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Strains of Glycopeptide-Resistant *Enterococcus faecium* Can Alter Their *van* Genotypes during an Outbreak

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Two isolates of *Enterococcus faecium* with VanA glycopeptide resistance were isolated during a hospital outbreak of *E. faecium* with plasmid-mediated VanB resistance. Both were found to be identical to the VanB outbreak strain by pulsed-field gel electrophoresis. The genotype of this strain changed from *vanB* to *vanA* through an intermediate isolate that contained both the *vanA* and *vanB* gene clusters on distinct plasmids.

Glycopeptide-resistant *Enterococcus faecium* (GREF) has emerged as a significant cause of nosocomial infections in patients in specialized hospital units, such as renal, bone marrow transplant (BMT), and intensive care units. Most of these enterococci display the VanA form of acquired glycopeptide resistance, but isolates displaying the VanB phenotype are encountered increasingly. Both forms of resistance may be transferred in vitro, in association either with conjugative or mobilizable plasmids or with transposable elements on plasmids or the chromosome (5).

We have reported previously on two clusters of colonization and infection caused by GREF at a leukemia/BMT unit (1). Throughout the first cluster, which lasted from October 1992 to July 1994, the majority of GREF isolates expressed the VanB phenotype and a predominant strain was recognized by pulsed-field gel electrophoresis (PFGE). However, in July 1993 two isolates that expressed the VanA phenotype were recovered from separate patients; no further VanA GREF isolates were recovered at this unit until the beginning of the second cluster in December 1994 (1). We report here the alteration of the glycopeptide resistance phenotype (and corresponding *van* genotype) expressed by the predominant strain from the first cluster from VanB to VanA, most probably through an intermediate isolate that contained both sets of resistance genes. To our knowledge, this is the first report of the isolation of an enterococcus in which the VanA and VanB glycopeptide resistance mechanisms coexisted, albeit on different plasmids.

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GREF isolates 1, 2, and 3 were all from different patients in the leukemia unit during 1993. The relatedness of these isolates was investigated by PFGE of *Sma*I-digested genomic DNA through 1.2% agarose gels in a contour-clamped homogeneous electric field DRII tank (Bio-Rad), with pulse times of 1 to 10 s for 30 h, followed by 10 to 40 s for 15 h as described previously (6). The *van* genotype was determined by amplification of fragments internal to the *vanA* and *vanB* genes, which encode D-alanyl-D-lactate ligases, by using published primers and cycling conditions (6). Plasmid DNA was extracted by alkaline lysis and analyzed on 0.8% agarose gels. Conjugal transfer of glycopeptide resistance to *E. faecium* GE-1 (Rif^r Fus^r) was investigated by cross-streak mating, and transconjugants were checked for teicoplanin resistance, *van* genotype, and plasmid profile. Plasmids were digested with *Eco*RV (Life Technologies, Paisley, United Kingdom), transferred to nylon membrane, and hybridized with digoxigenin-labeled probes specific for the *vanA* or *vanB* genes (6).

GREF isolates 1, 2, and 3 had indistinguishable macrorestriction digest patterns by PFGE (Fig. 1), indicating that they represented a single strain, the predominant strain isolated during the first cluster of GREF at this leukemia/BMT unit. Isolate 1 represented the major form of this predominant strain; it was sensitive to teicoplanin (MIC, 1 mg/liter) and had a typical VanB phenotype. In contrast, isolates 2 and 3 were both resistant to teicoplanin (MICs, >32 mg/liter) and so displayed a VanA phenotype. As the latter two isolates had emerged during a 22-month outbreak associated with VanB *E. faecium*, they were considered initially to be possible mu-

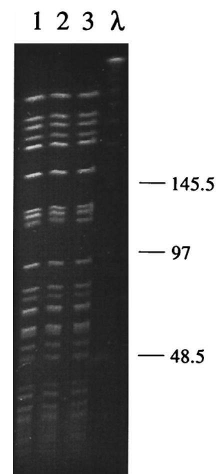

FIG. 1. Macrorestriction digest patterns of GREF isolates 1, 2, and 3 after PFGE. The size standard indicated (lane λ) is a concatamer of phage λ DNA. Sizes are given in kilobases.

