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Molecular Epidemiology of β-Lactamase-Producing Enterococci

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Plasmids from the first six reported β-lactamase-producing (Bla^+) enterococci were compared for genetic relatedness. Bla^+ enterococcal plasmids from strains isolated in Houston, Tex.; Philadelphia, Pa.; Connecticut; and Pittsburgh, Pa., had heterogeneous *Hae*III and *Msp*I-*Cla*I restriction endonuclease digestion patterns. A staphylococcal β-lactamase probe hybridized to all six Bla^+ enterococcal plasmids, but hybridization was detected on different *Hae*III and *Msp*I-*Cla*I fragments of the six plasmids. An enterococcal gentamicin resistance (Gm^r) probe hybridized to a common 3.9-kilobase *Hae*III fragment from the five Gm^r plasmids. The Houston plasmid was cross-hybridized to the other five strains, and moderate to extensive homology was demonstrated. Bla^+ enterococcal plasmids from a broad geographic range are heterogeneous with respect to size and restriction endonuclease digestion patterns but contain homologous genetic material, including Bla^+ and Gm^r determinants.

β-Lactamase production in enterococci was first reported from Houston, Tex., in 1983 (9) and subsequently from Philadelphia, Pa. (8). Three additional β-lactamase-producing (Bla^+) strains were reported from Connecticut (13, 14), and one was reported from Pittsburgh, Pa. (12). Five of these six strains demonstrate high-level gentamicin resistance (Gm^r) (MIC, ≥2,000 μg/ml). In previous studies, the two Bla^+ enterococcal plasmids from Houston and Philadelphia were shown to hybridize to a staphylococcal β-lactamase probe, suggesting a staphylococcal origin of this trait in enterococci (10).

In this study, we compare the genetic homogeneities and heterogeneities of the six Bla^+ enterococcal plasmids as determined by restriction endonuclease analysis and cross-DNA-hybridization of the Houston Bla^+ plasmid to other enterococcal Bla^+ plasmids. In addition, the plasmids from six strains were hybridized to an *Enterococcus faecalis* high-level Gm^r probe and to a staphylococcal Bla^+ probe to determine homology of resistance determinants.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. faecalis* HH22 was a urine isolate from Hermann Hospital, Houston, Tex., in 1981 (9). *E. faecalis* PA was isolated from blood cultures in Philadelphia, Pa., in 1983 (8). *E. faecalis* WH245, WH257, and WH571 were urine isolates from the West Haven Veterans Administration Hospital, West Haven, Conn., in 1986 and 1987 (13, 14). *E. faecalis* CH570 was isolated from a blood culture at Canonsburg Hospital, Canonsburg, Pa. (near Pittsburgh) (11, 12). Each strain demonstrated Gm^r except WH245 (gentamicin MIC, 12.5 μg/ml). Transconjugants (X) from strains HH22 (67X22) (7); PA (8); and WH245, WH257, and CH570 (12, 13), previously described, contain the plasmids used in this study and are shown in Table 1. A rifampin-resistant, fusidic acid-resistant mutant of enterococcal strain JH2-7 was used as the plasmid-free recipient for strains HH22 (9) and PA (8). A rifampin-resistant, fusidic acid-resistant mutant of enterococcal strain JH2-2 was used as the plasmid-free recipient for filter mating conjugation experiments with WH245, WH257, and CH570 as previously described (12, 13). The JH2-2 recipient was also used for filter mating conjugation experiments with WH571; a transconjugant obtained from these conjugation experiments was used in this study and is shown in Table 1.

Transconjugants contained single plasmids, except XWH245 and XWH571, which still contained multiple plasmids after serial conjugation studies. Plasmids pBEM10 from 67X22, pYN104 from XWH257, and pYN107 from XCH570 were previously characterized. Other antibiotic resistances previously described as transferring with Bla^+ (8, 9, 12, 13) are listed in Table 1.

