Characterization of Tn1546 in Vancomycin-Resistant Enterococcus faecium Isolated from Canine Urinary Tract Infections: Evidence of Gene Exchange between Human and Animal Enterococci

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Thirty-five enterococcal isolates were recovered from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital over a 2-year period (1996 to 1998). Isolated species included Enterococcus faecium (n = 13), Enterococcus faecalis (n = 7), Enterococcus gallinarum (n = 11), and Enterococcus casseliflavus (n = 4). Antimicrobial susceptibility testing revealed several different resistance phenotypes, with the majority of the enterococcal isolates exhibiting resistance to three or more antibiotics. One E. faecium isolate, CVM1869, displayed high-level resistance to vancomycin (MIC > 32 μg/ml) and gentamicin (MIC > 2,048 μg/ml). Molecular analysis of this isolate revealed the presence of Tn1546 (vanA), responsible for high-level vancomycin resistance, and Tn5281 carrying aac6’-aph2”, conferring high-level aminoglycoside resistance. Pulsed-field gel electrophoresis analysis revealed that CVM1869 was a canine E. faecium clone that had acquired Tn1546, perhaps from a human vancomycin-resistant E. faecium. Transposons Tn5281 and Tn1546 were located on two different conjugative plasmids. Sequence analysis revealed that in Tn1546, ORF1 had an 889-bp deletion and an IS1216V insertion at the 5’ end and an IS1251 insertion between vanS and vanH. To date, this particular form of Tn1546 has only been described in human clinical vancomycin-resistant enterococci isolates unique to the United States. Additionally, this is the first report of a vancomycin-resistant E. faecium isolated from a companion animal in the United States.

Enterococci cause serious illness in immunocompromised patients, including severely ill, hospitalized patients. Resistance to multiple antimicrobials, including resistance to ampicillin and vancomycin, has developed in the past decade, resulting in limited therapeutic options. One of the main reasons for this rapid rise in resistance is the capacity of enterococci to acquire and disseminate antimicrobial resistance determinants, including those that confer resistance to aminoglycosides and glycopeptides.

In susceptible strains, optimal therapy typically involves a combination of a cell wall-active agent and an aminoglycoside, usually gentamicin (28). High-level resistance to gentamicin is usually due to the presence of the bifunctional Aac6’-Aph2” aminoglycoside-modifying enzyme (11). This aminoglycoside-modifying enzyme is highly potent and confers resistance to all the clinically useful aminoglycosides with the exception of streptomycin. The aac6’-aph2” genes have, in most cases, been localized to plasmids. Studies by Hodel-Christian and Murray (17, 18) identified the aac6’-aph2” gene as part of transposon Tn5281. Association of aac6’-aph2” with a transposon further aids in the rapid dissemination of the resistance marker.

Recently there have been reports of truncated Tn5281 structures in enterococcal species (28, 29). The significance of these truncated Tn5281 structures is unknown, but they may represent remnants of incomplete transposon formation (28, 29).

The appearance of high-level gentamicin resistance in clinical medicine in 1979 had a substantial negative effect on the treatment of severe enterococcal infections. The only available antibiotic left thereafter in the mid-1990s for the successful treatment of enterococcal infections was vancomycin. However, over the past decade, there has been a rapid surge in the prevalence of vancomycin resistance among enterococci, predominantly of the vanA and vanH genotypes (38), and glycopeptide-resistant enterococci have emerged worldwide (6, 12, 16, 20, 26, 27). The vanA resistance locus, which is most prevalent, consists of a cluster of seven genes (vanS, vanR, vanH, vanA, vanX, vanY, and vanZ) present on Tn1546, a 10.8-kb transposon (2, 3).

