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NOTES

Quinupristin-Dalfopristin Resistance in *Enterococcus faecium* Isolates from Humans, Farm Animals, and Grocery Store Meat in the United States

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Three hundred sixty-one quinupristin-dalfopristin (Q-D)-resistant *Enterococcus faecium* (QDREF) isolates were isolated from humans, turkeys, chickens, swine, dairy and beef cattle from farms, chicken carcasses, and ground pork from grocery stores in the United States from 1995 to 2003. These isolates were evaluated by pulsed-field gel electrophoresis (PFGE) to determine possible commonality between QDREF isolates from human and animal sources. PCR was performed to detect the streptogramin resistance genes *vatD*, *vatE*, and *vgbA* and the macrolide resistance gene *ermB* to determine the genetic mechanism of resistance in these isolates. QDREF from humans did not have PFGE patterns similar to those from animal sources. *vatE* was found in 35%, 26%, and 2% of QDREF isolates from turkeys, chickens, and humans, respectively, and was not found in QDREF isolates from other sources. *ermB* was commonly found in QDREF isolates from all sources. Known streptogramin resistance genes were absent in the majority of isolates, suggesting the presence of other, as-yet-undetermined, mechanisms of Q-D resistance.

Enterococci are a common cause of nosocomial infections in the United States and are a particular concern in hospital intensive care units, where they are the third-leading cause of infections (9, 25). Treatment of patients with illness caused by *Enterococcus faecium* isolates poses a particular challenge due to their acquired resistance to multiple antimicrobial agents. Quinupristin-dalfopristin (Q-D) is a streptogramin antibiotic used to treat hospitalized patients infected with vancomycin-resistant *E. faecium* (8, 10, 14, 20, 21, 22, 23). Virginiamycin, a streptogramin compound that has been used in animal feed in the United States for growth promotion, has cross-resistance with Q-D. In this study, we evaluated Q-D-resistant *E. faecium* (QDREF) isolates from humans, food animals, and grocery store meat for strain relatedness. We also performed PCR to determine whether the streptogramin resistance genes previously found in enterococci, *vatD*, *vatE* (both encoding resistance to streptogramin A compounds), and *vgbA* (encoding resistance to streptogramin B compounds), and the macrolide resistance gene *ermB* were present in these isolates. The horizontal transfer of these genes has previously been demonstrated in vitro (12, 13, 18, 29, 30, 37) and, for *vatD*, in vivo (16). Gene linkage between both *vatD* and *ermB* and *vatE* and *ermB* has also been demonstrated previously (5, 13, 18, 37). The public health concern is that QDREF isolates from a food animal reservoir could potentially cause infections in humans or transfer streptogramin resistance determinants to *E. faecium* in humans. This could compromise the effectiveness of Q-D and further limit treatment options for patients with multiple-antimicrobial-resistant *E. faecium* infections.

Three hundred sixty-one *E. faecium* isolates obtained from 1995 to 2003 with a Q-D MIC of ≥4 μg/ml were evaluated. Fifty-seven isolates (45 inpatients and 12 outpatients) were obtained from human stool samples from Georgia, Illinois, Massachusetts, Michigan, Minnesota, Nevada, New York, Oregon, and Pennsylvania. Two hundred eighty-three QDREF isolates were obtained from farm animals in Michigan, Indiana, and Wisconsin (15). There were 105 turkey isolates, including 31 isolates cultured from individual cloacal swabs from turkeys on a farm that used virginiamycin (34). The remaining turkey isolates were obtained from cultures of manure drag swabs collected on seven farms, four of which used virginiamycin (15). The 62 chicken isolates were obtained from cultures of manure drag swabs collected on 10 chicken farms, 7 of which used virginiamycin (15). The 62 swine isolates, 51 dairy cattle isolates, and 3 beef cattle isolates were obtained from cultures of feces from individual animals at 10 swine, 13 dairy, and 3 beef farms, none of which used virginiamycin (15).

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TABLE 1. Q-D susceptibility testing, comparison of PFGE strain types, and streptogramin and macrolide resistance gene content for QDREF isolates from humans, farm animals, and grocery store meat sources

Source	Total no. of isolates	No. of isolates with Q-D MIC (μg/ml) of:			No. of PFGE groups[a]	No. of unique[b] PFGE types (%)	No. of isolates positive for *vatE* (%)	No. of isolates positive for *ermB* (%)
		4	8	≥16				
Humans	57	37	17	3	7	25 (44)	1 (2)	48 (84)
Hospitalized	45	27	17	1	6	15 (33)	0	43 (95)
Outpatients	12	10	0	2	1	10 (83)	1 (8)	5 (42)
Farm animals	283	100	81	102	61[c]	82[d]	53 (19)	198 (70)
Turkeys	105	13	31	61	24	27	37 (35)	71 (68)
Chickens	62	0	22	40	12	29	16 (26)	23 (37)
Swine	62	42	19	1	11	17	0	61 (98)
Dairy cattle	51	42	9	0	14	9	0	42 (82)
Beef cattle	3	3	0	0	3	3	0	1 (33)
Grocery store meat	21	3	4	14	6	10 (48)	0	11 (52)
Chicken	18	1	4	13	3	7 (39)	0	8 (44)
Ground pork	3	2	0	1	3	3 (100)	0	3 (100)
Total	361	140	102	119	74	117 (32)	54 (15)	257 (71)

