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Comparison of the Gentamicin Resistance Transposon Tn*5281* with Regions Encoding Gentamicin Resistance in *Enterococcus faecalis* Isolates from Diverse Geographic Locations

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The genetic determinant encoding gentamicin resistance (Gm^r) on the β-lactamase encoding plasmid pBEM10 of *Enterococcus faecalis* HH22 is carried on a transposon, termed Tn*5281*, that is highly related to the staphylococcal Gm^r transposons Tn*4001* found in Australian isolates of *Staphylococcus aureus* and Tn*4031* found in United States isolates of *Staphylococcus epidermidis*. We have now studied plasmid DNA from Gm^r strains of *E. faecalis* isolated from diverse geographical locations (Houston, Pennsylvania, Thailand, and Chile) by using restriction endonuclease analysis and DNA-DNA hybridization to determine whether other Gm^r *E. faecalis* carry Tn*5281* or a similar type of element. We also compared these enterococci to several United States isolates of *Staphylococcus aureus* with nonmobile Gm^r determinants. Three *E. faecalis* isolates (from Houston and Chile) carried Tn*5281*-like elements, whereas two isolates (from Houston and Pennsylvania) had restriction endonuclease and DNA-DNA hybridization patterns more similar to those of the Tn*4001*-IS*257* hybrid found in the nonmobile Gm^r determinants in United States isolates of *S. aureus*. A strain from Thailand had a third pattern unrelated to either Tn*5281* or the nonmobile Gm^r determinants present in United States isolates of *S. aureus*. Our results demonstrate that there is both similarity and diversity between the Gm^r determinant of strains of *E. faecalis* isolated in diverse geographic locations.

Enterococcus faecalis with high-level gentamicin resistance (Gm^r) (MIC, ≥2,000 μg/ml) was first reported in France in 1979 (14). Four years later, nine clinical isolates of *E. faecalis* from Houston, Texas, were reported to have high-level resistance to gentamicin and all other commercially available aminoglycosides, much of which was shown to be plasmid encoded (19). Since these initial reports, strains of *E. faecalis* with high-level Gm^r have been reported from a diversity of geographical locations, including Thailand, Chile, England, Italy, and throughout the United States (5, 22). High-level resistance eliminates the usefulness of synergistic treatment of enterococcal endocarditis with a combination of penicillin and a modified aminoglycoside.

An identical bifunctional aminoglycoside-modifying enzyme having 6′-acetyltransferase [AAC(6′)] and 2″-phosphotransferase [APH(2″)] activities is the cause of Gm^r in both *Staphylococcus aureus* and *E. faecalis* and also causes high-level resistance to and/or resistance to synergism with tobramycin, kanamycin, amikacin, sisomicin, and netilmicin (5, 7, 10, 18, 19, 23). Genetic determinants encoding this bifunctional enzyme are known to be carried on transposons in some Australian isolates of *S. aureus* (Tn*4001*) and United States isolates of *Staphylococcus epidermidis* (Tn*4031*) (17, 25). We recently have shown that a Gm^r transposon (Tn*5281*) also is present in *E. faecalis* on the conjugative Gm^r β-lactamase plasmid, pBEM10, located in the Houston isolate HH22 (12, 13). All three transposons (Tn*4001*, Tn*4031*, and Tn*5281*) are similar, if not identical, composite transposons composed of a 2.0-kb unique region encoding the *aacA-aphD* that is bounded on each side by insertion sequences 1.35 kb in length (IS*256* in Tn*4001*) for a total length of 4.7 kb. These elements contain symmetrically located *Hin*dIII (2.5 kb apart), *Cla*I (slightly greater than 2.5 kb apart), and *Hae*III (3.9 kb apart) sites (13, 16, 25). All three transposons are known to move in a *rec*-independent manner (13, 17, 25).