In Europe, the vanA transposable element has been identified in enterococci isolated in the community and from sewage, animal feces, and raw meat, suggesting that each of these may act as a reservoir of both resistant enterococci and the vanA gene (1, 4, 9, 37, 38, 40, 42). The occurrence of vanA outside of the hospital environment has been attributed to the use in Europe of the glycopeptide avoparcin as a growth promoter in pigs and poultry (23). In contrast, vanA has never been identified in the community in the United States. One reason that has been put forth is that glycopeptides have never been approved for food animals in the United States (8, 33). The concurrent appearance of vanA in both Europe and the United States, despite the lack of avoparcin use in the United States, suggests that the epidemiology and global dissemination of vanA are complex (38, 40). Tn1546 is often associated with plasmids, and several reports have emphasized the fact that
there are polymorphisms between Tn1546 elements due to insertions, deletions, and point mutations (10, 13, 14, 36–39, 42).

There have been several worldwide studies documenting the relatedness of vancomycin-resistant enterococci isolates and/or Tn1546 elements found in animals and humans (7, 21, 35, 36, 39, 42). Recently, 24 Tn1546 types were described in Europe and the United States. Some types were specific to human or animal vancomycin-resistant enterococcus isolates, while others were common to both human and animal vancomycin-resistant enterococcus isolates (36–38). Each study indicated a possible role of animals as a source of vancomycin-resistant enterococci, but none of these studies was conclusive.

Recent studies by Willems et al. (36) and Donabedian et al. (10) have shown that two specific forms of Tn1546 (designated F1 and F2) are unique to human vancomycin-resistant enterococcus isolates found only in the United States (Fig. 1). In isolates unique to the United States, Tn1546 shows a deletion of 889 bp in ORF1 and an insertion of IS1216V. In addition, there is an insertion of IS1251 between vanS and vanH. The only difference between the F1 and F2 types described by Willems et al. (36) was a single base change, C→T.

Epidemiological studies in Europe suggest that vancomycin-resistant enterococci are horizontally transmitted from animals to humans (30). However, there have been no reports of high-level vancomycin resistance (>32 μg/ml) in Enterococcus spp. from animal feces in the United States. In view of the possible involvement of companion animals in the spread of antibiotic-resistant enterococci to humans, the aim of the present study was to characterize gentamicin and vancomycin resistance among enterococci isolated from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital over a 2-year period (1996 to 1998).

**MATERIALS AND METHODS**

**Sample collection and isolation of enterococci.** Enterococci were isolated from urine samples taken from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital between 1996 and 1998. A 1-μl aliquot of urine was used to inoculate Columbia agar supplemented with 5% defibrinated sheep blood, 1% yeast extract and 1% horse serum. Inoculated plates were incubated at 36°C in a 5% CO2 environment for 24 h. Following incubation, single colonies characteristic of Enterococcus spp. were subcultured onto Columbia agar supplemented with 5% sheep blood to ensure purity and to check for hemolysis. These colonies were also tested for catalase activity, L-pyrrolidonyl-β-naphthylamide (PYR) production, and Gram stain reaction. Catalase-negative, gram-positive, PYR-positive isolates were confirmed as Enterococcus spp. with the AccuProbe Enterococcus identification test (Gen-Probe, Inc.). AccuProbe-positive isolates were identified to species level with the Vitek automated microbial identification system (bioMerieux Vetek, Inc.).

**Determination of antimicrobial susceptibility of enterococci.** MICs of various antimicrobials for enterococci were determined with the Pasco antimicrobial susceptibility system (Becton-Dickinson, Cockeysville, Md.) and interpreted according to the National Committee for Clinical Laboratory Standards standard for broth microdilution methods (24). Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, and Enterococcus faecalis ATCC 29212 were used as quality control microorganisms.

**DNA extraction, PCR studies, and DNA sequencing.** Total bacterial DNA was extracted with the guanidium thiocyanate method described previously (29) and used as the template for the PCR. Amplification of sacB-aprB2 and vanA was done via PCR with the oligonucleotide primers and PCR conditions previously described (28, 38). We used extended-PCR primers and conditions described previously to confirm the presence of Tn5281 and Tn1546 (29, 42). We further characterized the van4 transposon with 19 PCR primers (P1 to P19, Fig. 1) specific to the different regions of Tn1546 to determine the orientation of the various components of the transposon (38).