E. faecalis DNA was obtained by a sodium dodecyl sulfate-lysozyme lysis procedure (12, 16) and ethidium bromide-cesium chloride density centrifugation. Restriction endonucleases *Hae*III, *Msp*I, *Cla*I, and *Eco*RI were used according to the manufacturer's specifications. These enzymes were chosen to best isolate the β-lactamase gene, to generate smaller fragments, and to appreciate the differences in fragment sizes. DNA was analyzed by agarose gel electrophoresis and transferred to nylon membranes (Hybond-N; Amersham, Arlington Heights, Ill.) by alkaline fixation.

The enterococcal Gm^r gene probe pSF815A contained the 1.5-kilobase (kb) *Eco*RI-*Hin*dIII fragment of the gene for bifunctional 6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase from *E. faecalis* plasmid pIP1800 previously described by Ferretti et al. (2). An 840-base-pair *Hin*dIII-*Xba*I fragment of the recombinant plasmid pJM13 (6, 10), previously shown to code for the leader peptide and 80% of the mature staphylococcal β-lactamase, was used to probe for the β-lactamase gene. Plasmid pBEM10 was used for cross-hybridization to the other five strains (7).

Nick translation was performed with [^{32}P]dCTP and cold nucleotides by standard methods (5). ^{32}P-labeled probe DNA was hybridized to blotted DNA under conditions of high stringency followed by high-stringency washes (5, 10).

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TABLE 1. Transconjugants used in this study

E. faecalis transconjugant	Phenotype[a]	Plasmid	Reference
67X22	Bla$^+$ Gm^r	pBEM10	7
XPA	Bla$^+$ Cm^r Gm^r Sm^r		8
XWH245	Bla$^+$ Cm^r Em^r Sm^r Tc^r		13
XWH257	Bla$^+$ Cm^r Em^r Gm^r Sm^r	pYN104	12
XWH571	Bla$^+$ Cm^r Em^r Gm^r Sm^r Tc^r		This study
XCH570	Bla$^+$ Cm^r Em^r Gm^r Sm^r Tc^r	pYN107	12

[a] For aminoglycosides, resistance was defined by an MIC of ≥2,000 μg/ml. Resistance to gentamicin predicts resistance to amikacin, kanamycin, and tobramycin. Abbreviations: Bla$^+$, β-lactamase production; Cm^r, resistance to chloramphenicol; Em^r, resistance to erythromycin; Gm^r, resistance to gentamicin; Sm^r, resistance to streptomycin; Tc^r, resistance to tetracycline.

RESULTS

The transfer frequency of β-lactamase production in strain WH571 using streptomycin or tetracycline for selection ranged from 10^{-8} to 10^{-9}. Transfer frequencies of β-lactamase production in the other five strains have been reported elsewhere (8, 12) and ranged from 10^{-4} to 10^{-10}. A common cryptic plasmid was contained in donor strains WH245, WH257, and WH571 from the West Haven Veterans Administration Hospital (Fig. 1). Donor strains contained from one to five plasmids. Except for XWH245 and XWH571, transconjugants contained single plasmids. Plasmids in Bla$^+$ transconjugants from Houston, Philadelphia, Connecticut, and Pittsburgh strains had heterogeneous patterns using *Hae*III, *Msp*I-*Cla*I, *Eco*RI, and *Hin*dIII restriction endonuclease digestions, although some common fragments were observed. Results of *Hae*III and *Msp*I-*Cla*I digestions are shown in Fig. 2A.