North American isolates of Gm^r *S. aureus* share some homology with Tn*4001*, Tn*4031*, and Tn*5281*, as they contain a 2.5-kb *Hin*dIII fragment that hybridizes with Tn*4001*; however, they lack the *Hae*III sites located 3.9 kb apart and instead have symmetrically located *Bgl*II sites (~3.15 kb apart) not found in the three Gm^r transposons (16). Heteroduplex analysis showed that inverted repeats were present at the termini of the region surrounding the Gm^r determinants in the North American *S. aureus*; however, these termini were shorter than those present in Tn*4001* (16). Nucleotide sequencing data revealed that the region encoding Gm^r in the North American isolates of *S. aureus* is bounded on each side by 425 bp of the IS*256* elements with both sides missing the external 900 bp of IS*256* (3). In place of the external portion of IS*256*, these isolates have three copies of a commonly occurring staphylococcal insertion sequence, IS*257* (3). Studies have shown that these Tn*4001*-IS*257* hybrids are transposition deficient (25).

In this study, we compared Tn*5281* located on pBEM10 in *E. faecalis* HH22 with other Gm^r isolates of *E. faecalis* from Houston, Pennsylvania, Chile, and Thailand. Using restriction endonuclease analysis and DNA-DNA hybridization with three probes (from the Gm^r gene, from IS*256*, and from IS*257*), we investigated the relationship of other Gm^r enterococci to Tn*5281* and the North American isolates of *S. aureus* that have nonmobile Gm^r determinants.

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TABLE 1. Bacterial strains and plasmids utilized

Strain or plasmid	Relevant plasmid markers[a]	Plasmid size (kb)	Original host and/or origin (reference)	Known control transposons or IS elements
E. faecalis				
pBEM10 (host JH2-2)	Gm^r Bla$^+$ Tra$^+$	~70	HH22, Houston (19, 20)	Tn*5281*
X-Penn (host JH2-7)	Gm^r Bla$^+$ Tra$^+$	~70	Penn, Pennsylvania (15, 21)	
X-H181 (host JH2-7)	Gm^r Tra$^+$	>70	H181, Houston (19)	
X-H197 (host JH2-7)	Gm^r Tra$^+$	>70	H197, Houston (19)	
X-Th133 (host JH2-7)	Gm^r Tra$^+$	>70	Th133, Thailand (22)	
CE13	Gm^r	>70	CE13, Chile (22)	
X-CE30 (host JH2-2)	Gm^r Tra$^+$	>70	CE30, Chile (22)	
S. aureus				
L3711(pL3711)	Gm^r Tra$^+$	28	United States (6)	Tn*4001*-IS*257* hybrid
CRG1600(pCRG1600)	Gm^r Bla$^+$ Tra$^+$	52.9	United States (11)	Tn*4001*-IS*257* hybrid
G5(pGO5)	Gm^r Bla$^+$ Tra$^+$	55.2	United States (1)	Tn*4001*-IS*257* hybrid
E. coli clones				
SF815A(pSF815A)	Gm^r Amp^r	4.2	United States (10)	
GO137(pGO137)	Gm^r	6.7	United States (24)	
GO121(pGO121)[b]	Gm^r	14	United States (25)	Tn*4031* with IS*256*
GO156(pGO156)	Amp^r Tet^r	4.6	United States	IS*257*[c]

[a] Abbreviations: Amp, resistance to ampicillin; Gm, gentamicin; Tet, tetracycline; Bla, β-lactamase; and Tra, transfer function.
[b] Contains a 7.0-kb *Bgl*II fragment from *S. epidermidis* chromosome that contains Tn*4031*.
[c] Also known as IS*431*.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and their plasmids utilized in this study are listed in Table 1. Gm^r *E. faecalis* strains were isolated in Houston, Tex.; Pennsylvania; Thailand; and Chile; in most instances, the plasmids were isolated from transconjugants derived from matings with JH2-2 or JH2-7 (9, 19, 20, 22). All three *S. aureus* strains used were clinical isolates from the United States which contain large conjugative plasmids that confer resistance to gentamicin. Two of these plasmids, pCRG1600 and pGO5, also carry the gene encoding β-lactamase production, while *S. aureus* strain L3711 has a β-lactamase gene residing on the chromosome (26). The United States isolates of Gm^r *S. aureus* were kindly provided by M. L. Cohen (L3711) (6), R. V. Goering (CRG1600) (11), and G. L. Archer (G5) (1). Archer also provided the probe strains GO156 and GO121. All bacterial strains were grown at 37°C. Gentamicin concentrations used were 1,000 μg/ml for *E. faecalis* and 10 μg/ml for both *Escherichia coli* and *S. aureus*.

