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Mechanisms of Resistance to Quinupristin-Dalfopristin among Isolates of *Enterococcus faecium* from Animals, Raw Meat, and Hospital Patients in Western Europe

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Twenty-eight quinupristin-dalfopristin-resistant isolates of *Enterococcus faecium* from hospital patients and nonhuman sources in European countries were studied. High-level resistance (MICs, ≥32 μg/ml) was associated with the presence of *vat*(E) (*satG*) (14 isolates [50%]) or *vat*(D) (*satA*) (6 isolates [21%]). These genes were not detected in eight (29%) isolates with lower levels of quinupristin-dalfopristin resistance (MICs, 4 to 16 μg/ml). This suggests the presence of further mechanisms of resistance to quinupristin-dalfopristin in *E. faecium*.

Quinupristin-dalfopristin is a semisynthetic, injectable mixture of streptogramin A and B compounds which has recently been licensed for clinical use in the United States and in Europe for the treatment of infections caused by multiresistant gram-positive pathogens, including glycopeptide-resistant *Enterococcus faecium* (9, 17, 18). Virginiamycin, another streptogramin A and B combination, has been used as a growth promoter in animal feed for many years. It selects for virginiamycin-resistant strains of *E. faecium*, which are cross-resistant to quinupristin-dalfopristin (11, 12, 19) and which may pose a risk to public health. As a consequence, the use of virginiamycin has been banned in the European Union.

Resistance to mixtures of streptogramin A and B compounds was first reported among staphylococci (10) and requires only resistance to the A component (15), although resistance to both the A and B components may give a higher level of resistance (B. Bozdogan and R. Leclercq, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 841, 1999). Several genes conferring resistance to type A streptogramins have been identified in staphylococci (1–5, 7, 8). In this paper we adopt the nomenclature proposed recently by Roberts and colleagues (16). The staphylococcal genes *vat*(A), *vat*(B), and *vat*(C) encode acetyltransferases (2, 4, 7); *vga*(A) and *vga*(B) encode ATP-binding proteins involved in active efflux (3, 5). Among enterococci, *Enterococcus faecalis* is naturally resistant to streptogramin A-B combinations (15), whereas most isolates of *E. faecium* are susceptible. Two acetyltransferase genes, *satA* (15) and *satG* (20), have been characterized for strains of *E. faecium* resistant to streptogramin A-B combinations. These have been renamed *vat*(D) and *vat*(E), respectively (16). However, *vat*(D) occurs in only a minority of the resistant *E. faecium* organisms investigated in several countries in the European Union (11, 12), and the prevalence of *vat*(E) is unknown. In this study we investigated the basis of resistance to quinupristin-dalfopristin in a small, diverse group of *E. faecium* isolates.

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Twenty-eight quinupristin-dalfopristin-resistant (MICs, ≥4 μg/ml) isolates of *E. faecium* were collected in the United Kingdom (UK) and other European countries prior to both the licensing of quinupristin-dalfopristin and the ban on virginiamycin use: 18 from animals (15 from pigs and 3 from chickens), 4 from raw meat, 2 from sewage, and 4 from UK hospital patients. Isolates from hospital patients, raw meat, and sewage were from a collection of enterococci kept at the Antibiotic Resistance Monitoring and Reference Laboratory. Animal isolates were kindly provided by Pfizer Ltd., Reigate, UK). *E. faecium* strain BM4145 [*vat*(D)] (kindly supplied by P. Courvalin, Institut Pasteur, Paris, France) and three strains of staphylococci, BM3093 [*vat*(A) *vga*(A) *vgb*(A)], BM12235 [*vat*(B) *vga*(B)], and BM12392 [*vgb*(B) *vat*(C)] (kindly supplied by N. El-Solh, Institut Pasteur, Paris, France), were used as positive PCR controls. A *vat*(E)-positive control strain was not available for this study. The identity of all isolates was confirmed by amplification of the *E. faecium*-specific gene encoding D-alanyl-D-alanine ligase (21).

The MICs of quinupristin-dalfopristin (Rhone Poulenc Rorer, West Malling, UK) and virginiamycin (Pfizer, Rixensart, Belgium) were determined by agar incorporation in DST agar (Oxoid, Basingstoke, UK) containing 5% lysed horse blood, with an inoculum of 10^4 CFU per spot. Etest strips (Cambridge Diagnostic Services, Cambridge, UK) were used for some quinupristin-dalfopristin MIC determinations. Isolates were screened by PCR for *vat*(A), *vat*(B), *vat*(C), *vat*(D), *vat*(E), *vga*(A), *vga*(B), *vgb*(A), *vgb*(B), and *erm*(B). The primer sequences and cycling conditions are listed in Table 1. Degenerate primers M and N (Table 1), derived from motifs conserved within streptogramin A acetyltransferases (14), were used to detect putative genes encoding acetyltransferases in quinupristin-dalfopristin-resistant isolates. Plasmid DNA was isolated from *E. faecium* isolates by alkaline lysis (22), separated by electrophoresis through 0.8% agarose gels, and transferred to nylon membranes by vacuum blotting. Hybridization was performed at high stringency using digoxigenin-labeled *vat*(D)- and *vat*(E)-specific probes, which were prepared by incorporation of digoxigenin-11-dUTP (Roche, Lewes, UK) into PCR products. *Escherichia coli* strain 39R861 (NCTC 50192), an