The Gm^r gene probe hybridized to a common *Hae*III fragment of approximately 3.9 kb and to a different *Eco*RI-*Cla*I fragment in the five Gm^r plasmids (data not shown). The Gm^r probe did not hybridize with the Bla$^+$ plasmid from XWH245, which lacks Gm^r. The staphylococcal Bla$^+$ probe hybridized to different *Hae*III and *Msp*I-*Cla*I fragments of Bla$^+$ enterococcal plasmids (Fig. 2B). Hybridization occurred with a 7.5-kb *Hae*III fragment and a 6.6-kb *Msp*I-*Cla*I fragment of the plasmids from XWH245, a 7.5-kb *Hae*III fragment and a 6.2-kb *Msp*I-*Cla*I fragment of pYN107, a 7.0-kb *Hae*III fragment and a 5.8-kb *Msp*I-*Cla*I fragment of pBEM10, a 9.4-kb *Hae*III fragment and a 6.0-kb *Msp*I-*Cla*I fragment of XPA, a 2.7-kb *Hae*III fragment and a 10.1-kb *Msp*I-*Cla*I fragment of pYN104, and a 5.1-kb *Hae*III fragment and a 6.4-kb *Msp*I-*Cla*I fragment of XWH571. Although faint hybridization of the fragment from XWH571 is shown in lanes D′ and K′ of Fig. 2B, repeat studies appeared to show the same degree of hybridization as shown by the other plasmids with the staphylococcal Bla$^+$ probe.

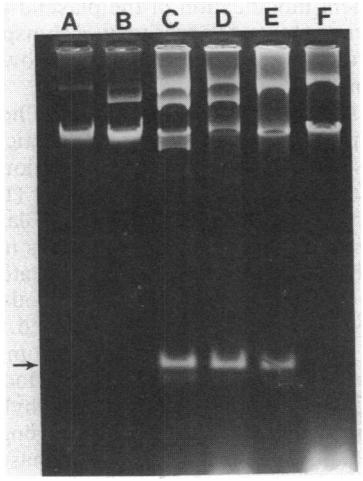

FIG. 1. Agarose gel electrophoresis of plasmid DNA. Lanes: A and B, molecular size standards RP4 (36 megadaltons) and R6K (26 megadaltons), respectively; C, D, and E, plasmids from strains WH245, WH257, and WH571, respectively, isolated at the West Haven Veterans Administration Hospital, West Haven, Conn. (arrow indicates common cryptic plasmid in these three strains); F, pYN107 from strain CH570 isolated at Canonsburg Hospital, Canonsburg, Pa.

