Variation within the vat(E) Allele of Enterococcus faecium Isolates from Retail Poultry Samples

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In a survey of retail meat samples, twelve quinupristin-dalfopristin-resistant (MICs, ≥4 mg/liter) Enterococcus faecium isolates that carried a vat(E) gene were recovered. DNA sequence comparison revealed five new variations in the vat(E) allele among 12 isolates, which were designated vat(E-4) through vat(E-8); two isolates had vat(E-1). There was no correlation between the number of base changes and the quinupristin-dalfopristin MIC.

Quinupristin-dalfopristin (Synercid), a semisynthetic streptogramin A and B mixture, was recently licensed for human use in both the United States and Europe for the treatment of multiresistant gram-positive pathogens, including Enterococcus faecium (4). A related streptogramin, virginiamycin, has been used as a growth promoter in animal husbandry for over 2 decades in both the United States and Europe. However, virginiamycin was banned in the European Union effective July 1999, along with several other growth promoters that belong to drug classes also used in human medicine.

Isolates of E. faecium resistant to virginiamycin display cross-resistance to quinupristin-dalfopristin. Several reports have described the isolation of streptogramin-resistant E. faecium from both animal and human sources (1, 4, 7). Resistance to streptogramin A in E. faecium is conferred by either of two genes encoding an acetyltransferase, vat(D) (satA) or vat(E) (satG) (3). Only one vat(D) allele has been reported to date, and several vat(E) alleles have recently been described. The vat(E) alleles deposited within GenBank have been designated vat(E-1) (accession numbers AF139735, AF229200, and AF242872), vat(E-2) (accession number AF153312), and vat(E-3) (accession numberAY008284) (5). Two other alleles have yet to be deposited in GenBank, but each allele differs from vat(E-1) by two nucleotides (5, 6). vat(E-2) and vat(E-3) have 95.5 and 97% amino acid identity with vat(E-1), respectively, and vat(E-3) has 96% amino acid identity with vat(E-2) (5).

We recently conducted a study to examine allelic variation in the vat(E) gene among enterococci isolated from retail poultry meats collected from the greater Washington, D.C., area in 1999. Thirty-three E. faecium isolates were recovered from a total of 43 chicken and 32 turkey retail samples. Antimicrobial MICs for the 33 isolates were determined with the Sensititre (NCCLS) guidelines for broth microdilution (2). The organisms used for quality control were Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, and Enterococcus faecalis ATCC 29212. Quinupristin-dalfopristin MICs for 27 of the 33 isolates were ≥4 mg/liter (NCCLS resistant breakpoint). These isolates were further tested for the presence of vat(D) and vat(E) genes by PCR using previously described primers and cycling conditions (4). vat(D) was not detected in any of the isolates, but vat(E)-like sequences were detected in 12 (44%) of the quinupristin-dalfopristin-resistant E. faecium isolates (chicken, n = 10; turkey, n = 2). As these primers amplified only a 512-bp internal region of the vat(E) allele, representing 80% of the coding region, we used primers described by Soltani et al. (5) to amplify the 3' end of the vat(E) allele. For amplification of the 5' end of the vat(E) allele, we used the primers 5'-TGG GTA CTA ACA TGA C-3' and 5'-ATT GTT GCC AAC ATG CAC CT-3', corresponding to nucleotides 3566 to 3867 of GenBank sequence AF242872. These three primer sets gave overlapping PCR products which spanned the entire vat(E) gene and in addition extended into both the upstream and downstream regions flanking the vat(E) gene. The DNA sequence of each overlapping amplicon was determined in both directions (SeqWright, Houston, Tex.) and compared to that of vat(E-1), vat(E-2), and vat(E-3).

Two of the E. faecium isolates (CVM3975 and CVM3983) possessed vat(E) DNA sequences that were 100% identical to vat(E-1). The remaining sequences all contained base substitutions resulting in amino acid changes. None of these base substitutions have been described previously. The five new vat(E) alleles found in this study have been designated vat(E-4) through vat(E-8).

One isolate (CVM3475) had two base substitutions, C45T→G (Ile15→Met) and A257→T (Ala85→Ile), and this allele was designated vat(E-5). Five of the isolates carried base pair inversions at position 37 and 38 (TC→CT), resulting in a single amino acid change (Ser15→Leu), and we designated this allele vat(E-6). One isolate showed the base 37–38 inversions along with an A507→T (Arg175→Ser in CVM3981), and the allele was designated vat(E-7). One isolate (CVM3976) had a single base change (A37→T) resulting in Lys13→Ile. This was desig-
nated vat(E-7). Finally, one isolate (CVM3982) contained 11 base substitutions resulting in 7 amino acid changes, and we designated the allele vat(E-8). All the amino acid substitutions are listed in Fig. 1. These sequence variations represent a 1 to 2% divergence from the vat(E-1) DNA sequence. Quinupristin-dalfopristin MICs for all isolates were between 8 and 32 μg/ml; however, there was no correlation between the number or position of the base changes and the quinupristin-dalfopristin MICs.

In contrast to the recent report of Soltani et al. (5), we found only two animal E. faecium strains that carried the vat(E-1) allele. None of the isolates harbored the vat(E-2) or vat(E-3) alleles, suggesting that there are regional variations among vat(E) alleles from isolates in Europe and the United States. The majority of the retail meat E. faecium isolates had the vat(E-5) allele, which showed only 0.4% divergence from the vat(E-1) allele. Additionally, it was not possible to distinguish between chicken and turkey isolates based on vat(E) sequence variation. We agree with Soltani et al. (5) that it is not entirely possible to trace the epidemiological spread of the vat(E) gene based on PCR results alone and that DNA sequencing information is necessary to obtain a more complete picture of vat(E) gene dissemination in veterinary and human environments.

**Nucleotide sequence accession numbers.** The sequences determined in this study have been deposited in GenBank under accession numbers AY043211 [vat(E-4)], AY043209 [vat(E-5)], AY043210 [vat(E-6)], AY043212 [vat(E-7)], and AY043213 [vat(E-8)].

**REFERENCES**