

A Clonal Lineage of VanA-Type *Enterococcus faecalis* Predominates in Vancomycin-Resistant Enterococci Isolated in New Zealand

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Avoparcin was used as a feed additive in New Zealand broiler production from 1977 until June 2000. We report here on the effects of the usage and discontinuation of avoparcin on the prevalence of vancomycin-resistant enterococci (VRE) in broilers. Eighty-two VRE isolates were recovered from poultry fecal samples between 2000 and mid-2001. VRE isolates were only obtained from broiler farms that were using, or had previously used, avoparcin as a dietary supplement. Of these VRE isolates, 73 (89%) were VanA-type *Enterococcus faecalis* and nine (11%) were VanA-type *Enterococcus faecium*. All *E. faecalis* isolates were found to have an identical or closely related pulsed-field gel electrophoresis (PFGE) pattern of *Sma*I-digested DNA and were susceptible to both ampicillin and gentamicin. The PFGE patterns of the nine *E. faecium* isolates were heterogeneous. All VRE contained both the *vanA* and *ermB* genes, which, regardless of species or PFGE pattern, resided on the same plasmid. Eighty-seven percent of the VRE isolates also harbored the *tet*(M) gene, while for 63 and 100%, respectively, of these isolates, the avilamycin and bacitracin MICs were high (≥ 256 $\mu\text{g/ml}$). Five of eight vancomycin-resistant *E. faecalis* isolates recovered from humans in New Zealand revealed a PFGE pattern identical or closely related to that of the *E. faecalis* poultry VRE isolates. Molecular characterization of Tn1546-like elements from the VRE showed that identical transposons were present in isolates from poultry and humans. Based on the findings presented here, a clonal lineage of VanA-type *E. faecalis* dominates in VRE isolated from poultry and humans in New Zealand.

Since their discovery in 1986 (34, 43), vancomycin-resistant enterococci (VRE) have emerged as a major cause of nosocomial infection. In the United States, the first VRE isolate was found in 1987 (37), and by 1997, more than 15% of nosocomial enterococcal infections in United States hospitals were due to VRE (14). Surveillance of VRE in the United States has shown that *Enterococcus faecium* is the dominant species identified (24). This is also the situation in Europe, where VanA-type *E. faecium* is the predominant phenotype isolated from animal, human, or environmental sources (3, 7, 11, 12, 18, 21, 22, 32, 38, 46). It is unclear why this particular species makes up the majority of VRE isolated.

A possible explanation for the emergence and spread of VRE in Europe has been the use of the growth promoter avoparcin in animal husbandry. Avoparcin is a glycopeptide produced by *Streptomyces candidus* and is closely related to vancomycin. The discovery in 1993, of VanA-type VRE in food animals in England (7), led to the postulation that food animals might be a potential reservoir for resistance genes. While the extent to which animal sources play a contribution to human infections remains a matter of debate, the use of avoparcin has been associated with an increase in the occurrence of VRE in broilers and pigs (6). Due to the potential for spread of resistance through the food chain, a European Union-wide ban was imposed in 1997 on avoparcin use in animal husbandry. Since this discontinuation, a decrease in the prevalence of VRE in Danish poultry has been observed (4); however, this trend has not been seen in Norway (10).

In New Zealand, VRE are uncommonly recovered from human sources, with only about 16 isolates being recovered prior to 2002 (unpublished data). In this country avoparcin has been used in poultry as a prophylactic for necrotic enteritis caused by *Clostridium perfringens* since 1977. Between 1999 and 2000, 2,195 kg of avoparcin was used. Because of the withdrawal of avoparcin from the market by the manufacturer in 1999, the use of avoparcin was discontinued in June 2000. We report here on the effect of usage and discontinuation of avoparcin on the prevalence of VRE in poultry animals and report the association of a distinct clone of vancomycin-resistant *E. faecalis* with both poultry and humans in New Zealand.

MATERIALS AND METHODS

Bacterial isolates. Poultry VRE were isolated from broilers from four poultry suppliers as described below. Human strains used in this study were isolates from the culture collections of the Institute of Environmental Science and Research: Health, Communicable Disease Centre, Porirua, New Zealand; Auckland District Health Board; and the Canterbury District Health Board. *E. faecium* BM4147, which contains Tn1546 on plasmid pIP816, was used in this study as a positive control in the long-template PCR amplification and digestion of Tn1546. *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 were used to generate intragenic probes for species identification. *E. faecalis* JH2-2 (25) and *E. faecium* GE-1 (20) were recipient strains used in conjugation experiments.

