, typically resulting in a lowering of the high MICs with large inocula to an MIC typical for beta-lactamase-negative strains (12, 20, 22, 23, 26). In animal models of endocarditis caused by Bla$^+$ enterococci, production of β-lactamase was detrimental to therapy with penicillin or ampicillin alone, although these agents did result in some decrease in the CFU per gram of vegetation (e.g., ~10^9 CFU/g in untreated animals versus ~10^7 CFU/g in animals treated with a penicillin alone) (9, 11). The addition of clavulanate or sulbactam further decreased the CFU per gram of vegetation by 3 to 4 $\log_{10}$, equivalent or slightly better than the effect of vancomycin (9, 11). In a prophylaxis study, ampicillin plus sulbactam was 65% effective in preventing enterococcal endocarditis following a challenge of 10^5 CFU of the Bla$^+$ strain HH22, while ampicillin alone was only 29% effective (1).

The animal studies confirm that β-lactamase production by enterococci is an important determinant for therapeutic outcome, at least in endocarditis. However, routine susceptibility tests cannot be relied upon to detect these strains. The experience of Wells et al. at a Veterans Administration (VA) hospital in Richmond is illustrative of the problems of the clinical laboratory in identifying Bla$^+$ enterococci without specific tests. This institution was in the midst of an outbreak of Bla$^+$ enterococci that was not recognized until 80 isolates were submitted to the Centers for Disease Control as part of a national enterococcal surveillance study; these isolates were then tested for β-lactamase, which revealed that 10 isolates (12.5%) were Bla$^+$ (39). Several β-lactamase tests, including Cefinase disks (BBL) (containing the chromogenic cephalosporin nitrocefin), Beta-Lactam (Remel), and DrySlide Beta-Lactamase (Difco Laboratories), have been used successfully (15). Whether all ampicillin-susceptible enterococci should be tested for β-lactamase production with a specific test is not clear, especially in areas where periodic screening fails to detect these strains. It seems reasonable, however, that isolates cultured from serious infections should be tested.

Handwerger et al. recently reported a strain of *E. faecalis* that was an exception to the above observations (8). This strain failed to hydrolyze nitrocefin but was shown to be Bla$^+$ by virtue of the fact that it almost completely inactivated penicillin by 2 h in a bioassay. Unlike strains previously reported, the strain did not escape detection since it was resistant to ampicillin by disk diffusion and by the Vitek automated susceptibility testing system and since the macrobroth MIC of ampicillin was shown to be 16 μg/ml at a 10^5-CFU/ml inoculum (8). This suggests that this β-lactamase is different from those previously described.

CLINICAL INFECTIONS AND EVIDENCE OF INTRA- AND INTERHOSPITAL SPREAD OF BLA$^+$ ENTEROCOCCI

Despite several reports of sporadic isolates of Bla$^+$ enterococci from the early to mid-1980s (19, 20, 26), the first large-scale occurrence of these organisms was recognized in July 1987 in an infant-toddler surgical ward in Boston, Mass. (27). Over the next 1½-year period, more than 75 patients were found to be colonized with Bla$^+$, highly gentamicin-resistant *E. faecalis*, isolated most often from their fecal flora. A number of samples from personnel and environmental samples were also culture positive for this organism, including one from a nurse with persistently positive fecal and hand cultures who appeared to be an epidemiologically important factor in the spread of these organisms. The same or a related plasmid was found in 10 of 10 isolates examined, suggesting that a single strain was present or predominated (27). Although the Boston enterococcal isolates were not implicated in definitive infections, Bla$^+$ enterococci have been isolated from patients with serious infections by others. Patterson et al. described a patient with possible endocarditis, determined on the basis of the presence of a murmur and a positive blood culture for a Bla$^+$, highly gentamicin-resistant strain (22). Among six Bla$^+$ enterococcal isolates at

a pediatric hospital in Buenos Aires, Argentina, two were cultured from blood, another was cultured from a subdiaphragmmatic abscess, and a fourth was obtained from cerebrospinal fluid (18). Wells et al. reported a large outbreak of infections due to Bla$^+$ enterococci at a VA hospital in Richmond, Va. (39); the outbreak was recognized initially in 1988 and continues through the current time. Over a 17-month period, 8% of 1,426 enterococci (114 isolates) were Bla$^+$, and all of these were highly resistant to gentamicin. In a case controlled study of infected versus colonized patients, sites of infection caused by Bla$^+$ enterococci were the urinary tract (62%), blood (10%), wounds (17%), vascular catheters (7%), and pancreatic abscesses (3%) (39).