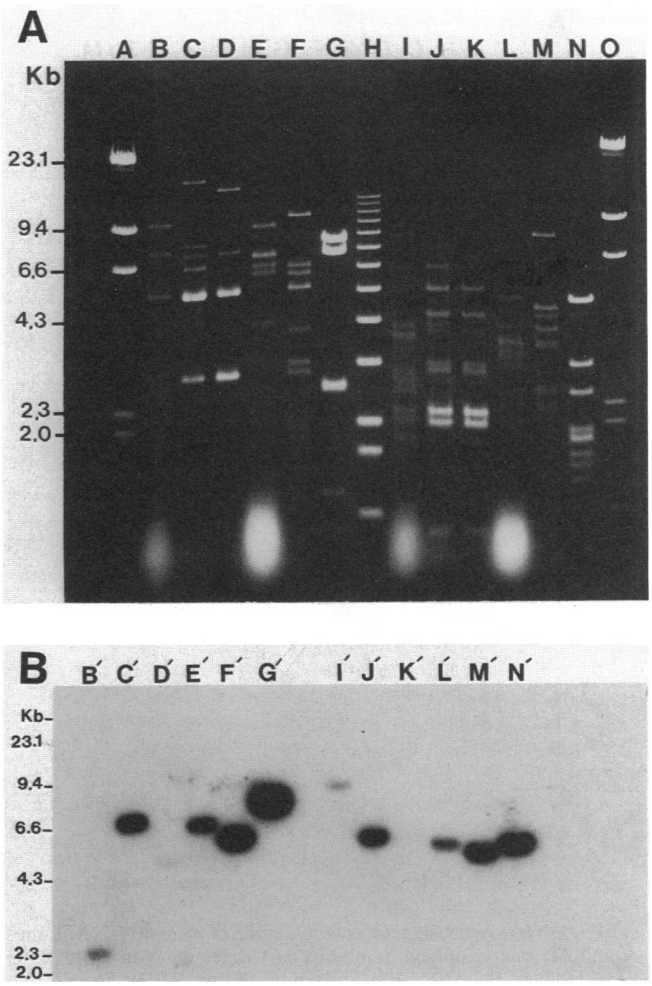

FIG. 2. (A) Agarose gel electrophoresis of plasmid DNA. Lanes: A and O, bacteriophage lambda DNA digested with *Hin*dIII; H, 1-kb ladder; B to G, Bla$^+$ plasmids from XWH257(pYN104), XWH245, XWH571, XCH570(pYN107), 67X22(pBEM10), and XPA, respectively, digested with *Hae*III; I to N, Bla$^+$ plasmids from XWH257(pYN104), XWH245, XWH571, XCH570(pYN107), 67X22(pBEM10), and XPA, respectively, digested with *Msp*I-*Cla*I. (B) Corresponding autoradiograph after hybridization with the staphylococcal β-lactamase gene probe. Lanes: B′ to G′, Bla$^+$ plasmids from XWH257(pYN104), XWH245, XWH571, XCH570 (pYN107), 67X22(pBEM10), and XPA, respectively, digested with *Hae*III; I′ to N′, Bla$^+$ plasmids from XWH257(pYN104), XWH245, XWH571, XCH570(pYN107), 67X22(pBEM10), and XPA, respectively, digested with *Msp*I-*Cla*I.

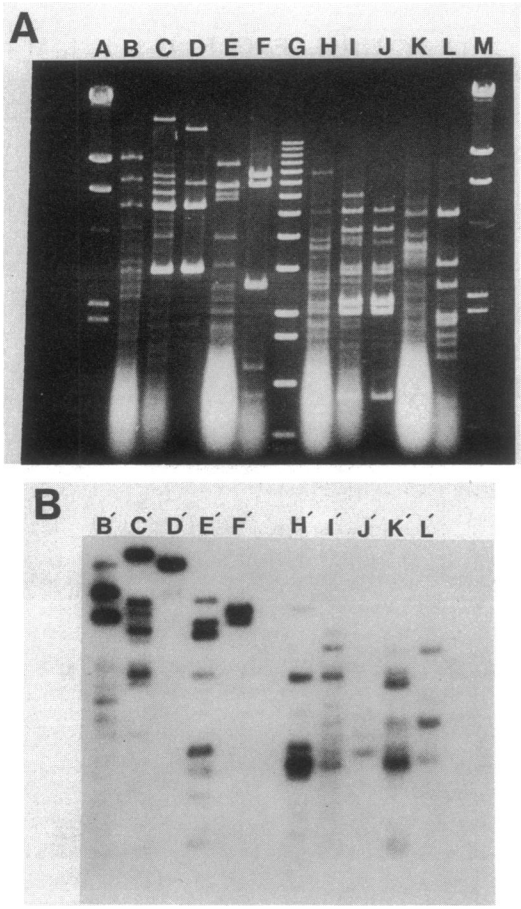

FIG. 3. (A) Agarose gel electrophoresis of plasmid DNA. Lanes: A and M, bacteriophage lambda DNA digested with *Hin*dIII; G, 1-kb ladder; B to F, Bla$^+$ plasmids from XWH257(pYN104), XWH245, XWH571, XCH570(pYN107), and XPA, respectively, digested with *Hae*III; H to L, Bla$^+$ plasmids from XWH257 (pYN104), XWH245, XWH571, XCH570(pYN107), and XPA, respectively, digested with *Msp*I-*Cla*I. (B) Corresponding autoradiograph after DNA-DNA hybridization with pBEM10. Lanes: B′ to F′, Bla$^+$ plasmids from XWH257(pYN104), XWH245, XWH571, XCH570(pYN107), and XPA, respectively, digested with *Hae*III; H′ to L′, Bla$^+$ plasmids from strains XWH257(pYN104), XWH245, XWH571, XCH570(pYN107), and XPA, respectively, digested with *Msp*I-*Cla*I.