**E. faecalis** strain HN-22, which carries Tn5281 on plasmid pBEM10, and **E. faecium** strain BM4147, which carries the Tn1546 transposon on plasmid...
TABLE 1. Primers used for detection of IS216V and IS251 in Tn1546 and primers designed to determine the presence of an 889-bp deletion of ORF1 in Tn1546

| Primer       | Primer sequence (5′ → 3′) | Position in gene |
|--------------|----------------------------|-----------------|
| IS216VF      | CACTGCTCTACACCGATTA        | 558–577         |
| ORF1CompR    | AAGCGAGGTGAGCTAAC          | 279–290         |
| ORF1DelR     | ATCATATGGAGGCTACG          | 1148–1127       |
| VanS         | GCTGCATACGGAGGAT           | 847–864         |
| IS251R       | CAAATGAGGTGACGTC          | 252–235         |
| IS251F       | TCAGAGGACCTTGAGGAC         | 1095–1112       |
| VanHR        | GTTCGCTACTGGCAAG           | 363–346         |

pIPS16, were used as positive controls. PCRs were performed with the AmpliTaq Gold PCR system (Perkin Elmer). In addition, several primers were designed (Table 1) to detect IS216V and IS251. Primers IS216VF and ORF1CompR were used to determine if the first 889 bp of ORF1 were present (expected product of 409 bp). Primers IS216VF and ORF1DelR were used to determine if the first 889 bp of ORF1 were deleted (expected product of 388 bp). In addition, primer vanS-IS251R was used to determine the presence of insertion of IS251 at the stop codon of vanS (expected product of 561 bp). Primers IS251F and vanHR were used to determine the presence of vanH at the stop codon of IS251 (expected product of 562 bp).

All PCR products obtained with the primers described in Table 1 were sequenced commercially (Seqwright, Houston, Tex.) to confirm the identity of the PCR products.

Pulsed-field gel electrophoresis (PFGE) was performed after DNA digestion with BsmII as described previously (32). To analyze the PFGE results for strain relatedness of the E. faecium canine isolates, we used the interpretive criteria of Tenover et al. (31). Comparisons of the PFGE fingerprinting were made with computer-assisted analysis (BioNumerics, Applied Maths).

Conjugation of gentamicin and vancomycin resistance determinants. The transfer frequency of the aminoglycoside and glycopeptide resistance determinants was determined by the filter mating method (29). The recipient strain used for the conjugation studies was E. faecium GE-1, which displays resistance to rifampin and fusidic acid. Following mating, transconjugants were selected on blood agar plates containing rifampin (50 μg/ml) or gentamicin (25 μg/ml) and fusidic acid (25 μg/ml) or a combination of vancomycin and gentamicin (16 and 250 μg/ml, respectively). From each mating, 10 transconjugants were selected, and their susceptibility profiles and plasmid contents were analyzed.

Plasmid analysis. Plasmid extraction was done by the alkaline lysis method described previously (29) to determine the presence and number of plasmids in E. faecalis (CVM1869) to determine the presence and number of plasmids in the canine urinary tract infections (Table 1) to detect IS216V and IS251. Primers IS216VF and ORF1CompR were used to determine if the first 889 bp of ORF1 were present (expected product of 409 bp). Primers IS216VF and ORF1DelR were used to determine if the first 889 bp of ORF1 were deleted (expected product of 388 bp). In addition, primer vanS-IS251R was used to determine the presence of insertion of IS251 at the stop codon of vanS (expected product of 561 bp). Primers IS251F and vanHR were used to determine the presence of vanH at the stop codon of IS251 (expected product of 562 bp). All PCR products obtained with the primers described in Table 1 were sequenced commercially (Seqwright, Houston, Tex.) to confirm the identity of the PCR products.

RESULTS

Bacterial isolates and antimicrobial susceptibility profiles. A total of 35 enterococcal isolates were recovered from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital. Species isolated included E. faecium (n = 13), E. faecalis (n = 7), Enterococcus gallinarum (n = 11), and Enterococcus casseliflavus (n = 4). Overall, E. faecium showed resistance to the largest number of antimicrobials tested, with 54% (6 of 13) displaying high-level gentamicin resistance (Table 2). A single E. faecium isolate (CVM1869) displayed resistance to vancomycin with an MIC of >32 μg/ml. Thirty-eight percent of the E. faecium isolates displayed resistance to erythromycin, and 84% displayed resistance to penicillin and tetracycline.