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TABLE 1. Primer sequences and PCR conditions used for streptogramin resistance genes

Primer	Sequence (5′–3′)	GenBank accession no. and/or gene	Position of amplicon	PCR conditions	Reference(s)
vat-1	TGGAGTGTGACAAGATAGGC	L07778	200–712	Same as for *vat*(D)	7, 11
vat-2	GTGACAACAGCTTCTGCAGC	*vat*(A)			
vatB-1	GGCCCTGATCCAAATAGCAT	U19459	76–634	1 cycle of 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C; and 1 cycle of 10 min at 72°C	2, 11
vatB-2	GTGCTGACCAATCCCACCAT	L38809, *vat*(B)			
vatC-O	ATGAATTCGCAAAATCAGCAAGG	AF015628	1307–1886	1 cycle of 3 min at 95°C and 2 min at 60°C; 30 cycles of 20 s at 72°C, 20 s at 95°C, and 20 s at 60°C; and 1 cycle of 1 min at 72°C	4
vatC-P	TCGTCTCGAGCTCTAGGTCC	*vat*(C)			
satA-1	GCTCAATAGGACCAGGTGTA	L12033	361–632	1 cycle of 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C; and 1 cycle of 10 min at 72°C	11, 15
satA-2	TCCAGCTAACATGTATGGCG	*vat*(D)			
satG-1	ACTATACCTGACGCAAATGC	AF139725	66–577	1 cycle of 5 min at 94°C; 30 cycles of 25 s at 94°C, 40 s at 52°C, and 50 s at 72°C; and 1 cycle of 6 min at 72°C	20
satG-2	GGTTCAAATCTTGGTCCG	*vat*(E)			
vga-1	AGTGGTGGTGAAGTAACACG	M90056	1149–1808	Same as for *vat*(D)	5, 11
vga-2	CTTGTCTCCTCCGCGAATAC	*vga*(A)			
vgaB-1	TGACAATATGAGTGGTGGTG	U82085	990–1566	1 cycle of 3 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C; and 1 cycle of 10 min at 72°C	3, 11
vgaB-2	GCGACCATGAAATTGCTCTC	*vga*(B)			
vgb-1	TACAGAGTACCCACTACCGA	M36022	781–1350	Same as for *vga*(B)	6, 11
vgb-2	TCAATTCCTGCTCCAGCAGT	*vgb*(A)			
vgbB-Q	CAGCAGTCTAGATCAGAGTGG	AF015628	512–1240	Same as for *vat*(C)	4
vgbB-R	CATACGGATCCATCTTTTCC	*vgb*(B)			
ermB-1	CATTTAACGACGAAACTGGC	M11180	836–1260	1 cycle of 3 min at 94°C; 25 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C; and 1 cycle of 10 min at 72°C	13
ermB-2	GGAACATCTGTGGTATGGCG	ermB-1			*erm*(B)
strep-M	ATHATGAAYGGIGCIAAYCAYMGIATG			1 cycle of 5 min at 95°C; 35 cycles of 2 min at 40°C, 90 s at 72°C, and 30 s at 95°C; and 1 cycle of 4 min at 40°C and 12 min at 72°C	14
strep-N	ICCDATCCAIACRTCRTTICC				

isolate containing plasmids of known size, was used as a source of molecular size markers.