Sampling and isolation of VRE from poultry. In April 2000, fecal samples were obtained from 66 individual broilers from four suppliers, A, B, C, and D in New Zealand. One year later (2001), 10 additional samples from each of four farms, A1, A2, A3, and A4, managed by original supplier A were received (40 samples in total). For each fecal sample, a pea-sized scoop was emulsified in 10-ml *Streptococcus faecalis* medium (Bacto SF medium; Difco Laboratories, Detroit, Mich.). After incubation for 48 h at 35°C, dilutions were made to 10^{-3} and 100 μl was spread onto bile esculin azide (BEA) agar plates (Becton Dickinson and Co., Sparks, Md.) to obtain single colonies. All BEA agar plates were incubated for 48 h at 35°C. The natural ability of enterococci to grow in the presence of bile and to hydrolyze esculin to form black colonies was used for their selection.

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TABLE 1. PCR primers used in this study

Primer	Sequence (5' → 3')	Specificity	Reference
efaAF	CGTTAGCTGCTTGCGGGAATC	<i>efaA</i>	40
efaAR	CCATACTACGTTTATCGACAC		
aac(6')-IIF	GCGGTAGCAGCGGTAGACCAAG	<i>aac(6')-II</i>	19
aac(6')-IIR	GCATTTGGTAAGACACCTACG		
VanABF	GTAGGCTGCGATATTCAAAGC	<i>vanA</i>	8
VanAR	CGATTCAATTGCGTAGTCCAA		
ermB-1	CATTTAACGACGAAACTGGC	<i>ermB</i>	28
ermB-2	GGAACATCTGTGGTATGGCG		
TetM-FW	ACAGAAAGCTTATTATATAAC	<i>tet(M)</i>	5
TetM-RV	TGGCGTGTCTATGATGTTTAC		
VanX1	ACTTGGGATAATTTACCCGG	<i>vanX</i>	27
VanX2	TGCGATTTTGCAGCTTCATTG		
ORF1-F1	AATCTTCATTAAGCTACCTGTCCG	<i>Tn1546</i>	17
VanY-R1	TATCTCATAACGAAGATTAGTCGGC		

Antimicrobial susceptibility testing. Any black esculin-positive colonies growing on BEA agar plates were assumed to be enterococci. One hundred enterococcus-like colonies were chosen randomly from each sample and inoculated onto brain heart infusion (BHI) agar plates (Becton Dickinson and Co.) containing 32 µg of vancomycin/ml. All plate contents were incubated at 37°C and examined for growth after 24 h. Any positive growth was assumed to represent VRE. Vancomycin, teicoplanin, gentamicin, and ampicillin MICs for these isolates were obtained using Mueller-Hinton agar (Difco) plates and E-test strips (AB BIODISK, Solna, Sweden) according to the manufacturer's instructions. All VRE were also examined for their sensitivity to erythromycin (Sigma-Aldrich Chemicals, St. Louis, Mo.), tetracycline (Sigma), avilamycin (Eli Lilly & Co., Indianapolis, Ind.), and zinc bacitracin (50,000 IU/g; Aldrich Chemical Co., Milwaukee, Wis.). The MICs of these antibiotics were determined by following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (36).

PCR amplification. All primer sequences and specificities used in this study are listed in Table 1. For species identification, intragenic probes for *efaA* and *aac(6')-II* were generated by PCR from *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434, respectively. An intragenic probe for *vanA* was generated from *E. faecium* BM4147, while probes for *ermB* and *tet(M)* were generated from an erythromycin- and tetracycline-resistant clinical VRE isolate. All PCR products were sequenced to ensure homology with the published sequence for these genes. Amplification of *vanX* was carried out using the primers VanX1 and VanX2. PCRs were performed in 100-µl volumes with 1 U of *Taq* DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany) in accordance with the manufacturer's instructions and the PCR program described previously (30). DNA template was prepared by dissolving a bacterial colony in 50 µl of water and freeze-thawing at -20°C. PCR products were purified with a PCR purification kit (Roche). *Tn1546*-like elements were amplified using an Expand Long Template PCR system (Roche) and the conditions recommended by the manufacturer. The primers used were ORF1-F1 and VanY-R1 (Table 1). Amplification consisted of one cycle at 94°C for 2 min and 10 cycles at 94°C for 10 s, 65°C for 30 s, and 68°C for 8 min. This was followed by 20 cycles at 94°C for 10 s, 65°C for 30 s, and 68°C for 8 min (with the elongation time increased by 10 s per cycle) and a final cycle at 68°C for 7 min.