Although most Bla$^+$ enterococci have been isolated from hospitalized patients, Patterson et al. reported three isolates from outpatients. All three isolates, however, were from older men with a history of chronic urinary problems including chronic prostatitis, carcinoma of the bladder, and staghorn calculi, and all had been hospitalized at the West Haven VA hospital at some time within the 2 years prior to isolation of the Bla$^+$ enterococcus (24). Although none was hospitalized at the same time or on the same ward, all three were monitored as outpatients in the same VA genitourinary clinic in West Haven, Conn.

A number of the isolates described above in the large outbreak of infection, as well as sporadic isolates from a number of locations, have been compared by pulsed-field gel electrophoresis of their genomic DNA. This technique, coupled with restriction endonuclease digestion using enzymes with infrequent recognition sites (e.g., 5-25) within the enterococcal chromosome, allows one to easily compare different isolates to determine the identity or similarity of the chromosomal restriction endonuclease digestion patterns. By this technique, isolates from the Richmond, Va., VA outbreak and from Houston, Philadelphia, Pittsburgh, and Delaware and two isolates from Florida appeared to represent the clonal dissemination of a single strain (21). On the other hand, isolates from Argentina, Boston, Connecticut, and Beirut were found to be distinct from this group as well as from each other (21). These conclusions were based upon the near identity of the chromosomal restriction endonuclease digestion patterns of the former isolates and the markedly dissimilar patterns of the other Bla$^+$ isolates as well as of other *E. faecalis* isolates. In addition, the six Argentina isolates all appear to represent a single, distinct strain, as do the three Connecticut isolates (18, 24). These observations demonstrate the clonal spread of some Bla$^+$ enterococci, as well as the emergence of this property in a number of distinct strains. It is not known whether the β-lactamase genes in these different enterococcal strains derive from a single genetic transfer event, presumably from staphylococci, with subsequent spread within the species, or whether multiple instances of transfer of β-lactamase genes into enterococci have occurred.

GENETICS AND EXPRESSION OF β-LACTAMASE PRODUCTION IN ENTEROCOCCI

β-Lactamase has been shown to be encoded on transferable plasmids in a number of *E. faecalis* strains, including the original strain, HH22, from Texas (19), isolates from Pennsylvania (37), Virginia (12), Argentina (18), and Connecticut (25), and to be chromosomally encoded in isolates from Boston (28). It is difficult to directly demonstrate the transfer of β-lactamase from one enterococcus to another. This is because a single bacterial cell, inoculated from mating mixtures onto a penicillin-containing surface, does not usually produce enough enzyme to survive on such surfaces. For this reason, most conjugation studies with Bla$^+$ enterococci have been done by selecting for transfer of another resistance trait, and colonies resistant to this agent have then been tested for penicillin resistance and β-lactamase production. The plasmid from HH22 codes for both penicillinase and high-level resistance to gentamicin, as do a number of other plasmids from Bla$^+$ *E. faecalis* (18, 19, 24, 39). The HH22 plasmid has also been shown to be a pheromone-responsive plasmid and to belong to the group of plasmids characterized by pAD1 (17). Pheromone-responsive plasmids are usually found only in *E. faecalis* and are characterized by high-frequency transfer from donor to recipients in broth as well as on agar surfaces.