[a] Each group contains more than one isolate with PFGE patterns with ≥80% similarity.
[b] Only one isolate with this PFGE pattern.
[c] One PFGE group of turkey isolates also contained one chicken isolate, and one strain type unique among turkeys had a related PFGE pattern compared to two chicken isolates.
[d] One PFGE group of chicken isolates also contained one turkey isolate, and two strain types unique among chickens contained turkey isolates.

Twenty-one QDREF isolates were cultured from grocery store meat samples (18 from chicken carcasses and 3 from ground pork) purchased in Georgia, Maryland, Michigan, Minnesota, and Oregon.

Human stools, farm animal samples (feces, cloacal swabs, and manure drag swabs), and grocery store meat samples were collected and cultured using previously described methods (7, 15, 22). Enterococcosel medium (BD Diagnostics, Sparks, MD) containing 4 μg/ml Q-D was used for selecting QDREF isolates (7, 15, 22). Isolates were identified as *E. faecium* by using standard biochemical reactions (11). In vitro MICs of Q-D (read at 16 to 20 h) were determined for all isolates using a standardized broth microdilution method and interpretive standards described by CLSI (formerly NCCLS) (24), with an MIC of ≥4 μg/ml being resistant.

Genomic DNA was prepared using a previously described method (6). BioNumerics software (Applied Maths, Kortrijk, Belgium) was used to calculate percent similarities (Dice coefficient) of pulsed-field gel electrophoresis (PFGE) patterns. Isolates were considered to be related if their PFGE patterns were ≥80% similar. PCR was performed to determine the presence of the known enterococcal streptogramin resistance genes, *vatD*, *vatE*, and *vgbA*, and the macrolide resistance gene *ermB* by using previously described methods (3, 27, 31, 35).

Q-D MICs ranged from 4 to ≥16 μg/ml (Table 1). Ninety-six percent of isolates with MICs of ≥16 μg/ml were isolated from turkeys on farms or grocery store samples of chicken or pork. Of the 45 isolates from hospitalized patients, 39 (87%) were also vancomycin resistant. None of the QDREF isolates from outpatients, farm animals, or grocery store meats were vancomycin resistant.

PFGE analysis produced 197 different patterns (<80% similarity) for the 361 QDREF isolates evaluated (Table 1). Seven PFGE groups of QDREF isolates from humans contained multiple isolates (Fig. 1), one with 12 isolates from four hospitals in one city in Michigan, one with eight isolates from hospitals in three cities in Michigan, one with two isolates from two outpatients in Michigan, one with three isolates from outpatients in three different states (Massachusetts, Michigan, and New York), one with two isolates from patients in one hospital

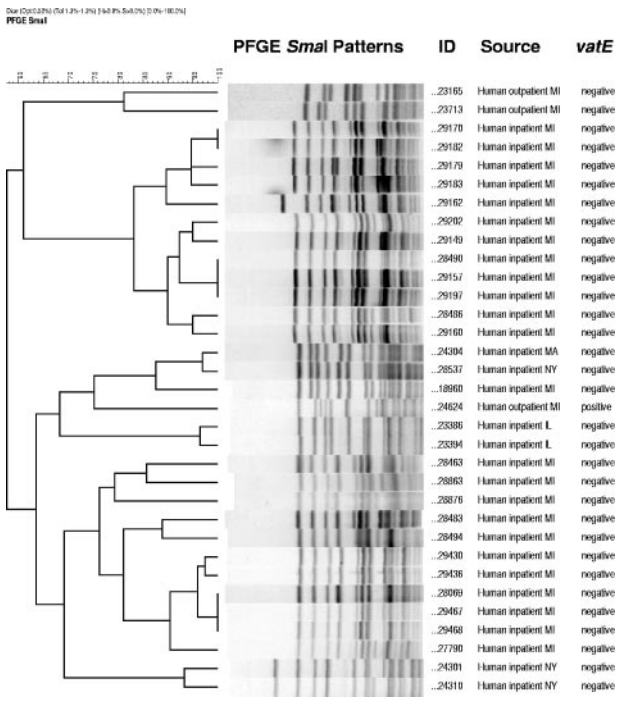

FIG. 1. Dendrogram showing PFGE groups for quinupristin-dalfopristin-resistant *E. faecium* isolates from human sources.

