ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2001, p. 2931–2932 Vol. 45, No. 10
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.10.2931–2932.2001

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

S. SIMJEE,* P. F. McDERMOTT, D. D. WAGNER, AND D. G. WHITE

Division of Animal and Food Microbiology, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, Maryland

Received 12 March 2001/Returned for modification 8 May 2001/Accepted 16 July 2001

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 number AY008284) (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 99.5 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 Automated Antimicrobial Susceptibility System (Trek Diagnostic Systems, Westlake, Ohio) and interpreted according to the National Committee for Clinical Laboratory Standards (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′-TCG GAG GTA CTA ACA TGA C-3′ and 5′-ATT GTT GCC AAT CGC 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, $C_{45}\rightarrow G$ ($Ile_{17}\rightarrow Met$) and $A_{47}\rightarrow T$ ($Ala_{18}\rightarrow Ile$), and this allele was designated *vat*(E-4). Five of the isolates carried base pair inversions at position 37 and 38 (TC→CT), resulting in a single amino acid change ($Ser_{15}\rightarrow Leu$), and we designated this allele *vat*(E-5). One isolate showed the base 37–38 inversions along with an $A_{507}\rightarrow T$ ($Arg_{175}\rightarrow Ser$ in CVM3981), and the allele was designated *vat*(E-6). One isolate (CVM3976) had a single base change ($A_{47}\rightarrow T$) resulting in $Lys_{18}\rightarrow Ile$. This was desig-

* Corresponding author. Mailing address: Office of Research, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, MD 20708. Phone: (301) 827-8046. Fax: (301) 827-8250. E-mail: ssimjee@cvm.fda.gov.

FIG. 1. Amino acid sequence variations encoded by the *vat*(E) alleles of the streptogramin A acetyltransferase gene found in *E. faecium* from poultry retail samples.

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

1. **Hammerum, A. M., L. B. Jensen, and F. M. Aarestrup.** 1998. Detection of the satA gene and transferability of virginiamycin resistance in *Enterococcus faecium* from food-animals. FEMS Microbiol. Lett. **168:**145–151.
2. **National Committee for Clinical Laboratory Standards.** 2000. M7–A5. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
3. **Rende-Fournier, R., R. Leclercq, M. Galimand, J. Duval, and P. Courvalin.** 1993. Identification of the *satA* gene encoding a streptogramin A acetyltransferase in *Enterococcus faecium* BM4145. Antimicrob. Agents Chemother. **37:**2119–2125.
4. **Soltani, M., D. Beighton, J. Philpott-Howard, and N. Woodford.** 2000. Mechanisms of resistance to quinupristin-dalfopristin among isolates of *Enterococcus faecium* from animals, raw meat, and hospital patients in Western Europe. Antimicrob. Agents Chemother. **44:**433–436.
5. **Soltani, M., D. Beighton, J. Philpott-Howard, and N. Woodford.** 2001. 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. Antimicrob. Agents Chemother. **45:**645–646.
6. **Werner, G., I. Klare, H. Heier, K. H. Hinz, G. Bohme, M. Wendt, and W. Witte.** 2000. Quinupristin/dalfopristin-resistant enterococci of the satA (vatD) and satG (vatE) genotypes from different ecological origins in Germany. Microb. Drug Resist. **6:**37–47.
7. **Werner, G., I. Klare, and W. Witte.** 1998. Association between quinupristin/dalfopristin resistance in glycopeptide-resistant *Enterococcus faecium* and the use of additives in animal feed. Eur. J. Clin. Microbiol. Infect. Dis. **17:** 401–402.