DNA collection, restriction endonuclease analysis, and gel electrophoresis. Plasmid DNA was collected by using the following methods: (i) modification for gram-positive bacteria of the Currier and Nester (8) cell lysis procedure for enterococci (26), (ii) modification of the Birnboim and Doly (2) alkaline lysis technique for *E. coli*, and (iii) an ethanol-acetone-lysostaphin technique for staphylococci (1). All enterococcal and staphylococcal plasmid DNA was separated from chromosomal DNA by cesium chloride-ethidium bromide density gradient centrifugation. Restriction endonucleases were utilized according to the supplier's (Bethesda Research Laboratories, Gaithersburg, Md.) recommendations, except digestions were allowed to proceed for up to 5 h. Separation of fragments generated by restriction digestion was done on agarose gels (0.7%) by using electrophoresis at 40V for 8 to 15 h. The gel and running buffer was 1× TBE (10× TBE is 1 M Tris-base, 0.89 M boric acid, and 0.5 mM EDTA [pH 8.0]).

Southern blotting and hybridization studies. DNA fragments were transferred from agarose gels to Hybond-N membrane filters (Amersham Corp., Arlington Heights, Ill.) according to the manufacturer's recommendations. The 1.5-kb *Eco*RI-*Hin*dIII fragment of pSF815A containing the *aacA-aphD* coding region was used as the Gm^r gene probe, as previously described (10, 12) (Fig. 1). A 1-kb *Hin*dIII-*Cla*I fragment of pGO121 containing the outer portion of IS*256* located in Tn*4031* was used as a probe for the IS*256* element (Fig. 1); according to Thomas and Archer, the *Hin*dIII site (not shown on Fig. 1) lies just outside of the left terminus of Tn*4031* (25). The *Cla*I site is located 986 bp interior to the left terminus of Tn*4031* and is within the IS*256* element (4). A 250-bp *Hin*dIII fragment from pGO156 was used as a probe

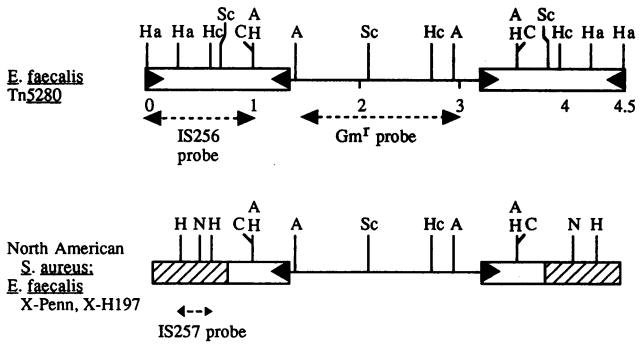

FIG. 1. Examples of gentamicin resistance elements and probes used. The top line shows Tn*5280* (13). The lower element is typical of Gm^r North American *S. aureus* and some enterococci (3, 16, 25). The clear rectangles represent IS*256* regions, and the hatched rectangles represent IS*257*. *Bgl*II sites (not shown), ~3.0 kb apart, are symmetrically located within the IS*257* elements, just outside the truncated IS*256* regions (3, 16). Restriction sites: A, *Alu*I; C, *Cla*I; H, *Hin*dIII; Ha, *Hae*III; Hc, *Hin*cII; N, *Nde*I; Sc, *Sca*I.

