Letters to the Editor

Are *Enterococcus faecalis* Strains with *vat*(E) in Poultry a Reservoir for Human Streptogramin Resistance? *vat*(E) Occurrence in Human Enterococcal Bloodstream Infections in North America (SENTRY Antimicrobial Surveillance Program, 2002)

Virginiamycin has been widely utilized in agriculture for nearly 3 decades but was removed from growth promotion and therapeutic use in the European Union in 1998 (1), generally in response to concerns about evolving resistance that might compromise streptogramin (quinupristin-dalfopristin) therapies in humans. Recent events affecting the future of streptogramins in human infection treatment include (i) Food and Drug Administration release of linezolid as an alternative agent for vancomycin-resistant *Enterococcus faecium* therapy, (ii) emerging resistances to streptogramins among human *E. faecium* strains, and (iii) elevated rates of adverse events compromising the wide clinical application of this class. Also, the discovery of *vat*(D)- and *vat*(E)-encoded streptogramin acetyltransferases associated with streptogramin resistance has been documented in enterococcal isolates of animal or poultry origin (4–6, 8; G. Werner and W. Witte, Letter, Antimicrob Agents Chemother. 43:1813–1814, 1999). Each of these latter reports describes the “possible” transfer of these organisms to the human gastrointestinal tract flora and subsequent direct causal effects of streptogramin-resistant organism infections or passage of the genetic resistance determinants to the human microbial flora (1).

Several publications have indicated that a reservoir of streptogramin resistance genes exists in poultry, in meat production environments, and on retail meat products, each associated with the consumption of virginiamycin (1, 2, 5–7, 8). The most recent report by Simjee and colleagues (5) further suggests the presence of a “potential public health hazard” in the form of a *vat*(E) reservoir among the intrinsically streptogramin-resistant *Enterococcus faecalis* strains isolated in 5 of 16 retail poultry samples from the Washington, D.C., area. To assess the level of this hazard, isolates of *E. faecalis* from unique human episodes of bloodstream infection (BSI; 159 strains from 32 different medical centers in North America, including the middle-Atlantic states) in the SENTRY Antimicrobial Surveillance Program were screened for *vat*(D) and *vat*(E) (4, 8;

Werner and Witte, letter). Also, 10 BSI strains of *E. faecium* were tested for which the quinupristin-dalfopristin MICs were elevated. Eight of the *E. faecium* strains were from North America (streptogramin MIC range, 2 to 8 µg/ml), and two isolates were from BSI episodes in European patients (streptogramin MICs, 4 and >8 µg/ml).

The 169 strains were tested by reference broth microdilution susceptibility methods against 27 antimicrobial agents (TREK Diagnostics, Cleveland, Ohio) according to procedures described by the National Committee for Clinical Laboratory Standards (3). However, only the results for eight drugs are reported here (Table 1). The enterococcal identification was confirmed by the Vitek System (bioMérieux, Hazelwood, Mo.). Determination of the streptogramin resistance genes was accomplished after genomic DNA extraction from broth cultures with a QIAamp DNA mini kit (QIAGEN, Inc.). *vat*(E) primers were prepared with the following primer sequences (6): *vatE* forward, 5'-ACT ATA CCT GAC GCA AAT GC-3'; *vatE* reverse, 5'-GGT TCA AAT CTT GGT CCG-3'. PCRs were performed with puReTaqReady-To-Go PCR beads (Amersham Biosciences, Inc., Piscataway, N.J.) in accordance with a protocol described by Werner and Witte (letter). The testing for *vat*(D) utilized the methods of Werner et al. (8). Following amplification, the PCR products were separated by electrophoresis with 2% agarose gel and visualized on a Gel Doc 2000 imaging system. *E. faecium* strains UW1965 and BM4145 were included in all procedures as a positive control for the *vat*(D) and *vat*(E) genes (4, 6; Werner and Witte, letter).

Table 1 summarizes the antimicrobial susceptibility pattern for the 159 screened bloodstream isolates of *E. faecalis*. All strains were susceptible to ampicillin, but 2.5 to 5.0% were resistant to the two glycopeptides (teicoplanin and/or vancomycin). All *vat*(D) tests were negative (data not shown). *vat*(E) was not detected in any of the North American *E. faecalis* or *E. faecium* strains (167 bacteremia isolates) tested by the same procedures utilized by Simjee et al. (5). These results from

TABLE 1. Antimicrobial activities of seven selected agents tested against 159 BSI strains of *E. faecalis* for which the quinupristin-dalfopristin MICs were ≥4 µg/ml (SENTRY Program, 2002, North America)

Antimicrobial agent	MIC (µg/ml)			% by category ^a	
	50% of strains	90% of strains	Range	Susceptible	Resistant
Ampicillin	≤2	≤2	≤2–8	100.0	0.0
Erythromycin	>8	>8	0.12–>8	3.1	76.7
Tetracycline	>8	>8	≤4–>8	39.0	61.0
Teicoplanin	≤0.12	0.25	≤0.12–>16	97.5	2.5
Vancomycin	1	2	1–>16	95.0	5.0
Gentamicin (HL) ^b	≤500	>1,000	≤500–>1,000	49.1 ^c	
Streptomycin (HL) ^b	≤1,000	>2,000	≤1,000–>2,000	53.5 ^c	

^a According to the susceptibility criteria of the NCCLS (3).

^b HL, high-level screen for resistance.

^c Percent susceptible indicates the proportion of strains for which there is likely to be synergy when the drug is used with an active β-lactam or glycopeptide (3).

invasive human infection isolates stand in marked contrast to the rates of *vat*(E) documented in *E. faecalis* from the small sample of retail poultry (31.3%) (5) and to the level of quinupristin-dalfopristin resistance (51 to 78%) reported in *E. faecium* from the poultry production environments on the eastern seaboard (2). Also, the findings of a non-*vat*(D) or -*vat*(E) mechanism of resistance in *E. faecium* stands in contrast to the occurrence rates published by Werner et al. (8) for Europe (Germany), where 32 (89%) of 36 isolates from hospitalized patients have a detectable acetyltransferase gene.

To address the possible public health risks of an endemic *vat*(E) reservoir in animal strains of *E. faecalis* (5), surveillance programs must be alert to document initial occurrences of these strains producing human infections or to the transfer of *vat*(E) to species (*E. faecium*) likely to receive human quinupristin-dalfopristin regimens. This investigation, stimulated by the results published by the U.S. Food and Drug Administration Center for Veterinary Medicine (5), suggests that a large comprehensive sample of contemporary (2002) human enterococcal bacteremia isolates does not harbor *vat*(E). Furthermore, BSI isolates of *E. faecium* (eight strains from North America) for which the quinupristin-dalfopristin MICs indicate nonsusceptibility were free of *vat*(D) and *vat*(E) as a cause of the documented resistance (3). Since other investigators have noted a high prevalence of virginiamycin-resistant strains in *E. faecium* of poultry origin in the United States (2, 5, 7), we must conclude that these strains remain extremely rare as the causative agent of invasive human enterococcal infection.

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