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Letters to the Editor

Plasmid-Mediated Coresistance to Streptogramins and Vancomycin in *Enterococcus faecium* HM1032

Enterococcus faecium has emerged as a major cause of nosocomial infections. Correspondingly, this species has become increasingly resistant to a broad range of antimicrobial agents, including aminoglycosides, penicillins, and glycopeptides (4). Quinupristin-dalfopristin, an injectable streptogramin, is proposed as an alternative drug for severe infections caused by multiply resistant *E. faecium* and in many cases could represent the only therapy available (3).

We have isolated from urine a strain of *E. faecium*, HM1032, which was resistant to both vancomycin and quinupristin-dalfopristin, and we have studied the localization of the genetic determinants for these resistances. *E. faecium* HM1032 was resistant to erythromycin (MIC > 128 μg/ml), quinupristin-dalfopristin (MIC = 16 μg/ml), vancomycin (MIC >128 μg/ml), and tetracycline. We found that resistance to macrolides and vancomycin was related to the presence of an *ermAM* (*ermB*)-like gene and a *vanA* gene, respectively, as shown by PCR experiments (2, 6). Resistance to quinupristin-dalfopristin was due to inactivation of quinupristin and dalfopristin (MICs = 64 μg/ml), as suggested by a microbiological screen test, Gots' test, and confirmed in PCR experiments by amplification of *vgb*- and *satA*-like genes responsible for acetylation and hydrolysis of quinupristin and dalfopristin, respectively. The nucleotide sequences of amplicons were nearly identical to those of the prototype *satA* and *vgb* genes (1, 5).

The resistances could not be transferred by mating on filters to *E. faecium* HM1070, a plasmid-free recipient strain, except for tetracycline resistance, which transferred with a frequency of 10^{-6} per donor colony. Transformation of *E. faecium* HM1070 with plasmid extracts of *E. faecium* HM1032 yielded clones coresistant to vancomycin, quinupristin-dalfopristin (MIC = 8 μg/ml), and erythromycin. However, the transformants inactivated dalfopristin but not quinupristin. One of the transformants, *E. faecium* HM1070SR, was studied further, and PCR experiments indicated that the strain contained the *vanA*, *ermAM*, and *satA* genes but not the *vgb* gene.

Total DNA, plasmid DNA of *E. faecium* HM1032, and plasmid DNA of *E. faecium* HM1070SR were analyzed by agarose gel electrophoresis and hybridization with *vanA*, *ermAM*, *vgb*, and *satA* probes (Fig. 1). *E. faecium* HM1032 contained at least six plasmids, three of which were present in *E. faecium* HM1070SR. The *ermAM*, *satA*, and *vanA* genes were localized on the same large plasmid (>60 kb), while the *vgb* gene was apparently chromosomal in the wild-type strain (lack of hybridization to plasmid bands with hybridization to the region with linearized DNA, including fragments of chromosomal DNA) and absent from the transformant. After plasmid digestion with *Eco*RI, *vanA*, *satA*, and *ermAM* probes hybridized to *Eco*RI fragments, of 5.5, 10, and 10 kb, respectively (data not shown).

Although uncommon, quinupristin-dalfopristin resistance in glycopeptide-resistant *E. faecium* has already been reported for human and animal isolates from Germany, the United Kingdom, and the United States (3, 7, 8). The presence of genes responsible for glycopeptide and streptogramin resistance linked on the same plasmid is an additional cause of concern if this plasmid happened to disseminate. Although we were unable to transfer by conjugation this resistance plasmid to a recipient strain, transformation experiments showed that the combination of genes for resistance to vancomycin and streptogramins borne by this plasmid was sufficient to confer resistance to both antibiotics.

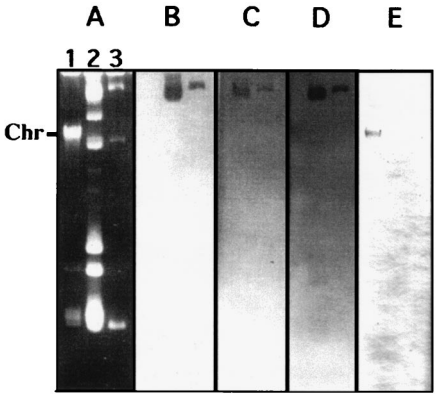

FIG. 1. Analysis of non-digested genomic DNA (lane 1) and plasmid DNA (lane 2) from *E. faecium* HM1032 and of nondigested plasmid DNA from the transformant *E. faecium* HM1070SR (lane 3) by agarose gel electrophoresis and hybridization. (A) Agarose gel electrophoresis of DNA; (B to E) hybridization to *ermAM*, *satA*, *vanA*, and *vgb* probes, respectively. Linear (chromosomal) DNA is indicated on the left of the gel (Chr). *ermAM*, *satA*, and *vanA* probes hybridized to the same large plasmid; the *vgb* probe hybridized to the chromosomal DNA of *E. faecium* HM1032.

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