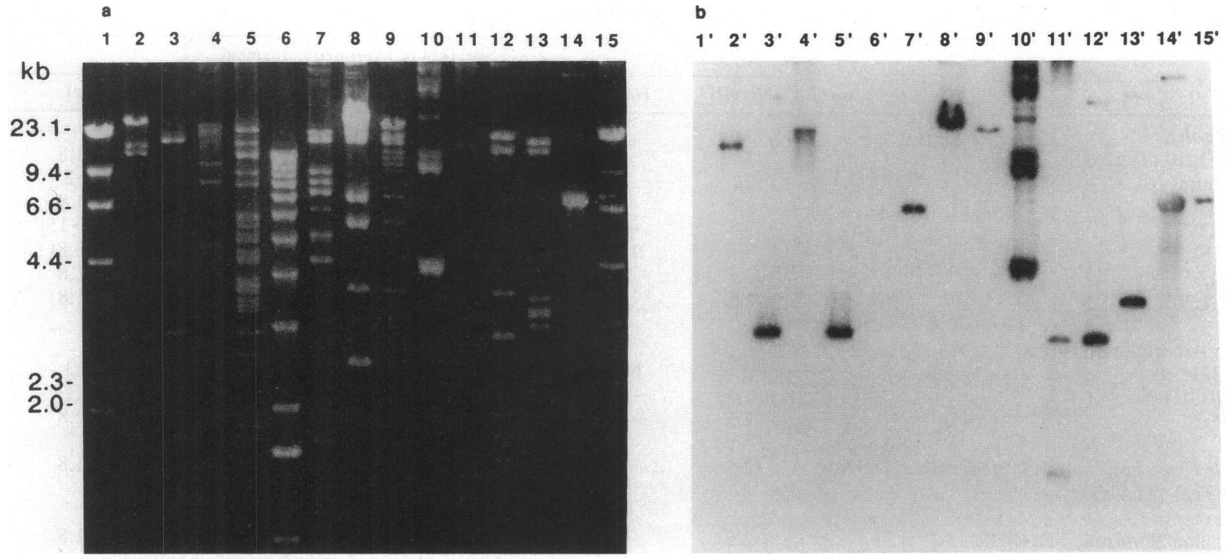

FIG. 2. (a) Agarose (0.7%) gel electrophoresis of restriction endonuclease digestions with *Bgl*II of pBEM10 (lane 2), X-Penn (lane 3), X-H181 (lane 4), X-H197 (lane 5), X-Th133 (lane 7), CE13 plasmid (lane 8), X-CE30 (lane 9), pGO137 (partially digested) (lane 10), pL3711 (lane 11), pCRG1600 (lane 12), pGO5 (lane 13), and pGO121 (lane 14). Molecular mass standards: lambda DNA digested with *Hin*dIII (lanes 1, 15) (lane 15 appears to have overflow from lane 14) and 1-kb ladder (lane 6). (b) Autoradiograph of filter of agarose gel on the left following hybridization with the ^{32}P-labeled Gm^r gene probe.

for the staphylococcal insertion sequence IS*431* (IS*257*) (Fig. 1). This fragment contains the region from nucleotide 259 to 501 on the IS*257* map (4). The probes were labeled by using the Random Primed DNA Labeling kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and [α-^{32}P]dCTP (Amersham). Prehybridization and hybridization solutions were as suggested by Amersham (5× SSPE [0.9 M NaCl, 5 mM EDTA, 50 mM $NaPO_4$], 5× Denhardt solution, 0.5% sodium dodecyl sulfate, 50% formamide, and 100 μg of denatured calf thymus DNA per ml). Hybridization was done under highly stringent conditions at 42°C as described in the Hybond-N protocol (Amersham).

