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Chromosomally Mediated β-Lactamase Production and Gentamicin Resistance in *Enterococcus faecalis*

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We have analyzed four distinct strains of multiply resistant, β-lactamase-producing enterococci isolated during an outbreak of colonization with these strains on an infant-toddler surgical ward at The Children's Hospital in Boston, Mass. All four strains were resistant to erythromycin, penicillin, and tetracycline and to high levels of gentamicin and streptomycin. One strain was also resistant to chloramphenicol. Plasmid profiles revealed four different plasmid patterns, with the number of identified plasmids ranging from zero to three. The gene coding for β-lactamase production could be transferred at low frequency ($<10^{-8}$) to an enterococcal recipient from one strain in conjunction with all of the other resistance determinants. Probes derived from the staphylococcal β-lactamase gene and gentamicin resistance gene failed to hybridize with any of the detectable plasmids, but both genes were present on restriction fragments of genomic DNA in all strains. Our results indicate that the β-lactamase genes and gentamicin resistance genes in these strains are integrated into the bacterial chromosome. The cotransmissibility of the resistance determinants raises the possibility of their incorporation into a multiresistance transposable genetic element.

The continued importance of the enterococcus as a nosocomial pathogen is due in large measure to its resistance to antimicrobial agents. Intrinsically resistant to all clinically available cephalosporins, enterococci are also 100-fold more resistant to penicillin than the streptococci with which they are usually compared. Moreover, the characteristic tolerance of these organisms to the activity of all cell wall-active agents necessitates the use of such agents in combination with aminoglycosides to achieve adequate bactericidal activity (15). As a result, the treatment of severe infections due to even relatively susceptible enterococci can pose significant therapeutic difficulties, especially in patients who are allergic to penicillins or who have compromised renal functions.

In addition to this intrinsic resistance, the development in enterococci of acquired resistance to aminoglycosides, chloramphenicol, erythromycin, and tetracycline rapidly followed clinical introduction of these antimicrobial agents (3, 4, 10). In marked contrast, β-lactamase-mediated penicillin resistance in enterococci did not appear until more than 40 years after the clinical introduction of penicillin and is still a distinctly rare finding despite intensive and widespread screening by certain groups (16, 17, 19). Although the gene responsible for enterococcal β-lactamase production is homologous with that found in *Staphylococcus aureus*, its phenotypic expression differs in that enterococcal β-lactamase is produced constitutively, is not excreted into the medium, and has only a minimal effect on the penicillin MIC at standard inoculum sizes (16). Genetic studies on five strains previously described have revealed the presence of the β-lactamase genes on conjugative plasmids in close association with genes encoding enzymes conferring high-level resistance to gentamicin (16, 19, 20).

We have recently analyzed an outbreak involving the colonization of a large number of patients with β-lactamase-producing enterococci resistant to high levels of gentamicin. The majority of these isolates appear to represent a single strain that spread rapidly among patients inhabiting an infant-toddler surgical ward (20a). A few strains, however, were isolated from patients with no definable epidemiologic relation to one another. The present study was undertaken to examine the phenotypic and genetic characteristics of these organisms.

MATERIALS AND METHODS

Bacterial strains. Study strains of *Enterococcus faecalis* were derived from clinical specimens at The Children's Hospital, Boston, Mass., between July 1987 and July 1988. CH 19, isolated from a wound culture, was the original isolate which appeared to spread throughout the ward. CX 19 was a β-lactamase-producing transcipient derived from the mating of CH 19 with JH2-7, a rifampin-resistant, fusidic acid-resistant, Thy^- strain of *E. faecalis* (5). CH 116 was a urine isolate obtained from a patient on a separate hospital ward who had a temporally distant exposure to the ward with the outbreak. CH 136 was a stool surveillance isolate obtained on the day of the patient's admission. An enterococcal strain had been isolated from this patient's urine during a prior admission which predated the isolation of CH 19 by 3 months. The susceptibility profile of this prior isolate is unknown. CH 188 was isolated as part of a mixed flora from a liver abscess in a postoperative liver transplant recipient who had never been exposed to the ward where the outbreak occurred. *E. faecalis* HH22 was kindly provided by Barbara E. Murray, Houston, Tex. The identification of clinical isolates was carried out by the API Rapidstrep system (API Analytab Products, Plainview, N.Y.). β-Lactamase production was ascertained with nitrocefin-impreg-

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nated disks (BBL Microbiology Systems, Cockeysville, Md.).