A lower prevalence of resistance was seen with E. faecalis isolates recovered from the canine urinary tract infections (Table 2). None of the E. faecalis isolates displayed high-level gentamicin resistance. Only 14% (1 of 35) were resistant to tetracycline, and no E. faecalis isolate displayed resistance to vancomycin, erythromycin, and penicillin. Six of 11 (55%) E. gallinarum isolates exhibited high-level resistance to gentamicin, whereas all 11 isolates demonstrated resistance to penicillin, tetracycline, and vancomycin (due to vanC-1). None of the E. gallinarum isolates was resistant to erythromycin. All four E. casseliflavus isolates demonstrated resistance to erythromycin and tetracycline. Three of the four E. casseliflavus isolates demonstrated resistance to penicillin. Resistance to vancomycin was also noted and can be accounted for by the presence of the intrinsic vanC-2.

Interestingly, one E. faecium isolate, CVM1869, displayed high-level resistance to both gentamicin (MIC, >2,046 μg/ml) and vancomycin (MIC, >32 μg/ml), as well as resistance to penicillin and tetracycline. This vancomycin-resistant high-level gentamicin-resistant isolate was examined further to characterize the molecular organization of the vancomycin resistance gene cluster.

Detection of vanA and aac6′-aph2′ by PCR. PCR studies confirmed that the high-level gentamicin resistance could be attributed to the presence of aac6′-aph2′, flanked by IS256 elements to form transposon Tn5281, while the presence of Tn1546 accounted for vancomycin resistance.

The orientation of the Tn1546 constituents was determined with primers P1 to P19. It was interesting that no PCR product was obtained with primers P1 and P2 (Fig. 1), suggesting that ORF1 was either missing or disrupted. In addition, no PCR products were obtained with primers P1 and P2 (Fig. 1), indicating disruptions or insertions in the intergenic spacer region between vanS and vanH. To investigate these possibilities further, additional PCR analyses were conducted with the primers described in Table 1.

No PCR product was obtained with the IS216VF and ORF1CompR primers, suggesting that ORF1 was truncated. However, when the IS216VF and ORF1DelR primers were used, a PCR product of 388 bp was obtained, suggesting that in E. faecium isolate CVM1969, the first 889 bp of ORF1 had been deleted. This was confirmed by sequencing the 388-bp PCR product, which showed that IS216V was inserted at a site in ORF1 corresponding to a deletion of the first 889 bp.

When PCR was carried out with the vanSF and IS251R primers and the IS251F and vanHR primers, PCR products of 561 bp and 562 bp were obtained, respectively. These PCR products suggest an insertion of IS251 into the intergenic spacer region between vanS and vanH, as was previously described by Willems et al. (36). This was confirmed by DNA sequencing of the two PCR products (data not shown).

Pulsed-field gel electrophoresis. Typing analysis was carried out on all 13 E. faecium isolates to determine if the canine vancomycin-resistant enterococcus isolate CVM1869 was of human origin or a canine strain that had acquired the Tn1546 transposon. The PFGE patterns were analyzed by computer-assisted analysis (BioNumerics, Applied Maths) and interpreted according to the criteria proposed by Tenover et al. (31). PFGE analysis produced 11 different PFGE types, which we designated PFGE groups A through K. The vancomycin-resistant enterococcus canine isolate CVM1869 was similar
to the canine *E. faecium* isolate CVM7669. Since these two isolates differed by only three bands, they were assigned to PFGE group A. Similarly, *E. faecium* isolates CVM7650 and CVM7689 varied by only one band and were assigned to PFGE group B. The remaining nine canine *E. faecium* isolates did not appear to be related by PFGE analysis and were assigned to PFGE groups C through K.