Genomic DNA preparation and restriction enzyme digestion. Genomic DNA embedded in agarose was prepared essentially as described by Keis et al. (30) for the preparation of clostridial genomic DNA, except that all steps were carried out aerobically and that bacteria were grown to an optical density at 650 nm of 0.6 in 10 ml of BHI broth. Genomic DNA was extracted from enterococci using the following method: bacterial cultures were grown overnight in 20 ml of BHI broth. Cells were pelleted and washed in 10 ml of Tris-EDTA (TE) buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) and were resuspended in 200 µl of TE-glucose buffer (50 mM glucose, 25 mM Tris-HCl [pH 8.0], and 10 mM EDTA) containing 12.5 mg of lysozyme/ml and 125 U of mutanolysin/ml. The suspension was incubated for 2 h at 37°C before addition of *N*-lauroyl sarcosine to a final concentration of 8% and 4 µg of RNase A/ml and incubation for a further 15 min at 37°C. To this mixture, 4 µg of pronase/ml was added and the solution was incubated for 30 min at 37°C. TE buffer was used to bring the volume up to 600 µl. The DNA was extracted twice with buffer-saturated phenol, three times with phenol-chloroform-isoamyl alcohol (25:24:1), and once with chloroform-isoamyl alcohol (24:1). DNA was precipitated with 1 volume of

isopropanol and 1/10 volume of 3 M sodium acetate, pH 5.2, washed twice with 70% ethanol, air dried, and resuspended in 100 µl of TE buffer.

Genomic DNA embedded in agarose was digested with the restriction endonucleases *SmaI* (Roche) and *I-CeuI* (New England Biolabs, Inc., Beverly, Mass.) by equilibrating slices of DNA plugs (10 by 2 mm) three times for 15 min in 100 µl of the restriction enzyme buffer recommended by the manufacturer before adding 10 U of the enzyme. DNA plugs digested with *SmaI* were preincubated overnight at 4°C prior to digestion at 25°C for 4 h. *I-CeuI* digests were incubated at 37°C for 4 h. For reproducibility, the *I-CeuI*-digested DNA plugs were treated after digestion with 0.5 mg of proteinase K/ml in EDTA-*N*-lauroyl sarcosine buffer (0.5 M EDTA [pH 9.0], 1% *N*-lauroyl sarcosine) as described previously (31). Long-template PCR products were digested with 10 U of *ClaI* (Roche), in the buffer recommended by the manufacturer, for 3 h at 37°C.

PFGE and agarose gel electrophoresis. Pulsed-field gel electrophoresis (PFGE) was performed by contour-clamped homogeneous electric field electrophoresis using the CHEF-DRIII system (Bio-Rad Laboratories, Richmond, Calif.). Agarose plugs were equilibrated three times in 0.5× Tris-borate-EDTA buffer (0.45 mM Tris-borate, 1 mM EDTA) for 15 min prior to electrophoresis. Gels were routinely run at 6 V/cm, 14°C, at an included angle of 120°, on a 1.2% agarose gel (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England) with pulse times of 5 to 25 s for 22 h. The Low Range PFG Marker (New England Biolabs, Inc.) containing lambda concatemers and lambda-digested *HindIII* fragments was used as a size standard. Long-template PCR products digested with *ClaI* were electrophoresed for 2 h at 70 V in 0.8% LE agarose (Roche).

Southern hybridization. The DNA from enterococcal colonies was bound to nitrocellulose filters as follows: overnight cultures, grown in BHI broth were stamped onto Hybond-N⁺ nylon membranes (Amersham) overlaid on BHI agar and were incubated for 24 h at 37°C. Colonies were fixed and lysed by soaking the membranes in denaturation solution (0.5 M NaOH, 1.5 M NaCl) for 40 min and by boiling twice in 0.5% sodium dodecyl sulfate for 20 min. Membranes were washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and blotted dry. DNA fragments separated by PFGE were transferred onto Hybond-N⁺ nylon membrane using a VacuGene (Pharmacia-LKB) vacuum blotting system, as described previously (31).