Susceptibility testing. Clinical isolates were tested by a reference method (24) with Mueller-Hinton agar (BBL Microbiology Systems) containing a range of antimicrobial concentrations in serial twofold dilutions. Standard dilutions of overnight broth cultures were inoculated onto antibiotic-containing plates by using a 32-prong inoculating device (Craft Machine, Inc., Chester, Pa.) to yield a final inoculum of 10^4 CFU per spot. The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth after 20 h of incubation at 35°C.

Selected strains were tested against penicillin at different inoculum sizes. Dilutions of overnight cultures were inoculated into 2 ml of dextrose phosphate broth (Scott Laboratories, Inc., Fiskeville, R.I.) containing serial twofold dilutions of penicillin at concentrations ranging from 256 to 0.25 μg/ml. Inoculum sizes tested included 10^4, 10^6, and 10^8 CFU/ml. The MIC was defined as the lowest concentration of penicillin which prevented visible turbidity after 20 h of incubation at 35°C. β-Lactamase inducibility was tested in one strain by using a broth macrodilution technique (9) to measure the MIC of penicillin after a 1-, 2-, or 3-h incubation of the strain with penicillin (0.5 U/ml), followed by a 1:1,000 dilution in dextrose phosphate broth (final inoculum, ca. 2×10^5 CFU/ml).

Antimicrobial agents. Antimicrobial agents tested included erythromycin and streptomycin (Eli Lilly & Co., Indianapolis, Ind.), gentamicin (Elkins-Sinn, Inc., Cherry Hill, N.J.), kanamycin (Lyphomed, Melrose Park, Ill.), and chloramphenicol, fusidic acid, rifampin, and tetracycline (Sigma Chemical Company, St. Louis, Mo.).

Conjugation experiments. Tests for transfer of resistance were carried out by using *E. faecalis* JH2-7 as the recipient. Matings were carried out by either cross-streaking or filter-mating techniques, with overnight incubation at 35°C as previously described (5, 7). Transcipients were selected on dextrose phosphate agar plates (1.7%) containing erythromycin (16 μg/ml), gentamicin (100 μg/ml), kanamycin (500 μg/ml), or tetracycline (16 μg/ml). Fusidic acid (25 μg/ml) and rifampin (100 μg/ml) were included in plates for counterselection.

DNA techniques. Plasmid DNA was isolated by a modification of the technique of Birnboim and Doly as previously described (5). Genomic DNA was obtained by the technique of Wilson (26), with the following modifications: the initial volume of cells was 500 ml of overnight culture in dextrose phosphate broth, and the sodium dodecyl sulfate concentration in the cell lysis step was 3% rather than 0.5%. The crude nucleic acid extracts were further purified either on a cesium chloride-ethidium bromide equilibrium density gradient (70.1 Ti fixed-angle rotor; 207,000 × *g* for 18 h) or by incubation with DNase-free RNase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) followed by sequential extractions with phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1).

Genomic DNA (10 μg) was digested with 10 to 20 U of restriction endonuclease *Eco*RI or *Bgl*I (Boehringer Mannheim Biochemicals) according to the specifications of the manufacturer.

Plasmid DNA preparations were separated by electrophoresis on 0.7% agarose gels, visualized with short-wave UV illumination after staining with ethidium bromide, and sized by comparison with standard plasmids. Digested genomic DNA was separated on 1% agarose gels overnight.

DNA was transferred to either nylon or nitrocellulose membranes (Sartorius Filters, Inc., Hayward, Calif.) by the technique of Southern (13). DNA-DNA hybridization was carried out with digoxigenin (Boehringer Mannheim Biochemicals)-labeled probes derived either from a 0.84-kb *Hin*dIII-*Xba*I fragment of plasmid pJM13 (14) containing the leader sequence and 80% of the structural gene coding for the staphylococcal β-lactamase or from a 1.5-kb *Hin*dIII-*Eco*RI fragment of plasmid pSF815a (6) encoding the entire 6′-aminoglycoside acetyltransferase–2″-aminoglycoside phosphotransferase (AAC 6′–APH 2″) bifunctional aminoglycoside resistance gene (known to confer gentamicin resistance in enterococci). Hybrids were detected by using an anti-digoxigenin-alkaline phosphatase conjugate with a chromogenic enzyme substrate.