To determine if PFGE group A isolates were related to any human vancomycin-resistant enterococcus isolates, they were compared to a database of vancomycin-resistant enterococci isolated from humans (10). This database consists of approximately 300 human vancomycin-resistant enterococcus isolates comprising 63 PFGE types isolated in Michigan over a 10-year period. None of the canine enterococcus isolates showed any similarities to the approximately 300 human vancomycin-resistant enterococcus isolates.

**Conjugation analysis.** We were able to transfer both the vancomycin resistance and high-level gentamicin resistance determinants by conjugation either together or independently to the plasmid-free *E. faecium* recipient strain GE-1. Transconjugants were visible after 24 h of incubation at 37°C. When selected on medium containing gentamicin, the conjugation frequency was $4.93 \times 10^{-3}$/recipient. With selection on medium containing vancomycin, the conjugation frequency was $6.24 \times 10^{-3}$/recipient, and on gentamicin-vancomycin-containing medium, the conjugation frequency was $9.18 \times 10^{-4}$/recipient. Ten transconjugants were selected from each mating and studied further to determine their plasmid and antimicrobial profiles.

Transconjugants selected in the presence of gentamicin did not always exhibit resistance to vancomycin. By the same token, transconjugants selected on vancomycin agar did not al-

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**TABLE 2. Antimicrobial susceptibility profiles of the 35 enterococcal isolates used in this study**

| Species and isolate no. | PFGE group | Gentamicin | Vancomycin | Erythromycin | Penicillin | Tetracycline |
|-------------------------|------------|------------|------------|--------------|------------|--------------|
| *E. faecium*             |            |            |            |              |            |              |
| 1869                    | A          | $>2.048$   | $>32$      | 2            | 128        | $>32$        |
| 7627                    | C          | 8          | 1          | $>32$        | 16         | $>32$        |
| 7637                    | D          | 512        | 1          | 2            | $>128$     | $>32$        |
| 7639                    | E          | 64         | 1          | $>32$        | 2          | $>32$        |
| 7650                    | B          | 256        | 1          | 2            | $>128$     | $>32$        |
| 7663                    | F          | 512        | 1          | 2            | 128        | $>32$        |
| 7666                    | G          | 64         | 1          | 4            | 128        | $>32$        |
| 7669                    | A          | $>2.048$   | 1          | $>32$        | $>128$     | 0.5          |
| 7670                    | H          | 256        | 1          | 1            | $>128$     | 32           |
| 7672                    | I          | 4          | $<0.5$     | $>32$        | 128        | $>32$        |
| 7674                    | J          | 4          | $<0.5$     | $<0.12$      | 0.25       | 8            |
| 7683                    | K          | 1,024      | 1          | $>32$        | $>128$     | $>32$        |
| 7689                    | B          | 256        | 1          | 2            | $>128$     | $>32$        |
| % Resistant             |            | 38         | 7          | 38           | 84         | 84           |
| *E. faecalis*            |            |            |            |              |            |              |
| 7624                    | 8          | 1          | 2          | 2            | 4          |
| 7648                    | 8          | 1          | 0.5        | 2            | 1          |
| 7665                    | 64         | 1          | 0.5        | 4            | 1          |
| 7676                    | 8          | 1          | 1          | 2            | 1          |
| 7678                    | 8          | 1          | 0.25       | 2            | 1          |
| 7681                    | 8          | 1          | 0.5        | 4            | 1          |
| 7684                    | 8          | 1          | 1          | 2            | 32         |
| % Resistant             |            | 0          | 0          | 0            | 0          | 14           |
| *E. gallinarum*          |            |            |            |              |            |              |
| 7617                    | 512        | 4          | 4          | 64           | $>32$      |
| 7619                    | 256        | 4          | 2          | 128          | $>32$      |
| 7620                    | 256        | 8          | 2          | $>128$       | $>32$      |
| 7638                    | 256        | 4          | 2          | $>128$       | $>32$      |
| 7641                    | 256        | 8          | 2          | $>128$       | $>32$      |
| 7643                    | 512        | 8          | 2          | $>128$       | 32         |
| 7644 $>2.048$           | 4          | 1          | $>128$     | $>32$        |
| 7662                    | 512        | 4          | 1          | $>128$       | 32         |
| 7668                    | 512        | 4          | 1          | $>128$       | 32         |
| 7671                    | 512        | 4          | 4          | $>128$       | $>32$      |
| 7680                    | 256        | 4          | 1          | $>128$       | $>32$      |
| % Resistant             | 54         | 100        | 0          | 100          | 100        |
| *E. casseliflavus*       |            |            |            |              |            |              |
| 7654                    | 8          | 4          | 2          | 128          | $>32$      |
| 7657                    | 8          | 4          | $>32$      | $>128$       | $>32$      |
| 7662                    | 512        | 8          | $>32$      | $>128$       | $>32$      |
| 7668                    | 256        | 4          | $>32$      | 128          | $>32$      |
| % Resistant             | 25         | 100        | 75         | 100          | 100        |