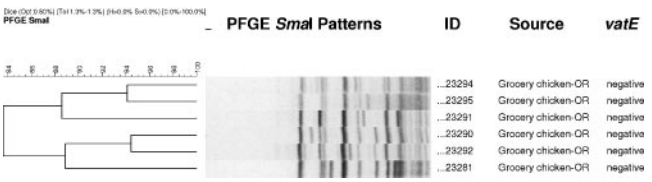

FIG. 2. Dendrogram showing related SmaI PFGE patterns (≥80% similarity) of QDREF isolates from grocery store chicken.

in Illinois, one with two hospitalized patients in New York, and one with isolates from three hospitalized patients one city in Michigan. QDREF isolates from humans did not share PFGE patterns with QDREF isolates from farm animals or grocery store meat. QDREF isolates from farm animal sources did not share PFGE patterns with QDREF isolates from grocery store meat sources. Three PFGE groups had both turkey and chicken isolates. These three groups consisted of four QDREF isolates from three turkey farms and five QDREF isolates from three chicken farms. Otherwise, related PFGE patterns were not observed in QDREF isolates from different species of farm animals. QDREF isolates from six samples of grocery store chicken from Oregon had related PFGE patterns (Fig. 2). Related PFGE patterns were commonly seen for QDREF isolates from farm animals of the same species on the same farm and on different farms. Six grocery store chicken carcasses from Oregon yielded QDREF isolates with related PFGE patterns. All other chicken and ground pork isolates had unique PFGE patterns.

PCR results for the detection of *vatE* and *ermB* can be found in Table 1. *vatE* was found in one QDREF isolate from a human source (outpatient), 35% of QDREF isolates from turkeys, and 26% of QDREF isolates from chickens but not in QDREF isolates from swine, dairy or beef cattle, hospitalized patients, or grocery store meats. A comparison of SmaI PFGE patterns for *vatE*-positive QDREF isolates is shown in Fig. 3. *ermB* was commonly found (range of 33 to 100%) in QDREF isolates from all types of strain sources (Table 1). *vatD* and *vgbA* were not detected in any isolates.

Although it is clear that the use of antimicrobial agents in animal feed contributes to the emergence and dissemination of antimicrobial resistance in enterococci isolated from farm animals, the human health consequence of this resistance continues to be debated (1, 2, 4, 26, 33). Virginiamycin is still used in food animals in the United States, including chickens, turkeys, swine, and cattle, and strain relatedness and resistance gene content of QDREF isolates has not been well described. We therefore evaluated QDREF isolates from humans, farm animals, and grocery store meats from multiple locations in the United States to gain a better understanding of the epidemiology of Q-D resistance. We found a diversity of PFGE patterns among the QDREF isolates from humans outside of the hospital setting; however, 2 of 12 isolates from outpatients from Michigan had related PFGE patterns (Fig. 1). There was evidence of clonal spread within and between hospitals, and 87% of QDREF isolates from hospitalized patients were also vancomycin resistant. One PFGE group contained eight isolates from three different cities (six hospitals) in Michigan, and two other groups contained multiple isolates from one of these cities (four hospitals). Another PFGE strain type of QDREF

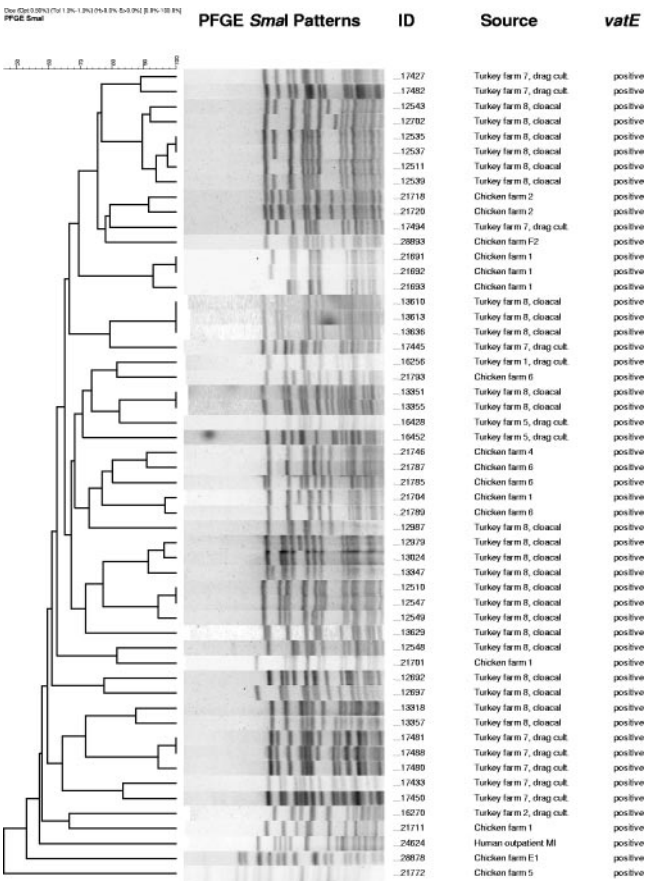

FIG. 3. Dendrogram showing SmaI PFGE patterns of quinupristin-dalfopristin-resistant *E. faecium* isolates positive for the streptogramin A resistance gene *vatE*.