When pBEM10 was labeled and hybridized with the other Bla$^+$ plasmids, between two and eight fragments of the other Bla$^+$ enterococcal plasmids showed moderate to extensive homology (Fig. 3). The most extensive homology was seen with pYN104, XWH245, and pYN107. Moderate homology was seen with XWH571 and XPA.

DISCUSSION

The first report of β-lactamase production in an enterococcus from Houston, Tex. (9), was followed by reports from different geographic areas, including Philadelphia, Pa. (8); Connecticut (11, 12); Pittsburgh, Pa. (13); and now Boston, Mass. (E. Rhinehart, C. Wennersten, E. Gorss, G. Eliopoulos, R. Moellering, N. Smith, and D. Goldmann, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1073, 1988). Because this trait has been transferable in each strain studied so far, the prevalence of these isolates is expected to increase. β-Lactamase production is transferred by Gm^r plasmids in most reported strains; the prevalence of plasmid-mediated Gm^r has increased markedly over the past decade and is now a global problem (3, 12).

The Houston and Philadelphia strains had previously been shown to hybridize with a specific staphylococcal β-lactamase probe (10), suggesting a staphylococcal origin of the enterococcal β-lactamase. In this study, the staphylococcal β-lactamase probe hybridized to each of the enterococcal plasmids from other geographic areas, further suggesting staphylococcal gene exchange. The five enterococcal plasmids expressing Gm^r contained homologous determinants, also suggesting a common origin of this resistance in these enterococci. Previous studies have shown that Gm^r in other enterococcal strains is determined by a fusion gene (6′-aminoglycoside acetyltransferase 2″-aminoglycoside phosphotransferase) which mediates gentamicin, tobramycin, kanamycin, and amikacin resistance (2). High-level streptomycin resistance may be transferred separately (4, 12). This Gm^r pattern was the phenotype of the five gentamicin-resistant strains, suggesting the presence of the fusion gene in these strains as well. Although Gm^r plasmids from different geographic areas appear to be physically distinct in this study and others (1, 8, 12), the Gm^r determinant in our strains appears to be the same.

Although Bla$^+$ enterococcal plasmids are heterogeneous by restriction endonuclease analysis, there is moderate to extensive cross-hybridization of strains from Philadelphia, Pittsburgh, and Connecticut with the Houston plasmid. This degree of homology cannot be explained on the basis of other common resistance determinants, such as erythromycin and tetracycline resistance determinants, since the plasmid used for cross-hybridization mediated only β-lactamase production and Gm^r. Thus, common cryptic genetic material other than the enterococcal resistance determinants appears to be contained in these plasmids.

Although a stable clonal plasmid was not demonstrated in these strains, the homologous plasmid fragments suggest that plasmid dissemination could have occurred initially, with subsequent modification of the plasmid in each unique selective environment. Alternatively, a transposon could be responsible for dissemination; there is a known β-lactamase transposon in *Staphylococcus aureus* (15).

The three strains isolated from the same hospital contain common cryptic plasmids, suggesting genetic exchange between these strains. Nosocomial dissemination of enterococcal Gm^r plasmids has been shown to occur (17).

The molecular epidemiology of these Bla$^+$ enterococci demonstrates that a single clonal plasmid is not responsible for the broad geographic range of these isolates; however, a moderate to extensive amount of homologous plasmid content suggests that the plasmids are related. Enterococcal β-lactamase determinants in plasmids from diverse geographic areas are homologous to a staphylococcal β-lactamase determinant, further suggesting a staphylococcal origin of this trait in enterococci over a broad geographic range. Gm^r determinants in enterococcal β-lactamase plasmids are homologous to the previously described fusion gene mediating gentamicin, tobramycin, kanamycin, and amikacin resistance in enterococci, a gene also found in staphylococci.

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