TABLE 1. Susceptibilities of clinical isolates

Isolate	MIC (μg/ml)[a]					
	PCN	CHLOR	EM	GM	SM	TET
CH 19	4	16	>16	>2,000	>2,000	64
CH 116	8	16	>16	>2,000	>2,000	64
CH 136	8	16	>16	>2,000	>2,000	64
CH 188	8	>64	>16	>2,000	>2,000	64
HH22	8	64	2	>2,000	>2,000	16

[a] Determined by agar dilution technique, with an inoculum of ca. 10^4 CFU per spot. Abbreviations: PCN, penicillin; CHLOR, chloramphenicol; EM, erythromycin; GM, gentamicin; SM, streptomycin; TET, tetracycline.

RESULTS

Organisms. All strains included in this study (CH 19, CH 116, CH 136, CH 188, HH22, and JH2-7) were identified as *E. faecalis* by the API Rapidstrep system. All strains (including CX 19) except JH2-7 were shown to produce β-lactamase by the nitrocefin test.

Susceptibility studies. The antimicrobial susceptibilities of the four isolates and of *E. faecalis* HH22 are listed in Table 1. All Children's Hospital isolates were resistant to erythromycin and tetracycline and exhibited high-level resistance (MIC > 2,000 μg/ml) to streptomycin, gentamicin, and kanamycin. Only CH 188 was resistant to chloramphenicol. At an inoculum size of ca. 10^6 CFU/ml, penicillin MICs ranged from 4 to 8 μg/ml. However, increasing the inoculum 100-fold resulted in an increase in the MIC to greater than 512 μg/ml for all strains except JH2-7. Preincubation with a subinhibitory concentration of penicillin did not alter the MIC for CH 19, suggesting that, like *E. faecalis* HH22, β-lactamase production by this strain was not inducible.

Conjugation experiments. Because of the insignificant effect of β-lactamase production on penicillin MICs at small inoculum sizes, we were unable to select directly for the transfer of the β-lactamase gene in conjugation experiments. Instead, we selected for the transfer of other resistance characteristics and tested these transcipients for β-lactamase production. We were able to obtain a small number of transcipients from two donor strains (CH 19 and CH 136) by the cross-streaking procedure, but we were unable to transfer resistance by filter-mating techniques, suggesting that the rate of transfer of erythromycin, gentamicin, streptomycin, and tetracycline resistance was no greater than 10^{-8} per donor cell. Transcipients all demonstrated the resistance to fusidic acid and rifampin of the JH2-7 recipient strain. Growth of donor colonies on control plates containing fusidic acid and rifampin was never observed. The majority of these transcipients expressed resistance to erythromycin,

TABLE 2. Resistance patterns of transcipients[a]

Donor strain	Donor pattern	Transcipient patterns
CH 19	Bla Em Gm Sm Tet	Bla Em Gm Sm Tet
		Em Gm Tet
		Tet
CH 136	Bla Em Gm Sm Tet	Em Gm Sm Tet
		Gm Sm Tet
		Em Gm Tet
		Sm

[a] Bla, β-Lactamase producing; Em, Gm, Sm, and Tet, resistance to erythromycin, gentamicin, streptomycin, and tetracycline, respectively.

gentamicin, streptomycin, or tetracycline in various combinations (Table 2). β-Lactamase production was detected in only one transcipient, CX 19, derived from a mating of CH 19 and JH2-7. The gene for β-lactamase production was transferred in conjunction with resistance to erythromycin, gentamicin, streptomycin, and tetracycline in this transcipient.

Plasmid analysis. The four strains selected for further study demonstrated four distinct plasmid patterns (Fig. 1). CH 19, the strain from which transfer of the β-lactamase production gene was observed, possessed a plasmid of approximately 36 MDa. CH 116, CH 136, and CH 188 had different plasmid patterns, with the number of plasmid bands ranging from zero to three. Transfer of the β-lactamase gene from these strains was not demonstrable. CX 19, the transcipient derived from mating CH 19 with JH2-7, had no detectable plasmids (Fig. 1).