was isolated from hospitalized patients in three states, suggesting broad dissemination between humans. Because these Q-D-resistant isolates were resistant to vancomycin in addition to Q-D, treatment of patients infected with these organisms will be a challenge, which highlights the need for precautions to control the further dissemination of Q-D- and vancomycin-resistant *E. faecium*.

A diversity of PFGE patterns was also found among the QDREF isolates from farm animals and grocery store meat. Although isolates from animals of the same species on the same farm often shared the same PFGE strain type and although those isolates from animals of the same species on different farms occasionally shared the same PFGE strain type, we rarely found QDREF isolates from different animal species with the same PFGE pattern. There were three PFGE groups that contained QDREF isolates from both turkeys and chickens (a total of four turkey and five chicken isolates from six farms). In our study, QDREF isolates with the same PFGE strain type were isolated from five chicken carcasses purchased from grocery stores in Oregon (Fig. 2); it is not known whether these chickens were obtained from more than one producer. Another study, which evaluated QDREF from retail poultry in the greater Washington, D.C., area, also found a diversity of PFGE patterns with some common PFGE strain types among retail chicken isolates and among retail turkey isolates (30).

We found no common PFGE strain types among QDREF isolates from humans, farm animals, and grocery store meats. Similar observations have been made in other studies, demonstrating a broad diversity of PFGE patterns among enterococci with various resistance phenotypes and suggesting that it is uncommon to find shared PFGE strain types among human and animal isolates of enterococci (1, 36, 38). Evaluation of larger numbers of isolates may be needed to further elucidate clonal relationships. Nonetheless, there have been several reports from Europe and the United States of antibiotic-resistant enterococci with similar or indistinguishable PFGE patterns isolated from humans and animals or humans and retail meat sources (7, 17, 19, 28). In the United States, for example, high-level gentamicin-resistant enterococci from one human and multiple ground pork samples from Michigan grocery stores had related PFGE patterns, and high-level gentamicin-resistant enterococci from one human and one chicken carcass from an Oregon grocery store had indistinguishable PFGE patterns (7). Transient colonization of QDREF isolates of animal origin in human volunteers has been shown previously (32), which may also have implications for the transfer of resistance genes in the human intestine. In the United States, *vatE* has been detected only in QDREF isolates from poultry, and *vatD* has not been found in isolates from any source (29, 30). We found *vatE* in 15% of QDREF isolates (from one human and from turkey and chicken farms) and did not find *vatD*. This differs from findings of European studies, where most QDREF has been attributed to *vatE* or *vatD* (31, 36). We found *vatE* mainly among QDREF isolates from turkeys and chickens. All but one *vatE*-containing Q-D-resistant isolate from farm animals originated from farms using virginiamycin for growth promotion. None of the QDREF isolates from swine or dairy or beef cattle contained the *vatE* gene, and none of these farms used virginiamycin for growth promotion. Importantly, we found *vatE* in one QDREF isolate from a human outpatient. This person had no history of hospital exposure or international travel and had never been treated with Q-D. The isolate from this person was resistant to high levels of Q-D (MIC $\geq$ 16 μg/ml) and yielded a unique PFGE pattern. This is the first report, to our knowledge, of a *vatE*-positive *E. faecium* isolate from a human in the United States.

In this study, we found that there were multiple PFGE strain types of QDREF responsible for Q-D resistance among humans, farm animals, and grocery store meat. We found evidence of animal-to-animal transmission, clonal strains among grocery store meat, and, importantly, the spread of a Q-D-resistant and vancomycin-resistant *E. faecium* strain to 22 patients in six different hospitals in southeastern Michigan. The extent of this spread of an *E. faecium* strain with resistance to both vancomycin and Q-D has not been previously described in U.S. hospitals and is of particular concern. *vatE* was detected commonly in animal isolates and was also found in one human strain, suggesting that horizontal transfer of streptogramin resistance elements is a potential mechanism by which Q-D resistance is spread between humans and animals. However, because only a small proportion of isolates in this study tested positive for previously described resistance genes, it will be of great importance to identify and characterize these new mechanisms of resistance in *E. faecium* in order to trace the origin, epidemiology, and mechanism of QDREF isolates from humans, farm animals, and grocery store meat in the United States.

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