Hybridization of DNA from Bla$^+$ enterococci from Houston, Pennsylvania, Boston, Argentina, Connecticut, Virginia, Beirut, Florida, and Delaware has shown that all have very strong homology to a β-lactamase gene from *S. aureus* (12, 18, 20, 33, 34, 37, 40). In staphylococci, there are at least four phenotypes (A, B, C, and D) of β-lactamases, and these enzymes are encoded by a family of closely related genes (4). The DNA sequence of the enterococcal β-lactamase gene of HH22 has been shown to be identical to that of the β-lactamase present on the staphylococcal plasmids pPC1 and pS1, as determined by East and Dyke (4), and varies by three nucleotides (none of which result in amino acid changes) from that of the β-lactamase on the staphylococcal plasmid pI258 (41). These staphylococcal plasmids code for a type A penicillinase, and the genes have previously been designated *blaZ* (4, 35). Immediately upstream of the staphylococcal β-lactamase structural gene and reading in the opposite direction is an open reading frame for the putative transmembrane antirepressor, and following that is an open reading frame for the putative repressor; together they are assumed to regulate β-lactamase production (30, 36). Marked differences in the restriction sites in the corresponding regions of two enterococcal Bla$^+$ plasmids suggest that these genes are not present in enterococci or are markedly altered (33). Sequencing upstream of the enterococcal β-lactamase gene of HH22 confirmed that only part of the antirepressor sequence is present, and hybridization studies have shown that there is no region in HH22 that is homologous to the repressor gene (33, 34, 42). The lack of a repressor presumably explains why production of this β-lactamase in enterococci is constitutive while it is inducible in staphylococci. It does not, however, explain why the amount of enzyme produced is smaller in enterococci.

Because of the identity between the enterococcal β-lactamase gene of HH22 and β-lactamase genes of staphylococci and because enterococci and staphylococci can support the replication of some of the same conjugative plasmids (32), the possibility that the enterococcal β-lactamase plasmid of HH22 might be directly derived from a common staphylococcal Bla$^+$ plasmid was considered. Several transferable Bla$^+$, gentamicin resistance-encoding plasmids from U.S. isolates of *S. aureus* as well as older, nonconjugative Bla$^+$ staphylococcal plasmids were compared after restriction endonuclease digestion and hybridization to the transferable Bla$^+$ gentamicin resistance-encoding plasmid of HH22. Only the β-lactamase genes and, for the gentamicin-resistant staphylococci, the gentamicin resistance genes showed cross-hybridization between these plasmids; none of the rest of the plasmids showed any homology to any other (37). On the other hand, the plasmid from HH22 showed extensive hybridization with the β-lactamase-encoding plasmid from a

Bla$^+$ enterococcus from Pennsylvania, indicating a close relationship between these plasmids (37). The subsequent observation that the HH22 plasmid was a pheromone-responsive plasmid also indicated that this plasmid did not transfer directly from staphylococci, since pheromone-responsive plasmids have a narrow host range and are specific for *E. faecalis*.

We have recently compared the restriction patterns of the *blaZ* region of enterococci from Philadelphia, Virginia, Florida, Beirut, and Buenos Aires, Argentina, to those of HH22 and several staphylococcal plasmids (33). The restriction sites present in the ~1400-bp region encompassing *blaZ* were the same, except for a downstream *Eco*RV site. This site was ~50 bp closer to the *Xba*I site near the end of *blaZ* in isolates from Florida, Virginia, and Philadelphia than it was in isolates from Houston (HH22), Beirut, and Buenos Aires or than it was in the staphylococcal Bla$^+$ transposon Tn*4201*. Since HH22 appears to be clonally related to the Florida, Virginia, and Philadelphia isolates by pulsed-field gel electrophoresis, the difference in the downstream restriction *Eco*RV site could indicate a small deletion between the *Xba*I and *Eco*RV sites after *blaZ* entered the ancestral progenitor of these isolates. A further difference between HH22 and the Philadelphia strain (PA) exists in the region past the *Eco*RV site, heading downstream with respect to *blaZ*. In this region, the β-lactamase-encoding plasmid from PA was shown to hybridize to the *Eco*RV-*Eco*RI region downstream of *blaZ* from the staphylococcal β-lactamase transposon Tn*4201*, while the plasmid from HH22 did not; this *Eco*RV-*Eco*RI fragment of Tn*4201* contains a 121-bp inverted repeat that may be important for transposition. Thus, the region downstream of the β-lactamase-encoding plasmid between the *Xba*I and *Eco*RV sites is shorter in PA than it is in Tn*4201* and HH22; nonetheless, on the basis of the hybridization results, PA has more homology to the *Eco*RV-*Eco*RI fragment from Tn*4201* containing this inverted repeat than HH22 (33). This suggests that *blaZ* in enterococci may have derived from a Tn*4201*-like transposon, with subsequent deletions in different areas. The history of the evolution of these various regions is not known, nor is it known whether the enterococcal *blaZ* is within a structure that can transpose. However, the report by Rice et al. of a chromosomal location of *blaZ* among isolates from the Boston outbreak suggests the possibility that transposition may have occurred to this location (28). This group has recently sequenced the region downstream of *blaZ* from a Boston strain and shown the presence of the 121-bp inverted repeat of Tn*4201* (29).