Radiolabeled PCR products were prepared by incorporation of [α -³²P]dCTP-labeled deoxyribonucleotides (Amersham) using the RTS Radprime DNA labeling kit (Gibco BRL Life Technologies, Gaithersburg, Md.). Prehybridization and hybridization were carried out in the same hybridization buffer (16) at 65°C for 18 to 24 h. After hybridization, membranes were washed stringently as described by Keis et al. (31).

DNA sequencing and analysis. PCR products were sequenced directly. Sequencing reactions were carried out by using a PRISM ready reaction DyeDeoxy terminator cycle sequencing kit (Applied Biosystems Inc., Warrington, United Kingdom) and a model ABI377 automated DNA sequencer (Applied Biosystems). DNA sequences were aligned using the LaserGene application software Megalign (DNASTAR, Inc.) for Apple Macintosh computers, which employed the algorithm CLUSTALV.

Conjugation and plasmid-curing experiments. Potential donor strains were mated with various recipient strains to determine whether antimicrobial resistance could be transferred. The recipient strains were *E. faecalis* JH2-2 (25) and *E. faecium* GE-1 (20), which are resistant to fusidic acid and rifampin. Transfer

in broth and transfer on plates were carried out as described earlier by Christie et al. (15) and Carias et al. (13), respectively. Transconjugants were selected on BHI agar plates containing vancomycin (32 µg/ml), rifampin (50 µg/ml), and fusidic acid (25 µg/ml).

Plasmid curing was carried out by subculturing an overnight culture grown in BHI broth at 37°C for 28 days.

RESULTS

Isolation and characterization of VRE isolates from poultry.

In 2000, a total of 500 enterococci were isolated from the feces of 66 individual broilers from four poultry suppliers in New Zealand. One of these suppliers (supplier A) was confirmed to have used avoparcin for an extended period of time, whereas the remaining three suppliers (B, C, and D) had never used avoparcin as a feed additive. No VRE were isolated from suppliers B, C, and D; however, 18 VRE out of a total of 200 enterococcus isolates tested were cultured from supplier A broiler fecal samples. All 18 VRE isolates had high-level resistance to vancomycin and teicoplanin (MICs \geq 256 µg/ml) but were susceptible to ampicillin and gentamicin. With species-specific intragenic probes, two of the VRE isolates were identified as *E. faecium* and the remaining 16 as *E. faecalis*. Colony hybridization with a PCR product specific for the *vanA* gene demonstrated that all VRE contained this gene. Furthermore, all VRE were PCR positive for *vanX* and exhibited the guanine base pair variation in the *vanX* gene at position 8234 (data not shown) as previously reported for poultry isolates (26).

Forty additional samples were received in 2001 from four farms, A1, A2, A3, and A4, belonging to supplier A. Four hundred enterococci were tested in 2001. In farm A1, no resistant enterococci were detected. In farm A2, 14 of 100 enterococci tested were found to be vancomycin resistant. DNA hybridization, using species-specific probes, identified two VRE isolates as *E. faecium* and the remaining 12 as *E. faecalis*. In farm A3, 10 of 100 enterococci were found to be VRE, with five vancomycin-resistant *E. faecalis* and five vancomycin-resistant *E. faecium* isolates cultured. Forty VRE isolates were cultured from farm A4. All VRE from this farm were *E. faecalis*. In total, 7 *E. faecium* and 57 *E. faecalis* vancomycin-resistant isolates were cultured from the four farms. All 64 VRE isolates revealed high-level resistance to vancomycin and teicoplanin (MICs \geq 256 µg/ml) but were susceptible to ampicillin and gentamicin. All contained the *vanA* gene.

All 82 VRE from both 2000 and 2001 were found to be resistant to erythromycin; MICs ranged from 50 to \geq 256 µg/ml. All contained the *ermB* gene. Seventy-one of the VRE isolates were found to be resistant to tetracycline (MICs \geq 20 µg/ml), and colony hybridization showed that all of these contained the tetracycline resistance gene *tet(M)*. For 52 of the VRE isolates, avilamycin MICs were \geq 256 µg/ml, and for all 82 VRE isolates, bacitracin MICs were \geq 256 µg/ml (data not shown).