RESULTS AND DISCUSSION

Restriction analysis of Gm^r plasmids from *E. faecalis*. *Hin*dIII digestion of the Gm^r plasmids revealed the presence of a 2.5-kb fragment common to all Gm^r isolates of *E. faecalis* from Houston, Pennsylvania, Thailand, and Chile. All 2.5-kb *Hin*dIII fragments hybridized with the Gm^r gene probe (Table 1; data not shown). All isolates also had a 1.5-kb *Alu*I fragment that hybridized with this probe, suggesting that all of these enterococci contain the same *aacA-aphD* coding region known to be located on a 1.5-kb *Alu*I fragment in the *E. faecalis* plasmid pIP800 (10). The *Alu*I sites are known to lie just exterior to the *aacA-aphD* coding region in pIP800.

*Hae*III digestion of plasmid DNA from Gm^r *E. faecalis* revealed that pBEM10 (containing Tn*5281*), X-H181 (Houston), CE13 (Chile), and X-CE30 (Chile) had a 3.9-kb fragment that hybridized with the Gm^r gene probe; this suggests that in these strains, Gm^r may reside on a transposon similar to Tn*5281*. However, larger *Hae*III fragments from X-Penn (Pennsylvania), X-H197 (Houston), and X-Th133 (Thailand) hybridized, suggesting that not all Gm^r *E. faecalis* contain a Tn*5281*-like transposon.

Since Tn*5281* does not have any *Bgl*II sites and the North American isolates of Gm^r *S. aureus* are reported to have symmetrically located *Bgl*II sites (3.15 kb apart), *Bgl*II was used to analyze the *E. faecalis* plasmid DNA. *Bgl*II digests of enterococcal plasmid DNA are shown in Fig. 2 (lanes 2 to 5 and 7 to 9). Both X-Penn and X-H197 have a *Bgl*II fragment about 3.0 kb in size (Fig. 2a, lanes 3 and 5), while none of the other enterococcal plasmids have a *Bgl*II fragment of this approximate size. Figure 2b shows that the *aacA-aphD* coding region is located on an ~3.0-kb fragment in both X-Penn and X-H197, as these fragments hybridized with the Gm^r gene probe, while the coding region was located on larger *Bgl*II fragments in the remaining plasmids.

Hybridization data obtained by using the Gm^r gene probe are summarized in Table 2. The Gm^r determinant in pBEM10 is known to reside on Tn*5281*, with the plasmids from *E. faecalis* X-H181, CE13, and X-CE30 all having restriction endonuclease digestion and DNA-DNA hybridization patterns indicating that their Gm^r determinants reside on Tn*5281*-like elements. It is not known whether these putative Tn*5281*-like elements are mobile. These *E. faecalis* strains were isolated in Houston and Chile, which suggests that Tn*5281*-like transposons can be found in diverse geographical locations in *E. faecalis*. However, our data also suggest that Gm^r determinants in *E. faecalis* are not always carried on Tn*5281*-like elements, since the Gm^r determinants in X-Penn, X-H197, and X-Th133 have restriction endonuclease digestion and DNA-DNA hybridization patterns different from those expected for Tn*5281*-like elements. Both X-Penn (from Pennsylvania) and X-H197 (from Houston) have similar patterns, whereas the Thai isolate (X-Th133) appears to have a pattern unrelated to the other enterococcal Gm^r patterns.

Comparison of enterococcal and staphylococcal Gm^r plasmids. Since the enterococcal transposon, Tn*5281*, previously had been shown to be like the staphylococcal transposons, Tn*4001* and Tn*4031*, and since the restriction and hybridization data obtained for some of the enterococcal strains appeared to correspond to those published for the North American isolates of Gm^r *S. aureus*, we investigated the