Although the spread of β-lactamase into enterococci apparently lagged long behind its appearance in staphylococci, the fact that it has finally done so should come as no surprise. Most resistance genes found in staphylococci, including most of the aminoglycoside resistance genes and the genes for erythromycin, chloramphenicol, and tetracycline resistance, are also found in enterococci. For example, we have recently shown that a gentamicin resistance transposon found in the Bla$^+$ isolate HH22 is highly related if not identical to gentamicin resistance transposons in staphylococci (10). In addition to the sharing of a number of antibiotic resistance genes and transposons by staphylococci, enterococci, and streptococci, certain plasmids, such as the broad-host-range conjugative plasmid pAMβ1, can transfer between these different genera (32). Thus, the identification of a β-lactamase in enterococci identical to one in staphylococci follows the pattern of other properties in gram-positive organisms. The real question is why this property did not appear in enterococci sooner and why it is not now more common. It also reminds us of the possibility that this gene might spread to other organisms such as streptococci, pneumococci, or listeria, where its presence could have devastating consequences. Clinical laboratories and basic science investigators should maintain an awareness of this possibility. The appearance of β-lactamase in enterococci serves as a reminder that the absence of a particular resistance trait, even in the face of 40+ years of use of an antibiotic, does not preclude its eventual emergence.

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REFERENCES

1. **Bayer, A. S., and J. Tu.** 1990. Chemoprophylactic efficacy against experimental endocarditis caused by β-lactamase-producing, aminoglycoside-resistant enterococci is associated with prolonged serum inhibitory activity. Antimicrob. Agents Chemother. **34**:1068–1074.
2. **Chirurgi, V. A., S. E. Oster, A. A. Goldberg, M. J. Zervos, and R. E. McCabe.** 1991. Ampicillin-resistant *Enterococcus raffinosus* in an acute-care hospital: case-control study and antimicrobial susceptibilities. J. Clin. Microbiol. **29**:2663–2665.
3. **Coudron, P. E., S. M. Markowitz, and E. S. Wong.** 1992. Isolation of a β-lactamase-producing, aminoglycoside-resistant strain of *Enterococcus faecium*. Antimicrob. Agents Chemother. **36**:1125–1126.
4. **East, A. K., and G. H. Dyke.** 1989. Cloning and sequence determination of six *Staphylococcus aureus* β-lactamases and their expression in *Escherichia coli* and *Staphylococcus aureus*. J. Gen. Microbiol. **135**:1001–1015.
5. **Gransden, W. R., A. King, T. M. S. Barkham, S. J. Eykyn, and I. Phillips.** 1991. Enterococcal bacteremia 1969—90. Changing clinical and microbiological patterns, abstr. 961, p. 262. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.
6. **Grayson, M. L., G. M. Eliopoulos, C. B. Wennersten, K. L. Ruoff, P. C. de Girolami, M.-J. Ferraro, and R. C. Moellering, Jr.** 1991. Increasing resistance to β-lactam antibiotics among clinical isolates of *Enterococcus faecium*: a 22-year review at one institution. Antimicrob. Agents Chemother. **35**:2180–2184.
7. **Grayson, M. L., G. M. Eliopoulos, C. B. Wennersten, K. L. Ruoff, K. Klimm, F. L. Sapico, A. S. Bayer, and R. C. Moellering, Jr.** 1991. Comparison of *Enterococcus raffinosus* with *Enterococcus avium* on the basis of penicillin susceptibility, penicillin-binding protein analysis, and high-level aminoglycoside resistance. Antimicrob. Agents Chemother. **35**:1408–1412.
8. **Handwerger, S., D. C. Perlman, D. Altarac, and V. McAuliffe.** 1992. Concomitant high-level vancomycin and penicillin resistance in clinical isolates of enterococci. Clin. Infect. Dis. **14**:655–661.
9. **Hindes, R. G., S. H. Willey, G. M. Eliopoulos, L. B. Rice, C. T. Eliopoulos, B. E. Murray, and R. C. Moellering, Jr.** 1989. Treatment of experimental endocarditis caused by a β-lactamase-producing strain of *Enterococcus faecalis* with high-level resistance to gentamicin. Antimicrob. Agents Chemother. **33**:1019–1022.
10. **Hodel-Christian, S. L., and B. E. Murray.** 1991. Characterization of the gentamicin resistance transposon Tn*5281* from *Enterococcus faecalis* and comparison to staphylococcal transposons Tn*4001* and Tn*4031*. Antimicrob. Agents Chemother. **35**:1147–1152.
11. **Ingerman, M., P. G. Pitsakis, A. Rosenberg, M. T. Hessen, E. Abrutyn, B. E. Murray, and M. E. Levison.** 1987. β-lactamase production in experimental endocarditis due to aminoglycoside-resistant *Streptococcus faecalis*. J. Infect. Dis. **155**:1226–1232.