DNA fingerprinting of VRE from poultry. DNA fingerprinting of the poultry VRE was carried out by comparing the *Sma*I digestion patterns obtained after PFGE, using the criteria described by Tenover et al. (42), for interpreting chromosomal DNA restriction patterns produced by PFGE. In total, seven PFGE patterns were found among the 82 VRE isolated in 2000 and 2001 from supplier A. VRE isolated in 2000 exhibited one

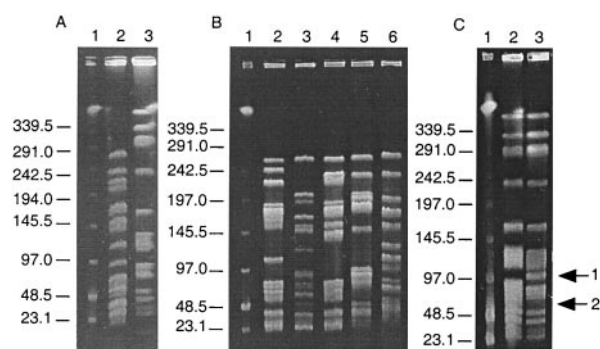


FIG. 1. Representative *Sma*I PFGE patterns among VRE isolated from broilers. (A) VRE isolated in 2000. Lane 1, lambda DNA ladder standard; lane 2, PFGE pattern 2; lane 3, PFGE pattern 1a. (B) Vancomycin-resistant *E. faecium* isolated in 2001. Lane 1, lambda DNA ladder standard; lane 2, PFGE pattern 7; lane 3, PFGE pattern 6; lane 4, PFGE pattern 5; lane 5, PFGE pattern 4; lane 6, PFGE pattern 3. (C) Vancomycin-resistant *E. faecalis* isolated in 2001. Lane 1, lambda DNA ladder standard; lane 2, PFGE pattern 1a; lane 3, PFGE pattern 1b. Arrow 1 indicates the presence of an additional band, while arrow 2 denotes the absence of two bands when compared with PFGE pattern 1a. Sizes indicated are in kilobases.

of two PFGE patterns, depending on the species to which the isolates belonged. All 16 *E. faecalis* isolates had an identical PFGE pattern (designated 1a), whereas both *E. faecium* isolates had the same PFGE pattern (designated 2) (Fig. 1A). A further five PFGE patterns (designated 3 to 7) were identified for *E. faecium* isolates recovered in 2001 (Fig. 1B) and appeared quite unrelated, with differences of more than seven bands. Both *E. faecium* isolates from farm A2 belonged to PFGE pattern 3, while at farm A3 a total of four PFGE patterns were identified, namely, 4, 5, 6, and 7 for the five *E. faecium* strains (Fig. 1B). In contrast to the vancomycin-resistant *E. faecium* isolates, for which no identical PFGE patterns were found from different farms, all vancomycin-resistant *E. faecalis* strains from farms A2, A3, and A4 had the same DNA fingerprint (Fig. 1C). Of 12 randomly chosen vancomycin-resistant *E. faecalis* isolates from farm A4, one belonged to PFGE pattern 1a, while the remaining 11 had a closely related PFGE pattern (designated 1b). This contained one additional band, and two fragments were absent when compared to pattern 1a (Fig. 1C).

Location of the resistance genes. *I-Ceu*I is a restriction enzyme that recognizes a specific site in the 23S rRNA operon of bacterial genomes and cleaves only chromosomal DNA. Hybridization of *I-Ceu*I-digested DNA and nondigested DNA can therefore determine whether a gene is present on the chromosome or is plasmid borne. *I-Ceu*I digestion of genomic DNA was completed on each of the seven poultry VRE PFGE patterns. In all isolates, the same band in both digested and uncut lanes hybridized to both the *vanA* and *ermB* gene probes (Fig. 2 shows a representative pattern for two VRE isolates). In all PFGE patterns, the *vanA* and *ermB* genes appeared to be plasmid borne, hybridizing to bands varying in size from 44.5 to 83.7 kb (Table 2). In PFGE pattern 2 a second plasmid band (approximately 204.7 kb) hybridized to the *ermB* probe (Fig. 2B).

The location of the *tet(M)* gene was also investigated by

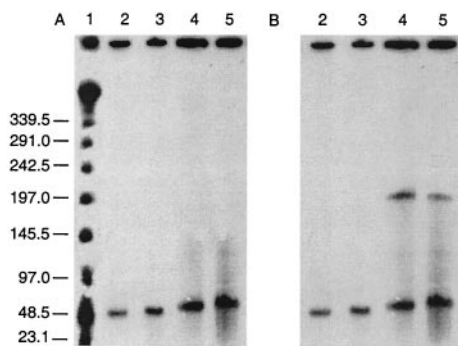


FIG. 2. Hybridization with *vanA* (A) and *ermB* (B) probes to *I-CeuI*-digested genomic DNA of two VRE isolates. Two lanes of genomic DNA from each isolate are shown. The first in each pair was not digested with *I-CeuI*, while the second was incubated with the enzyme. Lane 1, lambda DNA ladder standard; lanes 2 and 3, poultry PFGE pattern 1a; lanes 4 and 5, poultry PFGE pattern 2. Sizes indicated are in kilobases.