TABLE 2. Summary of hybridization with the Gm^r gene probe

Plasmid or strain	Size (kb) of hybridizing fragment						
	*Hin*dIII	*Hae*III	*Bgl*II	*Cla*I	*Hin*cII	*Sca*I	*Alu*I
E. faecalis							
pBEM10 (Tn*5281*)	2.5	3.9	>15	2.5	1.2, 2.2	1.5, 1.8	1.5
X-Penn	2.5	7.5	3.0	2.5	2.4 (6.6)[a]	4.6, 12	1.5
X-H181	2.5	3.9	>15	2.5	1.2, 2.2	1.5, 1.8	1.5
X-H197	2.5	~9	3.0	2.5	2.4 (9.4)	7.0 (>15)	1.5
X-Th133	2.5	9–10	6	3.0	2.2 (5.5)	1.5 (6.8)	1.5
CE13	2.5	3.9	>15	2.5	1.2, 2.2	1.5, 1.8	1.5
X-CE30	2.5	3.9	>15	2.5	1.2, 2.2	1.5, 1.8	1.5
North American *S. aureus*							
pL3711	2.5	ND[b]	1.4, 3.0	1.2, 2.5	2.4	2.8, 23	ND
pCRG1600	2.5	~12	3.0	2.5	1.8, 2.4	2.8, 11	ND
pGO5	2.5	~11	3.5	2.5	1.8, 4.4	2.3, 2.8	ND
E. coli clone containing *S. epidermidis* DNA, pGO121 (Tn*4031*)	2.5	3.9	7.0	2.5	1.2, 2.2	1.5, 1.8	1.5
Australian *S. aureus*, Tn*4001*[c]	2.5	3.9	~17	2.5	1.2, 2.2	1.5, 1.8	1.5

[a] Fragment sizes listed in parenthesis appeared to weakly hybridize with the Gm gene probe.
[b] ND, not determined.
[c] Tn*4001* fragments listed as showing hybridization were deduced from the published nucleotide sequence (4, 10, 23).

relatedness of regions encoding Gm^r in *E. faecalis* strains isolated in diverse geographical locations, several Gm^r North American *S. aureus*, and Tn*4031* from *S. epidermidis* by using restriction endonucleases and DNA-DNA hybridization analysis. Restriction enzymes were chosen on the basis of the known nucleotide sequence of Tn*4001* from *S. aureus* (4, 23). Table 2 summarizes the hybridization data for the isolates studied and expected hybridization results for Tn*4001* (*S. aureus*) based on published restriction maps and the nucleotide sequence (4, 23).

As with the enterococci, the staphylococcal Gm^r plasmids also contained a 2.5-kb *Hin*dIII fragment that hybridized with the Gm^r gene probe. The three Gm^r transposons (Tn*4001*, Tn*4031*, and Tn*5281*) are known to contain symmetrical *Cla*I sites located 2.5 kb apart, positioned just 15 bp outside of the symmetrical *Hin*dIII sites, and all but one (X-Th133) of the enterococcal and staphylococcal Gm^r isolates tested had a 2.5-kb *Cla*I fragment that hybridized with the Gm^r gene probe. The *S. aureus* plasmids pL3711 and pCRG1600, like X-Penn and X-H197, have an ~3.0-kb *Bgl*II fragment that hybridized with the Gm^r gene probe (Fig. 2, lanes 11 and 12). The 3.0-kb *Bgl*II fragment is slightly smaller than the 3.15-kb fragment reported by Lyon and coworkers (16); however, the size of the *Bgl*II fragment observed in these two *E. faecalis* plasmids was the same as that seen in the United States *S. aureus* isolates pL3711 and pCRG1600 (Fig. 2 and Table 2) and the same size as the sequence reported by Byrne et al. (4).

On the basis of the nucleotide sequence of Tn*4001*, two *Hin*cII fragments (1.2 and 2.2 kb) should hybridize with the Gm^r gene probe if the regions surrounding the Gm^r determinants are related to Tn*4001*-like transposons. This hybridization pattern was seen with Tn*4031* and all but three *E. faecalis* isolates (X-Penn, X-H197, and X-Th133). In addition, none of the United States isolates of *S. aureus* had *Hin*cII fragments of the size expected for Tn*4001*-like elements (Table 2). Similar data were obtained by using *Sca*I, which, for Tn*4001*-like elements, should show hybridization of two fragments (1.5 and 1.8 kb). Again, the same three *E. faecalis* isolates (X-Penn, X-H197, and X-Th133) and the United States isolates of *S. aureus* all lacked the Tn*4001*-like pattern.