12. **Markowitz, S. M., V. D. Wells, D. S. Williams, C. G. Stuart, P. E. Coudron, and E. S. Wong.** 1991. Antimicrobial susceptibility and molecular epidemiology of β-lactamase-producing, aminoglycoside-resistant isolates of *Enterococcus faecalis*. Antimicrob. Agents Chemother. **35**:1075–1080.
13. **Mazzulli, T., S. M. King, and S. E. Richardson.** 1992. Bacteremia due to β-lactamase-producing *Enterococcus faecalis* with high-level resistance to gentamicin in a child with Wiskott-Aldrich Syndrome. Clin. Infect. Dis. **14**:780–781.
14. **Mederski-Samoraj, B. D., and B. E. Murray.** 1983. High-level resistance to gentamicin in clinical isolates of enterococci. J. Infect. Dis. **147**:751–757.
15. **Meszaros, A., L. Strenkoski, and R. Firstenberg-Eden.** 1991. A comparative evaluation of three beta-lactamase test systems, abstr. C-125, p. 363. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
16. **Murray, B. E.** 1990. The life and times of the enterococcus. Clin. Microbiol. Rev. **3**:46–65.
17. **Murray, B. E., F. Y. An, and D. B. Clewell.** 1986. Plasmids and pheromone response of the β-lactamase producer *Streptococcus (Enterococcus) faecalis* HH22. Antimicrob. Agents Chemother. **32**:547–551.
18. **Murray, B. E., H. A. Lopardo, E. A. Rubeglio, M. Frosolono, and K. V. Singh.** 1992. Intrahospital spread of a single gentamicin-resistant, β-lactamase-producing strain of *Enterococcus faecalis* in Argentina. Antimicrob. Agents Chemother. **36**:230–232.
19. **Murray, B. E., and B. Mederski-Samoraj.** 1983. Transferable β-lactamase: a new mechanism for *in vitro* penicillin resistance in *Streptococcus faecalis*. J. Clin. Invest. **72**:1168–1171.
20. **Murray, B. E., B. Mederski-Samoraj, S. K. Foster, J. L. Brunton, and P. Harford.** 1986. *In vitro* studies of plasmid-mediated penicillinase from *Streptococcus faecalis* suggest a staphylococcal origin. J. Clin. Invest. **77**:289–293.
21. **Murray, B. E., K. V. Singh, S. M. Markowitz, H. A. Lopardo, J. E. Patterson, M. J. Zervos, E. Rubeglio, G. M. Eliopoulos, L. B. Rice, F. W. Goldstein, S. G. Jenkins, G. M. Caputo, R. Nasnass, L. S. Moore, E. S. Wong, and G. Weinstock.** 1991. Evidence for clonal spread of a single strain of β-lactamase-producing *Enterococcus faecalis* to six hospitals in five states. J. Infect. Dis. **163**:780–785.
22. **Patterson, J. E., S. M. Colodny, and M. J. Zervos.** 1988. Serious infection due to β-lactamase-producing *Streptococcus faecalis* with high-level resistance to gentamicin. J. Infect. Dis. **158**: 1144–1145.
23. **Patterson, J. E., B. L. Masecar, and M. J. Zervos.** 1988. Characterization and comparison of two penicillinase-producing strains of *Streptococcus (Enterococcus) faecalis*. Antimicrob. Agents Chemother. **32**:122–124.
24. **Patterson, J. E., K. V. Singh, and B. E. Murray.** 1991. Epidemiology of an endemic strain of β-lactamase-producing *Enterococcus faecalis*. J. Clin. Microbiol. **29**:2513–2511.
25. **Patterson, J. E., A. Wanger, K. K. Zscheck, M. J. Zervos, and B. E. Murray.** 1990. Molecular epidemiology of β-lactamase-producing enterococci. Antimicrob. Agents Chemother. **34**:302–305.
26. **Patterson, J. E., and M. J. Zervos.** 1989. Susceptibility and bactericidal activity studies of four β-lactamase-producing enterococci. Antimicrob. Agents Chemother. **33**:251–253.