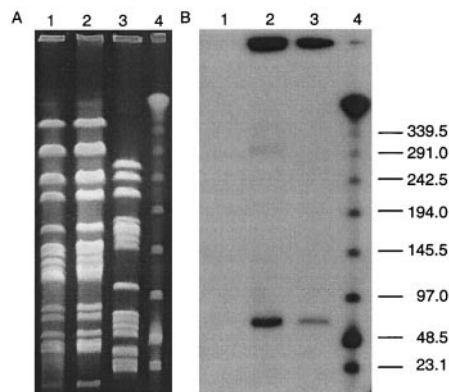


FIG. 3. Conjugative transfer of vancomycin resistance. (A) *SmaI* macrorestriction patterns of strains involved in conjugative transfer of vancomycin resistance; (B) corresponding Southern blot hybridized with a *vanA* gene probe. Lane 1, recipient strain JH2-2; lane 2, transconjugant strain JH2-129; lane 3, donor strain 12-9VP; lane 4, lambda DNA ladder standard. Sizes are indicated in kilobases.

using *I-CeuI* digestion (data not shown). In PFGE patterns 1a, 1b, 2, and 4, the resistance genes appeared to be located on plasmids ranging in size from 51.9 to 204.7 kb (Table 2). In PFGE pattern 2, the *tet(M)* probe hybridized to the same large 204.7-kb fragment that had hybridized to the *ermB* probe. The *tet(M)* genes appeared to be chromosomally located in PFGE patterns 3, 5, and 7, with hybridization occurring to *I-CeuI*-digested bands greater than 339.5 kb. No hybridization was seen with PFGE pattern 6.

The ability of VRE isolates, representative of the different PFGE patterns, to transfer resistance by conjugation was assessed by using both broth and plate mating. Transfer of erythromycin and vancomycin resistance was demonstrated for an *E. faecium* isolate (12-9VP) belonging to PFGE pattern 5. The resulting transconjugant was designated JH2-129. Transfer occurred in broth mating with a frequency of conjugation of 9.0×10^{-10} per recipient to *E. faecalis* JH2-2 but not to *E. faecium* GE-1 ($<10^{-10}$ per recipient). No transfer was observed using plate mating for any of the VRE isolates. Transfer of the plasmid from donor to transconjugant was confirmed by PFGE and hybridization. The *ermB* and *vanA* probes hybridized to a *SmaI* fragment of approximately 68.7 kb in both the donor and transconjugant, illustrating the presence of a transferable ele-

ment (Fig. 3). It is also worth noting that four vancomycin-resistant *E. faecium* isolates, PFGE patterns 3, 4, 6, and 7, became vancomycin susceptible after subculturing twice on BHI agar plates without vancomycin. When further examined, these isolates no longer contained either the *vanA* or *ermB* gene (data not shown), suggesting the loss of the resistance plasmid. The stability of resistance in the vancomycin-resistant *E. faecalis* PFGE pattern 1a was tested by subculturing a representative strain through a series of fresh BHI broth daily for 28 days. No loss of resistance was seen after this time, indicating stability of the plasmid.

Comparison of poultry VRE with clinical VRE in New Zealand. We chose 12 recent human clinical VRE isolates for a comparative analysis with our poultry VRE. Of these 12 isolates, eight were VanA-type *E. faecalis* and four were VanA-type *E. faecium*. All clinical isolates were resistant to gentamicin; MICs of $\geq 256 \mu\text{g/ml}$ were recorded, while only the four *E. faecium* isolates were resistant to ampicillin (MICs $\geq 256 \mu\text{g/ml}$). For six of the clinical VRE isolates, bacitracin MICs were $\geq 256 \mu\text{g/ml}$. The 12 clinical isolates were a heterogeneous group with seven distinct PFGE patterns. However, 5 of the 12 isolates, all VanA-type *E. faecalis*, belonged to a single PFGE pattern, indistinguishable from or closely related to the dominant VanA-type *E. faecalis* pattern found in poultry (Fig. 4A, lanes 4 to 8). PCR amplification and DNA sequencing of the *vanX* gene in the 12 human VRE isolates revealed that the base pair variation at position 8234 was guanine in all eight *E. faecalis* isolates. Thymine was found in position 8234 in the four *E. faecium* isolates. Colony hybridization revealed that all clinical isolates also contained the *ermB* gene conveying erythromycin resistance. The location of the resistance genes was examined in one PFGE pattern 1 clinical isolate, and the *ermB* and *vanA* genes were found to hybridize to a plasmid band of approximately 61 kb (data not shown).