DNA-DNA hybridization. The staphylococcal β-lactamase probe failed to hybridize to plasmids visualized in the clinical isolates (Fig. 1). Restriction digestions of these plasmids also failed to demonstrate hybridization. Likewise, the probe derived from the bifunctional aminoglycoside resistance gene, known to be responsible for gentamicin resistance in enterococci, did not hybridize to the plasmids in our clinical isolates (data not shown). The β-lactamase probe hybridized

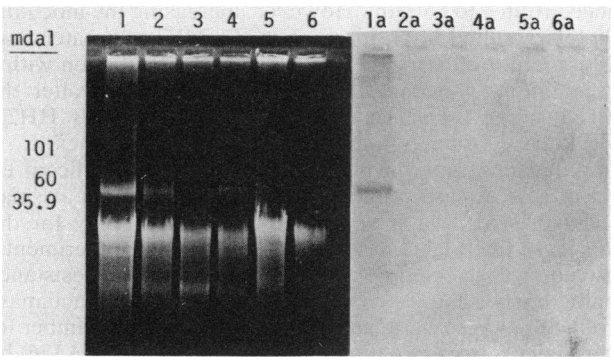

FIG. 1. (Lanes 1 through 6) Agarose gel electrophoresis of plasmid preparations from study strains and from *E. faecalis* HH22. Lanes: 1, HH22 (the two plasmids previously described [17] are not well separated on this gel); 2, CH 19; 3, CH 116; 4, CH 136; 5, CH 188 (the two lower-molecular-weight plasmids are not well separated on this gel); 6, CX 19. (Lanes 1a through 6a) DNA-DNA hybridization of a Southern transfer of the gel shown in the left panel, with a digoxigenin-labeled 0.84-kb *Hin*dIII-*Xba*I fragment of plasmid pJM13 containing the leader sequence and 80% of the structural staphylococcal β-lactamase gene used as a probe.

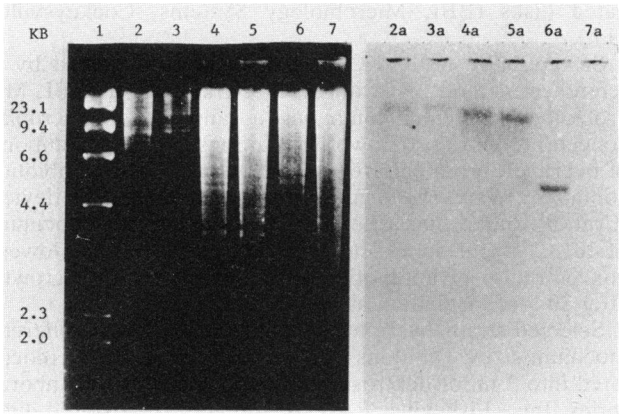

FIG. 2. (Lanes 1 through 7) Agarose gel electrophoresis of *Eco*RI restriction digestions of genomic DNA from strains examined in this study. Lanes: 1, bacteriophage lambda digested with *Hin*dIII (used as a sizing standard); 2, CH 19; 3, CH 116; 4, CH 136; 5, CH 188; 6, *E. faecalis* HH22; 7, *E. faecalis* JH2-7. (Lanes 2a through 7a) DNA-DNA hybridization of a Southern transfer of the gel shown in the left panel, with a digoxigenin-labeled 0.84-kb *Hin*dIII-*Xba*I fragment of plasmid pJM13 containing the leader sequence and 80% of the structural staphylococcal β-lactamase gene used as a probe.

to an approximately 23-kb *Eco*RI digestion fragment of genomic DNA from CH 19 and CH 116 and to slightly smaller fragments of genomic DNA from CH 136 and CH 188 (Fig. 2). The gentamicin resistance gene probe hybridized to a slightly larger fragment in CH 19 (data not shown). Digestion of genomic DNA from CH 19 and CX 19 with *Eco*RI revealed the β-lactamase gene on fragments of similar size. However, the β-lactamase gene was identified on an 18-kb fragment in CH 19 and an approximately 7-kb fragment in CX 19 after digestions with *Bgl*I. The gentamicin resistance genes of CH 19 and CX 19 were located on *Bgl*I fragments of approximately 5 kb (data not shown).

DISCUSSION

The transfer of resistance determinants among enterococci proceeds along several different pathways. The most common route is thought to be via the conjugal transfer of resistance plasmids. Transfer of some enterococcal plasmids utilizes an intricate system of pheromone production and response which facilitates contact between cells and transfer of genetic information among different strains (2). Since the first antibiotic resistance plasmid was described in enterococci in 1972 (3), a large number of distinct plasmids conferring resistance to one or more antibiotics have been described. Enterococci are also known to carry antibiotic resistance genes on mobilizable genetic elements (transposons), some of which are able to code for their own transfer functions in the absence of conjugative plasmids (7). The ability of transposons to integrate into either conjugative plasmids or the bacterial chromosome enhances both the transferability and the stability of a given resistance determinant. It now appears that enterococci are also capable of transferring resistance determinants via transduction. By using a strain described in this study (CH 19), we have recently demonstrated transduction of gentamicin resistance among enterococci (27).