27. **Rhinehart, E., N. E. Smith, C. Wennersten, E. Gorss, J. Freeman, G. M. Eliopoulos, R. C. Moellering, Jr., and D. A. Goldmann.** 1990. Rapid dissemination of β-lactamase-producing, aminoglycoside-resistant *Enterococcus faecalis* among patients and staff on an infant-toddler surgical ward. N. Engl. J. Med. **323**:1814–1818.
28. **Rice, L. B., G. M. Eliopoulos, C. Wennersten, D. Goldmann, G. A. Jacoby, and R. C. Moellering, Jr.** 1991. Chromosomally mediated β-lactamase production and gentamicin resistance in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **35**:272–276.
29. **Rice, L. B., and S. H. Marshall.** 1992. Evidence of incorporation of the chromosomal β-lactamase gene of *Enterococcus faecalis* CH19 into a transposon derived from staphylococci. Antimicrob. Agents Chemother. **36**:1843–1846.
30. **Rowland, S.-J., and K. G. H. Dyke.** 1990. Tn*552*, a novel transposable element from *Staphylococcus aureus*. Mol. Microbiol. **4**:961–975.
31. **Schaberg, D. R., D. H. Culver, and R. P. Gaynes.** 1991. Major trends in the microbial etiology of nosocomial infection. Am. J. Med. **91**(Suppl. 3B):72S–75S.
32. **Schaberg, D. R., and M. J. Zervos.** 1986. Intergeneric and interspecies gene exchange in gram-positive cocci. Antimicrob. Agents Chemother. **30**:817–822.
33. **Smith, M. C., and B. E. Murray.** 1992. Comparison of enterococcal and staphylococcal β-lactamase-encoding fragments. Antimicrob. Agents Chemother. **36**:273–276.
34. **Smith, M. C., and B. E. Murray.** 1992. Sequence analysis of the β-lactamase repressor from *Staphylococcus aureus* and hybridization studies with two β-lactamase-producing isolates of *Enterococcus faecalis*. Antimicrob. Agents Chemother. **36**:2265–2269.
35. **Wang, P.-Z., and R. P. Novick.** 1987. Nucleotide sequence and expression of the β-lactamase gene from *Staphylococcus aureus* plasmid pI258 in *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. J. Bacteriol. **169**:1763–1766.
36. **Wang, P.-Z., S. J. Projan, and R. P. Novick.** 1991. Nucleotide sequence of β-lactamase regulatory genes from staphylococcal plasmid p1258. Nucleic Acids Res. **19**:4000.
37. **Wanger, A. R., and B. E. Murray.** 1990. Comparison of enterococcal and staphylococcal beta-lactamase plasmids. J. Infect. Dis. **161**:54–58.
38. **Watanakunakorn, C.** 1989. The prevalence of high-level aminoglycoside resistance among enterococci isolated from blood cultures during 1980—1988. J. Antimicrob. Chemother. **24**:63–69.
39. **Wells, V. D., E. S. Wong, B. E. Murray, P. E. Coudron, D. S. Williams, and D. M. Markowitz.** 1992. Infections due to beta-lactamase-producing, high-level gentamicin-resistant *Enterococcus faecalis*. Ann. Intern. Med. **116**:285–292.
40. **Zervos, M. J., A. E. Bacon, J. E. Patterson, D. R. Schaberg, and C. A. Kauffman.** 1988. Enterococcal superinfection in patients treated with ciprofloxacin. J. Antimicrob. Chemother. **21**:113–115.
41. **Zscheck, K., and B. E. Murray.** 1991. Nucleotide sequence of the β-lactamase gene from *Enterococcus faecalis* HH22 and its similarity to staphylococcal β-lactamase genes. Antimicrob. Agents Chemother. **35**:1736–1740.
42. **Zscheck, K. K., M. C. Smith, and B. E. Murray.** 1992. Elements involved in the regulation of beta-lactamase production in enterococci and staphylococci. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.