***ClaI* RFLP analysis of Tn1546 elements.** To further examine the relationship between our poultry and human VRE isolates, *ClaI* restriction fragment length polymorphism (RFLP) analysis of Tn1546 elements was carried out. Identical Tn1546 types among human and animal isolates infer horizontal trans-

TABLE 2. Locations and sizes of hybridized *I-CeuI* fragments probed with *vanA*, *ermB*, and *tet(M)* gene products

Strain	PFGE pattern	<i>I-CeuI</i> fragment (length in kb) hybridized with antibiotic resistance genes		
		<i>vanA</i>	<i>ermB</i>	<i>tet(M)</i>
5A-13	1a	P ^a (61.0)	P (61.0)	P (55.5)
1B-10-96	1b	P (66.3)	P (66.3)	P (79.3)
7-11-7	2	P (62.8)	P (62.8, 204.7)	P (204.7)
10-9A	3	P (44.5)	P (44.5)	C ^b
11-3A	4	P (52.8)	P (52.8)	P (82.8)
12-9VP	5	P (68.7)	P (68.7)	C
12-8VP	6	P (75.6)	P (75.6)	ND ^c
12-7VP	7	P (83.7)	P (83.7)	C

^a P, plasmid.
^b C, chromosome.
^c ND, not detected.

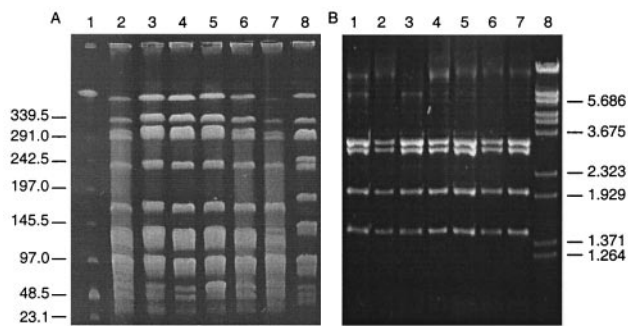


FIG. 4. Comparison of poultry and human VRE in New Zealand. (A) PFGE of *Sma*I macrorestriction patterns in vancomycin-resistant *E. faecalis*. Lane 1, lambda DNA ladder standard; lanes 2 and 3, poultry isolates; lanes 4 to 8, human clinical isolates. (B) Long-template PCR *Cla*I RFLP patterns of Tn1546 elements. Lane 1, *E. faecium* BM4147; lanes 2 and 3, poultry VRE isolates; lanes 4 to 7, human clinical isolates; lane 8, lambda DNA cut with *Bst*EII. *Cla*I fragments are 3,228, 2,968, 2,009, and 1,471 bp. Marker sizes are indicated in kilobases.

fer from a common resistance gene pool. Tn1546 was amplified from *E. faecium* BM4147 and was digested with *Cla*I. Four restriction fragments were obtained, and these were consistent with the predicted sizes of 3,228, 2,968, 2,009, and 1,471 bp from the DNA sequence. This method was applied to all 12 human isolates plus one poultry isolate from each of the seven PFGE patterns. For two *E. faecium* isolates, one poultry and one human, no long-template PCR product was obtained after repeated attempts. Digestion of the remaining 17 positive long-template PCR products gave identical results to BM4147 (Fig. 4B). This observation suggests a degree of conservation between the Tn1546 elements in New Zealand VRE isolates of animal and human origin.