In addition to transferring resistance determinants within the genus, enterococci share determinants with other gen-

era. Examples of this phenomenon include the presence in enterococci of the practically ubiquitous Tet M determinant, the staphylococcal AAC 6′–APH 2″ aminoglycoside-modifying-enzyme gene, and the APH 3′ aminoglycoside phosphotransferase gene found in *Campylobacter coli* (6, 11, 23). The appearance of the staphylococcal β-lactamase gene in enterococci serves as yet another example of intergeneric antibiotic resistance transfer involving enterococci (18). The findings of this study confirm the previously demonstrated homology between staphylococcal and enterococcal β-lactamases.

Intercellular transfer of resistance determinants in staphylococci is somewhat more complicated. In vitro studies have documented transfer of staphylococcal antibiotic resistance genes by transduction, transformation, bacteriophage-mediated conjugation, and plasmid-mediated conjugation (12). Chromosomal β-lactamase production in staphylococci was first described in 1966 (1). Further analysis of the strain for which this was described, PS 80, led to the discovery of the staphylococcal penicillinase transposon, Tn*552* (21). Other staphylococcal penicillinase transposons have since been described, one of which bears extensive structural homology to Tn*552* (Tn*4002*) and one of which does not (Tn*4201*) (12, 25). β-Lactamase genes have also been found integrated into the staphylococcal chromosome as components of wholly integrated plasmids (8). In some cases, this plasmid integration has been promoted by the previous integration of specific prophages next to which the plasmid preferentially inserts (22). The coexistence of a chromosomally integrated β-lactamase gene and a transducing phage in our strains raises the interesting possibility that a similar phenomenon may now be occurring in enterococci.

The chromosomal location of the β-lactamase gene in our strains, combined with our ability to transfer this gene (along with other antibiotic resistance genes), suggests the possibility of its incorporation into a mobilizable genetic element possessing determinants for resistance to multiple antimicrobial agents. Further studies designed to characterize the genetic element within which the enterococcal β-lactamase resides, using techniques such as restriction enzyme mapping, mobilization with plasmid pAD1 (2), and sequential transfer to recombination-deficient mutant pUV202 (7), are currently under way or planned for the near future.

It is unlikely that the presence of a single transposon coding for multiple antibiotic resistances will explain all of our findings. The abilities to transfer gentamicin and tetracycline resistance independently of β-lactamase and to transfer tetracycline resistance independently of gentamicin resistance suggest that other mechanisms of transfer are involved. It is possible that more than one transposon is involved or that bacteriophage-mediated transduction is occurring in parallel with conjugal transfer of antibiotic resistance.

It is intriguing that the β-lactamase gene involved in this outbreak was found among different enterococcal isolates within the hospital. Conjugal transfer in vitro was a distinctly rare event. In contrast, all previously described enterococcal β-lactamases have been found in single isolates despite their incorporation into plasmids which exhibit a high frequency of in vitro conjugal transfer. Factors affecting the transmissibility and stability of antibiotic resistance genes in vivo are undoubtedly more complex than those that are operative during conjugal transfer on a blood agar plate. The elucidation of these factors may contribute to our understanding of the processes involved in the widespread dissemination of resistance traits among nosocomial populations of bacterial pathogens and, ultimately, to our ability to prevent such dissemination.