*Values are MICs (in micrograms/milliliter). NCCLS breakpoints are as follows: for gentamicin, $>500 \mu$g/ml; for vancomycin, $>32 \mu$g/ml; for erythromycin, $>8 \mu$g/ml; for penicillin, $>16 \mu$g/ml; and for tetracycline, $>16 \mu$g/ml.*
ways exhibit gentamicin resistance. When transconjugants were selected on medium containing both gentamicin and vancomycin, high-level resistance to both vancomycin and gentamicin was observed. This suggests that the two resistance loci are present on separate plasmids. The antimicrobial susceptibility profiles of the transconjugants are shown in Table 3.

**Plasmid analysis.** Plasmid preparations were made from the *E. faecium* recipient strain GE-1, the donor strain CVM1869, and all the transconjugants obtained in this study. The parent strain, CVM1869, harbored three plasmids (ca. 70, 25, and 3.5 kb). Although the plasmid profiles of the transconjugants varied, all those that displayed resistance to vancomycin carried the 70-kb plasmid and those resistant to gentamicin harbored the 25-kb plasmid. The transconjugants selected on gentamicin- or vancomycin-selective medium contained both the ca. 70-kb and ca. 25-kb plasmids. In addition, all the transconjugants examined harbored a 3.5-kb plasmid of unknown function.

**DISCUSSION**

The present study documents the phenotypic and genotypic characterization of 35 enterococci isolated over a 2-year period (1996 to 1998) from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital. The predominant species were *E. faecium* (37%) and *E. gallinarum* (31.5%), with *E. faecalis* accounting for 20% of the isolates being recovered and *E. casseliflavus* accounting for 11.5% of the infections. A high incidence of resistance was observed to gentamicin, penicillin, and tetracycline, whereas all the *E. faecalis* and *E. gallinarum* strains were susceptible to erythromycin.

There have been numerous reports from Europe documenting the presence of vancomycin-resistant *E. faecium* in farm animals (9, 19, 25, 38) and companion animals (5, 9). However, each of the reports documenting vancomycin-resistant *E. faecium* in companion animals involved dogs that were resident on farms where vancomycin-resistant *E. faecium* was also prevalent among other farm animals, such as chickens and pigs. In the present study, one isolate, CVM1869, displayed high-level resistance to vancomycin (MIC, 32 μg/ml) and gentamicin (MIC, >2,048 μg/ml). It is uncertain if the dog from which *E. faecium* CVM1869 was recovered was resident on or had visited a farm. However, as avoparcin has never been approved for animal use in the United States and there have never been any data documenting the recovery of vancomycin-resistant enterococci from farm animals in the United States, we can assume that the scenario present in European farms, which is the exchange of vancomycin-resistant enterococci in farm animals, did not take place in the present situation. To our knowledge, this is the first report of isolation of a vancomycin-resistant *E. faecium* strain from a companion animal in the United States.