DISCUSSION

This is the first published report of VRE in food animals (in this case broilers) in New Zealand. Resistance to vancomycin in the 82 VRE strains isolated (73 *E. faecalis* and 9 *E. faecium* strains) was always due to the presence of the *vanA* gene and occurred only in enterococci recovered from the poultry supplier that had previously used avoparcin. The dominance of VanA-type *E. faecalis* in our isolates is unusual, as in Europe VanA-type *E. faecium* appears to prevail among animals, including poultry, that are fed avoparcin (2, 33). In fact, European studies have found that, in VRE isolated from human, animal or environmental sources, the *vanA* genotype and species *E. faecium* predominate (3, 7, 12, 18, 21, 22, 32, 38, 46). For example, of 90 VRE strains isolated from poultry carcasses in Norway, none were *E. faecalis* (11). Likewise, a study on vancomycin susceptibility in Denmark among *E. faecium* and *E. faecalis* isolates found 221 vancomycin-resistant *E. faecium* and only 2 vancomycin-resistant *E. faecalis* strains (3). It should be noted that speciation was carried out on our isolates using species-specific probes, rather than by biochemical methods, which can sometimes give misleading results (41). All 73 vancomycin-resistant *E. faecalis* strains included in the present study revealed PFGE patterns ranging from identical to closely related, indicating some degree of clonality among the study

isolates of this species. Conversely, the vancomycin-resistant *E. faecium* isolates revealed a range of distinct PFGE patterns that appeared to dominate at particular farms. This is similar to the findings of Aarestrup et al. (1), who also found heterogeneous *E. faecium* PFGE patterns among broilers.

In our studies, the continued occurrence of VRE in poultry from supplier A 1 year after avoparcin was eliminated as a food additive is not a new finding. As previously documented in pigs in Denmark (1), this may happen if the genes responsible for resistance occur on DNA elements containing genes encoding resistance to other antimicrobials that continue to be included in the animal's diet. All VRE isolates from New Zealand poultry contained both *vanA* and *ermB* genes, which, regardless of species or PFGE pattern, were found residing together on the same plasmid. This genetic linkage of erythromycin and vancomycin resistance has been seen previously in both animal (1, 9) and human (35, 44) isolates. The macrolide tylan is still used in New Zealand poultry animals, and its use could theoretically coselect for vancomycin-resistant bacteria. However, it seems that the farms from which the 2001 samples were taken had not used tylan in the previous 6 months but had used zinc bacitracin. Could it be that the use of zinc bacitracin in the diet is the coselecting force or that VRE clones are somehow better adapted for environmental survival (23) and/or subsequent recolonization of new animals? In this context, vancomycin resistance was lost upon subculturing without selection pressure in four *E. faecium* isolates but was retained in all vancomycin-resistant *E. faecalis* isolates. After subculturing of one *E. faecalis* strain continuously for 28 days, no loss of plasmid was seen. It is possible that the dominance of this vancomycin-resistant *E. faecalis* clone is due to its ability to retain the resistance plasmid in the absence of glycopeptide (i.e., avoparcin) selection pressure. It is also worth noting that the low frequency of transfer of vancomycin resistance seen in this study is likely to add to the dominance of this clonal lineage of vancomycin-resistant *E. faecalis*. Future studies are aimed at establishing the selection factors involved in plasmid maintenance in a glycopeptide-free environment. These data may provide answers as to why this vancomycin-resistant *E. faecalis* clone dominates in VRE isolated in New Zealand.

Although VRE presently appear uncommon in hospitalized patients in New Zealand, a striking factor of recorded cases has been the dominance of VanA-type *E. faecalis* among the isolates. Of the eight vancomycin-resistant *E. faecalis* strains of human origin that we have studied, five appear to be indistinguishable from or genetically related to the dominant poultry vancomycin-resistant *E. faecalis* clone. Indistinguishable PFGE patterns have previously been seen in vancomycin-resistant *E. faecium* from a Dutch farmer and one of his turkeys (45) and in a Danish woman who harbored an isolate very similar to the VRE clone that is predominantly found in Danish pigs (29). In the present study, both human and animal isolates that were typeable through RFLP of the Tn1546 element had an identical arrangement of genes, suggesting a common resistance gene pool and perhaps indicating horizontal transfer among different hosts. In a study by Schouten et al. (39), geographical association of different transposon types was suggested to be a result of differences in meat consumption between European countries. Because New Zealand is geographically isolated, it is not surprising that the resistance genes appear to have a com-

mon origin. Identical Tn1546 types have previously been found in humans and animals (17, 47–49). Because an identical VRE clone is found in poultry and retail chicken portions (unpublished data), current studies are aimed at investigating the prevalence of fecal VRE colonization in healthy humans in New Zealand.

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