The 28 quinupristin-dalfopristin-resistant isolates of *E. faecium* studied were divided into three categories: 6 (21%) isolates (from live pigs in Belgium, Germany, Spain, and The Netherlands) contained *vat*(D), 14 (50%) isolates (from UK hospital patients, from live pigs and poultry, and from raw poultry meat in Spain, Germany, and the UK) contained *vat*(E), and 8 (29%) isolates (from live pigs, raw poultry meat, and sewage in Belgium, Spain, and the UK) contained neither of these genes (Table 2). The MICs of both quinupristin-dalfopristin and virginiamycin were ≥32 μg/ml for all *vat*(D)- or *vat*(E)-positive isolates. In contrast, the eight *vat*(D)-negative, *vat*(E)-negative isolates had lower levels of resistance to quinupristin-dalfopristin (MICs, 4 to 16 μg/ml) and virginiamycin (MICs, 2 to 4 μg/ml). None of the isolates carried the *vat*(A), *vat*(B), *vat*(C), *vga*(A), and *vga*(B) streptogramin A resistance genes or the *vgb*(A) and *vgb*(B) streptogramin B resistance genes (4, 6) previously detected in staphylococci. The *erm*(B) gene, conferring macrolide-lincosamide-streptogramin B resistance (13), was detected in 82% of the isolates tested and in ≥75% of isolates in each quinupristin-dalfopristin resistance category (Table 2). The absence of *erm*(B) from some *vat*(D)- or *vat*(E)-positive isolates does not rule out the

TABLE 2. Categories of quinupristin-dalfopristin-resistant *E. faecium* based on amplification of acetyltransferase genes

Source or group	No. (%) in PCR group			
	vat(D)$^+$	*vat*(E)$^+$	*vat*(D) and *vat*(E) negative[a]	Total
Human	0	4	0	4
Sewage	0	0	2	2
Animal	6	7	5	18
Raw meat	0	3	1	4
Total	6 (21)	14 (50)	8 (29)	28 (100)
erm(B)$^+$ isolates	6 (100)	11 (79)	6 (75)	23 (82)

[a] Did not give a PCR product with M and N, degenerate primers for genes that encode streptogramin A acetyltransferases.

presence of other genes conferring resistance to streptogramin B compounds; it has been reported recently that resistance to both the A and B components is necessary to confer high-level resistance to streptogramin combinations on *E. faecium* (Bozdogan and Leclercq, 39th ICAAC).

When degenerate primers M and N were used to detect genes likely to encode streptogramin A acetyltransferases, amplicons of ca. 150 bp were obtained from the 20 *vat*(D)- or *vat*(E)-positive isolates but from none of the 8 negative isolates. When the DNA was blotted onto nylon membranes, the M and N amplicons from *vat*(D)-positive strains hybridized only with a *vat*(D)-specific probe, and those from *vat*(E)-positive isolates hybridized only with a *vat*(E)-specific probe. Plasmid DNA prepared from the eight *vat*(D)-negative, *vat*(E)-negative isolates failed to hybridize with either *vat*(D)- or *vat*(E)-specific probes. The *vat*(E)-specific probe hybridized with plasmids in the range of 35 to 100 MDa in all *vat*(E)-positive isolates (not shown).

The proportion of quinupristin-dalfopristin-resistant isolates shown to contain *vat*(D) (21%) is similar to that reported by other workers (11, 12). The majority of *vat*(D)-negative isolates (63% [14 of 22]) carried the recently described *vat*(E) gene (20). Our data indicate wide dissemination of *vat*(E), which we confirmed to be plasmid mediated, among enterococci from diverse sources and countries. One of the resistance plasmids now known to carry *vat*(E) could be transferred in vitro to a sensitive laboratory strain of *E. faecium* (23). Eight diverse isolates expressed lower levels of streptogramin resistance but contained no detectable acetyltransferase genes. The degenerate primers used yielded products with the five genes, *vat*(A) to *vat*(E), known to encode streptogramin A acetyltransferases, but we cannot rule out the possibility that these isolates contain unrelated acetyltransferases. However, their streptogramin resistance may have resulted from a mechanism unrelated to acetyltransferase production. Active efflux associated with ATP-binding cassette transporters (3, 5) is the only other mechanism reported to confer resistance to streptogramin A compounds, and it is possible that these isolates have such a mechanism, although the demonstration of this requires further study.

In conclusion, we have shown that distinct categories of quinupristin-dalfopristin resistance exist in isolates of *E. faecium*. The Vat(D) and Vat(E) acetyltransferases occurred in enterococci from different European countries and conferred resistance to both quinupristin-dalfopristin and virginiamycin. An undefined mechanism conferred higher levels of resistance to quinupristin-dalfopristin than to virginiamycin. A wide distribution of *vat*(D) in strains from human and nonhuman sources has been reported previously (11, 12, 19). Similarly, in this study we detected *vat*(E) on plasmids in *E. faecium* isolated from farm animals, meat, and UK hospital patients; comparable findings have been reported in Germany (20). None of the UK patients had received quinupristin-dalfopristin. It therefore seems likely that exchange of resistant strains or resistance genes may occur between *E. faecium* isolates from nonhuman and human sources. Until such time as these fears are proven to be unfounded, the occurrence of virginiamycin-resistant *E. faecium* in animals must be considered to remain a potential risk to public health, the extent of which has yet to be quantified.

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