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# Identification of *vat*(E-3), a Novel Gene Encoding Resistance to Quinupristin-Dalfopristin in a Strain of *Enterococcus faecium* from a Hospital Patient in the United Kingdom

Quinupristin-dalfopristin is a mixture of semisynthetic streptogramins A and B that was recently licensed for clinical use in the United States and Europe (3). A related antibiotic, virginiamycin, has been used as a growth promoter for production animals in Europe and the United States, although its use was banned in the European Union in July 1999. Virginiamycin-resistant *Enterococcus faecium* strains have been isolated from exposed farm animals, raw meat, and hospital patients and are cross-resistant to quinupristin-dalfopristin (1, 2, 5; L. B. Jensen, A. M. Hammerum, F. M. Aarestrup, A. E. van den Bogaard, and E. E. Stobberingh, Letter, Antimicrob. Agents Chemother. **42:**3330–3331, 1998; G. Werner, I. Klare, and W. Witte, Letter, Eur. J. Clin. Microbiol. Infect. Dis. **17:**401–402, 1998). Resistance to streptogramin A is a prerequisite for resistance to quinupristin-dalfopristin and virginiamycin and is mediated in *E. faecium* by *vat*(D) (previously *satA*) or *vat*(E) (previously *satG*), two plasmid-mediated genes that encode acetyltransferases that inactivate streptogramin A (4; G. Werner and W. Witte, Letter, Antimicrob. Agents Chemother. **43:**1813–1814, 1999). Available *vat*(E) sequences are not identical, and we propose designating the alleles in the order of their deposition in the GenBank database: *vat*(E-1) (accession numbers AF139735, AF229200, and AF242872) and *vat*(E-2) (AF153312). *vat*(E-2) differs from *vat*(E-1) by three nucleotides (99.5% identity), which are predicted to result in two amino acid substitutions (Fig. 1). Two other alleles, each differing from *vat*(E-1) by two nucleotides, have been reported but have not yet been deposited in GenBank (6).

We have previously detected *vat*(E) by PCR in isolates of quinupristin-dalfopristin-resistant *E. faecium* (MIC $\geq$ 32 μg/ml) from animals and raw meat ($n$ = 10) and also from hospital patients in the United Kingdom ($n$ = 4) (5). A 512-bp internal fragment of *vat*(E) was amplified from these isolates (5) and subjected to direct cycle sequencing using an ALFexpress DNA sequencer (Amersham Pharmacia Biotech, St. Albans, United Kingdom) and a Thermo Sequenase fluorescence-labeled primer cycle sequencing kit (Amersham Pharmacia Biotech). The sequences, which represented 80% of the *vat*(E) gene, were compared with those of *vat*(E-1) and *vat*(E-2). The sequences of the PCR products from 13 isolates were identical to *vat*(E-1). However, one isolate, designated *E. faecium* A41, from a hospital patient, yielded a distinct sequence. Two overlapping fragments of the *vat*(E) allele from this strain were amplified and cloned into pCR2.1-TOPO (Invitrogen, Groningen, The Netherlands) to yield recombinant plasmid pARL00.31, containing the 512-bp fragment, and pARL00.38, containing a 300-bp fragment spanning the 3′ end of *vat*(E) and extending 137 bp downstream of the stop codon. The latter fragment was amplified with primers 5′-CCA ATT CAA CTC ATC GGA CC-3′ and 5′-TAC GAG TAG AGT ACC GCC AG-3′ and corresponded to nucleotides 4063 to 4362 of GenBank sequence AF242872. For each fragment, the inserts of three separate clones were sequenced in both directions using a Dye-Labeled ddNTP Terminator Cycle Sequencing Kit (Beckman Coulter UK Ltd., High Wycombe, United Kingdom) and samples were analyzed on a CEQ 2000 automated sequencer (Beckman). Fragments were assembled with ContigExpress (InforMax Inc., Oxford, United Kingdom).

The *vat*(E) allele from *E. faecium* A41 has been designated *vat*(E-3) and deposited in GenBank under the accession num-

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                    *        20         *        40         *        60
Vat(E-1) : MTIPDANAIYPNSAIKEVVFIKNVIKSPNIEIGDYTYYDDPVNPTDFEKHVTHHYEFLGD
Vat(E-2) : ..........H.....................................................
Vat(E-3) : ~...........P.....................................................


                    *        80         *       100         *       120
Vat(E-1) : KLIIGKFCSIASGIEFIMNGANHVMKGISTYPFNILGGDWQQYTPELTDLPLKGDTVVGN
Vat(E-2) : .........L.....................................................
Vat(E-3) : ........................................K.....................


                    *       140         *       160         *       180
Vat(E-1) : DVWFGQNVTVLPGVKIGDGAIIGANSVVTKDVAPYTIVGGNPIQLIGPRFEPEVIQALEN
Vat(E-2) : .....................................................................
Vat(E-3) : .....................................................................


                    *       200         *
Vat(E-1) : LAWWNKDIEWITANVPKLMQTTPTLELINSLMEK
Vat(E-2) : ...................................
Vat(E-3) : .......V....V...........V.........
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FIG. 1. Comparison of the amino acid sequences of alleles of the Vat(E) streptogramin A acetyltransferase (see the text for GenBank accession numbers).

ber AY008284. It had 20 nucleotide changes (4% divergence) compared with *vat*(E-1). Fifteen of these changes were silent, but the others resulted in five previously undescribed amino acid substitutions (Fig. 1). The predicted Vat(E-3) peptide had 97% amino acid identity with Vat(E-1) and 96% identity with Vat(E-2). In comparison with sequences downstream of *vat*(E-1) and *vat*(E-2), the sequence immediately downstream of the *vat*(E-3) stop codon had a single base insertion (an additional C after nucleotide 4235 of GenBank sequence AF242872) and two substitutions (both T→C changes at nucleotides 4227 and 4253 of GenBank sequence AF242872).

We have confirmed the allelic nature of *vat*(E) apparent in GenBank submissions and previous reports (2, 6). The *vat*(E-1) allele was present in all *vat*(E) PCR-positive *E. faecium* strains from nonhuman sources studied here and in three of the four clinical isolates. The fourth clinical isolate harbored *vat*(E-3), which showed greater sequence divergence from *vat*(E-1) than other previously reported alleles (20 versus 2 or 3 nucleotide changes). In conclusion, isolates of quinupristin-dalfopristin- and virginiamycin-resistant *E. faecium* that give a *vat*(E)-specific PCR product should not be assumed to carry identical alleles. Furthermore, we suggest that the epidemiological significance of a *vat*(E)-positive PCR result cannot be judged accurately in the absence of sequence data.

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