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REFERENCES

1. **Ashehov, E. H.** 1966. Chromosomal location of the genetic elements controlling penicillinase production in *Staphylococcus aureus*. Nature (London) **210**:804–806.
2. **Clewell, D. B.** 1981. Plasmids, drug resistance, and gene transfer in the genus *Streptococcus*. Microbiol. Rev. **45**:409–436.
3. **Courvalin, P. M., C. Carlier, and Y. A. Chabbert.** 1972. Plasmid-linked tetracycline and erythromycin resistance in Group D Streptococci. Ann. Inst. Pasteur. (Paris) **123**:755–759.
4. **Courvalin, P. M., W. V. Shaw, and A. E. Jacob.** 1978. Plasmid-mediated mechanisms of resistance to aminoglycoside-aminocyclitol antibiotics and to chloramphenicol in group D streptococci. Antimicrob. Agents Chemother. **13**:716–725.
5. **Eliopoulos, G. M., C. Wennersten, S. Zighelboim-Daum, E. Reiszner, D. Goldmann, and R. C. Moellering, Jr.** 1988. High-level resistance to gentamicin in clinical isolates of *Streptococcus* (*Enterococcus*) *faecium*. Antimicrob. Agents Chemother. **32**:1528–1532.
6. **Ferretti, J. J., K. S. Gilmore, and P. Courvalin.** 1986. Nucleotide sequence analysis of the gene specifying the bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase enzyme in *Streptococcus faecalis* and identification and cloning of gene regions specifying the two activities. J. Bacteriol. **167**:631–638.
7. **Franke, A. E., and D. B. Clewell.** 1981. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J. Bacteriol. **145**:494–502.
8. **Johnston, L. H., and K. G. H. Dyke.** 1974. Staphylococcal penicillinase plasmids: studies on the reversion of a temperature sensitive replication mutant to temperature stability. J. Gen. Microbiol. **82**:308–317.
9. **Jones, R. N., A. L. Barry, T. L. Gavan, and J. A. Washington II.** 1985. Susceptibility tests: microdilution and macrodilution broth procedures, p. 972–977. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
10. **LeBlanc, D. L., J. M. Inamine, and L. N. Lee.** 1986. Broad geographical distribution of homologous erythromycin, kanamycin, and streptomycin resistance determinants among group D streptococci of human and animal origin. Antimicrob. Agents Chemother. **29**:549–555.
11. **LeBlanc, D. L., L. N. Lee, M. Banai, L. D. Rollins, and J. M. Inamine.** 1987. Dissemination of streptoccal antibiotic resistance determinants in the natural environment, p. 43–53. *In* J. J. Ferretti and R. Curtiss III (ed.), Streptococcal genetics. American Society for Microbiology, Washington, D.C.
12. **Lyon, B. R., and R. Skurray.** 1987. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol. Rev. **51**:88–134.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 382–386. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **McLaughlin, R. W., H. M. Sweeney, and J. C. Rabinowitz.** 1981. Unique features of the ribosomal binding site sequence of the gram-positive *Staphylococcus aureus* β-lactamase gene. J. Biol. Chem. **256**:11283–11291.
15. **Moellering, R. C., Jr., C. Wennersten, and A. N. Weinberg.** 1971. Synergy of penicillin and gentamicin against enterococci. J. Infect. Dis. **124**(Suppl.):S207–S209.
16. **Murray, B. E., D. A. Church, A. Wanger, K. Zscheck, M. E. Levison, M. J. Ingerman, E. Abrutyn, and B. Mederski-Samoraj.**

1986. Comparison of two β-lactamase-producing strains of *Streptococcus faecalis*. Antimicrob. Agents Chemother. **30:** 861–864.

17. **Murray, B. E., and B. D. Mederski-Samoraj.** 1983. Transferable beta-lactamase—a new mechanism for in vitro penicillin resistance in *Streptococcus faecalis*. J. Clin. Invest. **72:**1168–1171.
18. **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.
19. **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.
20. **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.

20a. **Rhinehart, E., N. E. Smith, C. Wennersten, E. Gorss, J. Freeman, G. M. Eliopoulos, R. C. Moellering, and D. A. Goldmann.** N. Engl. J. Med., in press.

21. **Rowland, S. J., and K. G. H. Dyke.** 1989. Characterization of the staphylococcal β-lactamase transposon Tn*552*. EMBO J. **8:**2761–2773.
22. **Schwesinger, M. D., and R. D. Novick.** 1975. Prophage-dependent plasmid integration in *Staphylococcus aureus*. J. Bacteriol. **123:**724–738.
23. **Trieu-Cuot, P., M. Arthur, and P. Courvalin.** 1987. Transfer of genetic information between gram-positive and gram-negative bacteria under natural conditions, p. 65–68. *In* J. J. Ferretti and R. Curtiss III (ed.), Streptococcal genetics. American Society for Microbiology, Washington, D.C.
24. **Washington, J. A., II.** 1985. Susceptibility tests: agar dilution, p. 967–971. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
25. **Weber, D. A., and R. V. Goering.** 1988. Tn*4201*, a β-lactamase transposon in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **32:**1164–1169.
26. **Wilson, K.** 1987. Large-scale CsCl prep of bacterial genomic DNA, p. 2.4.3–2.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Green Publishing Associates, Brooklyn, N.Y.
27. **Zighelboim-Daum, S., C. Wennersten, G. M. Eliopoulos, and R. C. Moellering, Jr.** 1988. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1257.