As expected, the *aac6'-aph2"* genes were amplified from *E. faecium* strain CVM1869 and found to be part of transposon Tn5281. PCR confirmed that high-level vancomycin resistance could be accounted for by the presence of *vanA* on Tn1546. However, when we tried to characterize the Tn1546 transposon, we could not amplify the left-hand inverse repeat/ORF1 by either PCR or extend-PCR. The absence of this region of Tn1546 has been reported previously by Woodford et al. (38) in vancomycin-resistant *E. faecium* isolates collected from noncompanion animals and sewage. Detailed molecular characterization of Tn1546 from *E. faecium* CVM1869 showed that Tn1546 had an 889-bp deletion in ORF1 and an insertion of IS2166. In addition, IS251 was present between *vanS* and *vanH*. This particular form of Tn1546 has only been reported to date in human clinical vancomycin-resistant *E. faecium* isolates in the United States.

Conjugation analysis showed that in *E. faecium* CVM1869, Tn5281 and Tn1546 were located on separate plasmids. Tn5281 was present on a ca. 25-kb plasmid. PCR studies and conjugation analysis confirmed that the *vanA* cluster was present within transposon Tn1546, being carried on a ca. 70-kb plasmid.

As *E. faecium* CVM1869 contained a Tn1546 transposon indistinguishable from Tn1546 found in human vancomycin-resistant
resistant *E. faecium* unique to the United States, all PFGE group A isolates were compared to a PFGE database consisting of human vancomycin-resistant *E. faecium* isolates found in the state of Michigan. This database, containing almost 300 human vancomycin-resistant *E. faecium* isolates comprising 63 different PFGE types, failed to yield any human vancomycin-resistant *E. faecium* PFGE patterns comparable to our PFGE group A pattern. This would suggest that canine PFGE group A isolates have yet to be described in human vancomycin-resistant *E. faecium* infections or else, more likely, PFGE group A is a unique canine clone, with CVM1869 acquiring Tn1546 from an external source.

PFGE group A contains two isolates, CVM1869 (Van* Gen*) and CVM7669 (Van* Gen*). Plasmid analysis showed that isolate CVM7669 contained two plasmids (ca. 70 kb, 25 kb, and 3.5 kb) while isolate CVM1869 contained three plasmids (ca. 70 kb, 25 kb, and 3.5 kb) and showed decreased susceptibility to tetracycline. Thus, the resistance determinants for tetracycline in addition to those for vancomycin may be present on the ca. 70-kb plasmid.

To our knowledge, there are only two reports documenting the genetic linkage of high-level gentamicin and vancomycin resistance. The first study by Woodford et al. (41) identified a 55-Mda plasmid in a clinical *E. faecalis* isolate. However, this plasmid carried the vanB gene, not vanA, along with aac(6′)-aph(2″). A second study by Cercenado et al. reported high-level gentamicin and vancomycin resistance in *E. faecium* and *Enterococcus durans* isolated in a pediatric hospital (7). Both these isolates carried a 40-kDa plasmid. Hybridization studies confirmed that the vanA gene was present on this plasmid, and although not conclusively shown by hybridization studies, the authors concluded that aac(6′)-aph(2″) was also probably present on the same plasmid.

Several speculations can be made as to how a canine *E. faecium* strain may have acquired a Tn1546 which, to date, has only been described in vancomycin-resistant *E. faecium* strains isolated from humans in the United States. Although direct selection pressure would be the most likely cause of acquisition of Tn1546, there is no record of the dog’s being administered vancomycin for treatment of its urinary tract infection. Additionally, it may be possible that either the dog owner or a member of the veterinary hospital staff attending the dog was the source of the vancomycin-resistant *E. faecium* transposon, perhaps on the 70-kb plasmid. Although this seems like a feasible route, no samples from either the dog owner or attending hospital staff are available for analysis.

In summary, we have described the first U.S. report of a Tn1546 transposon in a vancomycin-resistant *E. faecium* canine isolate that is indistinguishable from Tn1546 found in vancomycin-resistant *E. faecium* human isolates. These data demonstrate that exchange of resistance determinants between human and canine enterococcal strains can occur. The potential role that companion animals may play in the dissemination of genes conferring clinically relevant resistance among enterococci requires further study.

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