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TABLE 1. Glycopeptide resistance in three isolates of *E. faecium* with identical PFGE profiles

Isolate	MIC (mg/liter) of:		Van phenotype	Genotype		Location of:	
	Vancomycin	Teicoplanin		Inferred	Actual	*vanB*	*vanA*
1	>32	1	VanB	Inducible *vanB*	*vanB*	60-MDa plasmid	Absent
2	>32	>32	VanA	Constitutive *vanB*	*vanA* + *vanB*	60-MDa plasmid	24-MDa plasmid
3	>32	>32	VanA	Constitutive *vanB*	*vanA*	Absent	24-MDa plasmid

tants in which the *vanB* gene was expressed constitutively (2). However, although isolate 1 contained the *vanB* gene, isolate 3 contained only the *vanA* gene, while isolate 2 contained both the *vanA* and *vanB* genes (Table 1). The *vanB* genes in isolates 1 and 2 were associated with a transferable plasmid of ca. 60 MDa (Fig. 2 and 3). Transconjugants acquiring this plasmid from either isolate displayed a VanB phenotype. The *vanB* plasmids from isolates 1 and 2 were indistinguishable after digestion with *Eco*RV (data not shown), and the *vanB* gene was located on a 2.1-kb *Eco*RV fragment. An identical hybridization pattern has been reported previously for diverse VanB-encoding plasmids in strains of *E. faecium* and *Enterococcus faecalis* (6) and is consistent with the sequence of the *vanB* gene cluster on transposon Tn*1547* (3). VanA resistance in isolates 2 and 3 was associated with a transferable plasmid of ca. 24 MDa (Fig. 3), and the *vanA* gene was located on a 3.7-kb *Eco*RV fragment of this plasmid (data not shown). The plasmid contents of isolates 1 and 2 differed by the presence, in isolate 2, of the 24-MDa *vanA* plasmid and two small plasmids. As these three plasmids could be transferred and/or mobilized en bloc by conjugation (Fig. 2), we consider that their acquisition was likely to have resulted from a single mating event. Although isolate 1 also carried a plasmid of ca. 24 MDa, this plasmid did not hybridize with either the *vanA* or *vanB* probe (Fig. 3). The plasmid contents of isolates 2 and 3 differed only by the absence of the 60-MDa *vanB* plasmid from isolate 3 (Fig. 2).

In summary, during a cluster of GREF at this leukemia/BMT unit, the glycopeptide resistance genotype of the outbreak strain changed from *vanB* to *vanA*. Based upon the identical PFGE profiles but altered glycopeptide resistance phenotypes of the isolates, it was initially assumed (incorrectly) that constitutive *vanB* expression mutants had emerged in vivo, conferring teicoplanin resistance. However, resistance genotyping correctly identified the switch in genotype, which occurred presumably through an intermediate isolate, such as isolate 2 described here, that contained both the *vanA* and *vanB* gene clusters on distinct plasmids. Although isolates 1, 2, and 3 were from separate patients, a GREF isolate indistinguishable from isolate 1 (*vanB* genotype) had been recovered 7 months previously from the same patient from whom isolate 2 (*vanA vanB* genotype) was obtained. We suggest that isolate 2 may have arisen when this first isolate acquired a 24-MDa plasmid carrying the *vanA* gene cluster, together with other mobilizable plasmids, and that the subsequent loss of the 60-MDa *vanB* plasmid resulted in an isolate comparable to isolate 3 (*vanA* genotype). The origin of the 24-MDa *vanA* plasmid is unknown. No VanA enterococci had been isolated at the unit prior to July 1993, and they were not isolated again until the second cluster of GREF in December 1994. The VanA enterococci causing this second cluster were distinct from strains causing the first VanB cluster and were probably introduced from a nearby hospital with a preexisting VanA problem at a renal unit (1).