Hybridization studies with the IS*256* probe. According to sequencing data, Tn*4001* is essentially composed of a 3.9-kb *Hae*III fragment bounded by 0.3-kb *Hae*III fragments (4, 23); both related elements, Tn*4031* and Tn*5281*, generate the same restriction pattern (13, 25). The 3.9-kb *Hae*III fragment contains the Gm^r gene and about 1.3 kb of the surrounding IS*256* elements, whereas the 0.3-kb *Hae*III fragment consists entirely of IS*256* sequences. Therefore, a probe specific for a 1-kb region of the IS*256* element containing the terminal domain of Tn*4031* starting at the *Cla*I site and heading away from the Gm^r coding region was used to determine whether any of the Gm^r isolates had the 0.3-kb *Hae*III fragments that lie at the termini of related transposons. Hybridization showed that 0.3-kb *Hae*III fragments from *E. faecalis* pBEM10, X-H181, CE13, and X-CE30 plasmid DNA hybridized with the probe (data not shown), as did the 3.9-kb *Hae*III fragment containing the Gm^r gene. Presence of the IS*256* element adjacent to the Gm^r coding region in these plasmids is further evidence that they contain a Tn*4001*-like element. The Gm^r plasmids from three isolates of *E. faecalis* (X-Penn, X-H197, and X-Th133) had fragments of >3.9 kb that hybridized and lacked the 0.3-kb *Hae*III fragment, confirming earlier observations that the Gm^r determinants in these plasmids are not located on a Tn*4001*-like element. Since multiple copies of IS*256* are present on both staphylococcal plasmid and chromosomal DNA without the presence of Tn*4001*, several restriction fragments generated by *Hae*III digestion of the *S. aureus* plasmid DNA hybridized with the IS*256* probe; however, none yielded a Tn*4001*-like pattern (data not shown).

Hybridization studies with the IS*257* probe. Byrne and coworkers have reported that the region encoding the Gm^r gene in the North American isolates of *S. aureus* contains only partial copies of IS*256* and have three intact IS*257* elements in the adjoining region, creating a Tn*4001*-IS*257* hybrid (3). Since three Gm^r isolates of *E. faecalis* apparently do not contain Tn*4001*-like elements and because two of these isolates have ~3.0-kb *Bgl*II fragments, we used a

TABLE 3. Comparison of fragments hybridizing with Gm^r gene probe and IS*257* probe

Plasmid or strain	Size (kb) of hybridization fragments					
	*Hae*III		*Sca*I		*Nde*I	
	Gm^r probe	IS*257* probe	Gm^r probe	IS*257* probe	Gm^r probe	IS*257* probe
E. faecalis						
pBEM10	3.9	~10	1.5, 1.8	~21	>15	>15
X-Penn	**7.5**[a]	**7.5**	**4.6, 12**	**4.6, 12**	**3.5**	1.5, 1.9, **3.5**
X-H181	3.9	None	1.5, 1.8	None	9.4	None
X-H197	**9.4**	**9.4**	**7 (>15)**[b]	**7 (>15)**[b]	**3.5**	1, 2.8, **3.5**, 5, 6.6
S. aureus						
pL3711	ND[c]	ND	**2.8, 23**	**2.8**, 4.4, ~21, **23**	**3.5**	1.9, 2.8, **3.5**, 3.9, 7.5
pCRG1600	~12	ND	**2.8, ~11**	**2.8**, 4.4, **~11**, >15	**3.5**	1.9, 2.1, 2.8, **3.5**, 3.9, 7.5
pGO5	~11	ND	**2.3, 2.8**	**2.3, 2.8**, >15, >23	**3.5**	0.8, 1.9, 2.8, **3.5**, 3.9, 7.5
E. coli clone containing *S. epidermidis* DNA, pGO121	3.9	ND	1.5, 1.8	4.0, 7.0	6	1.9, 4.4, 6