PFGE has become widely accepted as the standard method for molecular typing of enterococci. Although the criteria (num-

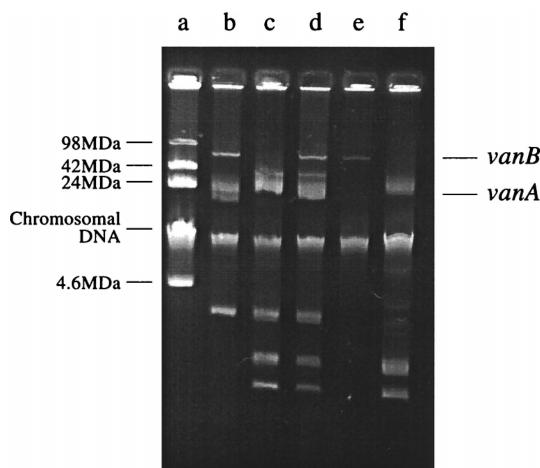

FIG. 2. Plasmid content of GREF. Lanes: a, control strain *Escherichia coli* 39R861; b, isolate 1 (*vanB*); c, isolate 3 (*vanA*); d, isolate 2 (*vanA vanB*); e, *vanB* transconjugant derived from isolate 2; f, *vanA* transconjugant derived from isolate 2.

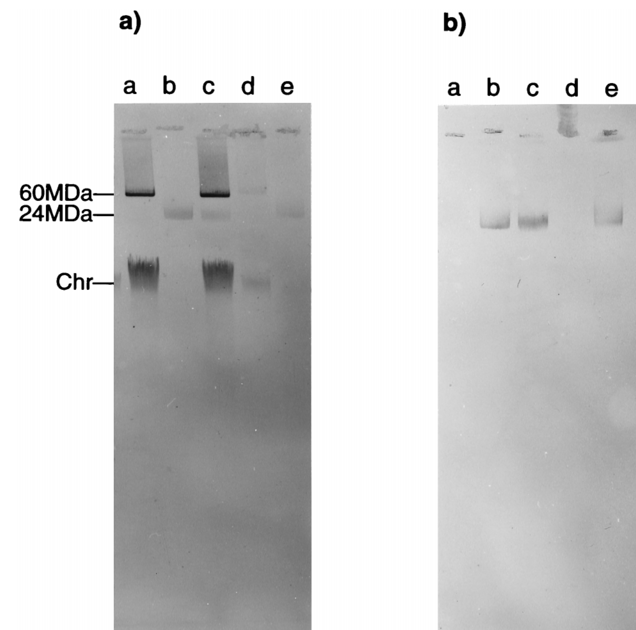

FIG. 3. Southern hybridization of GREF with a mixture of probes specific for both *vanA* and *vanB* (a) and a *vanA*-specific probe only (b). Lanes (each panel): a, isolate 1 (*vanB*); b, isolate 3 (*vanA*); c, isolate 2 (*vanA vanB*); d, *vanB* transconjugant derived from isolate 2; e, *vanA* transconjugant derived from isolate 2. Hybridization of the *vanB* probe with chromosomal DNA in lanes a, c, and d in panel a may indicate a chromosomal copy of the gene or plasmid linearized during extraction.

ber of band differences) required to define different strains is still debated, guidelines for interpreting the banding patterns have been proposed (4). The differences observed among the plasmid contents of the three isolates described here included gain and loss of plasmids of up to 60 MDa (approximately 90 kb) but did not result in any changes in their PFGE patterns. We conclude that PFGE analysis and the resistance phenotypes of GREF from hospital clusters may be insufficient to define fully the epidemiology of these problematic bacteria and should be used in conjunction with resistance genotyping.

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