[a] Bold numbers indicate the same fragment hybridized with both probes.
[b] There were partial digests present when *Sca*I was used.
[c] ND, not determined.

probe specific for a 250-bp internal segment of IS*257* (IS*431*) to determine whether any of the enterococcal plasmids contained copies of IS*257* in the area surrounding the Gm^r gene. IS*257* (IS*431*) is a commonly occurring insertion sequence that is known to be present in multiple copies on the staphylococcal chromosome and some staphylococcal plasmids. Byrne et al. (3) have sequenced all three IS*257* elements from a Tn*4001*-IS*257* hybrid and have shown that the three elements are not identical to each other or to other IS*257* elements that have been sequenced (89 to 97% homology). Since the sequences are varied, the three copies of IS*257* present in a Tn*4001*-IS*257* hybrid do not generate the same restriction endonuclease pattern. Our initial hybridization data were obtained by using plasmids digested with restriction endonucleases having recognition sites within the Gm^r coding region known to be the same in both the enterococci and staphylococci. Table 3 shows a comparison between the hybridization data obtained by using the Gm^r probe and the IS*257* probe for several enterococcal and staphylococcal isolates. Fragments hybridizing with both probes suggested that the Gm^r gene and at least a portion of the IS*257* element were located close to each other but did not indicate whether the regions were contiguous. Both X-Penn and X-H197 had *Hae*III and *Sca*I fragments that hybridized with both probes, whereas different fragments of pBEM10 (containing Tn*5281*) hybridized with the probes. On the basis of probe data, it appears that X-Penn and X-H197 each contain at least two copies of IS*257*, one on each side of the Gm^r gene. Although hybridization to the IS*257* probe indicates that pBEM10 contains at least part of an IS*257* element, it does not appear to be adjoining Tn*5281*, the transposon carrying the Gm^r determinant in this plasmid.

Analysis of the nucleotide sequence data available for the different IS*257* elements revealed that all of the IS*257* elements contained a unique *Nde*I recognition site (3). *Nde*I digestion of Tn*4001*-IS*257* hybrids should generate a 3.5-kb fragment that would contain the Gm^r gene, the two residual remnants of IS*256* (each 425 bp in length), and two copies of part of IS*257*. If the enterococcal Gm^r determinants are located on a Tn*4001*-IS*257* hybrid, they would be expected to generate a 3.5-kb *Nde*I fragment that would hybridize with both the Gm^r gene probe and the IS*257* probe. Our hybridization data suggest that two enterococcal isolates, X-Penn and X-H197, contain a Tn*4001*-IS*257* hybrid, as each yielded the expected hybridization results (Table 3). None of the other enterococcal isolates appear to contain a Tn*4001*-IS*257* hybrid; however, three of the plasmids (pBEM10, CE13, and X-CE30) contain both a Tn*4001*-like element and at least part of IS*257*. Therefore, it appears that the commonly occurring staphylococcal insertion element (IS*257*) also may be common in enterococci; however, IS*257* was not found on enterococcal plasmids in multiple copies as it is frequently found in staphylococci.

In conclusion, the Gm^r determinants in various *E. faecalis* isolates from diverse geographic locations share some similarities (common patterns for *Hin*dIII, *Cla*I, and *Alu*I) but also are divergent from each other. The Gm^r determinants in three isolates of *E. faecalis* (H181 from Houston and CE13 and CE30 from Chile) were shown to be located on a region similar to Tn*5281* which in turn is similar, if not identical, to the staphylococcal transposons Tn*4001* and Tn*4031*. The Gm^r determinants in two strains of *E. faecalis* (Penn from Pennsylvania and H197 from Houston) have restriction endonuclease and DNA-DNA hybridization patterns more similar to those of the nonmobile Gm^r determinants in North American isolates of *S. aureus* and apparently contain a Tn*4001*-IS*257* hybrid. A third pattern also exists, as an isolate from Thailand (Th133) was found to differ in the region surrounding the Gm^r gene from either of the other two patterns.

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