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Antibiotic-Resistant *Enterococcus faecalis* in Abattoir Pigs and Plasmid Colocalization and Cotransfer of *tet*(M) and *erm*(B) Genes

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ABSTRACT

This study was conducted to determine plasmid colocalization and transferability of both *erm*(B) and *tet*(M) genes in *Enterococcus faecalis* isolates from abattoir pigs in Canada. A total of 124 *E. faecalis* isolates from cecal contents of abattoir pigs were examined for antibiotic susceptibility. High percentages of resistance to macrolides and tetracyclines were found. Two predominant multiresistance patterns of *E. faecalis* were examined by PCR and sequencing for the presence of genes encoding antibiotic resistance. Various combinations of antibiotic resistance genes were detected; *erm*(B) and *tet*(M) were the most common genes. Plasmid profiling and hybridization revealed that both genes were colocated on a ~9-kb transferable plasmid in six strains with the two predominant multiresistant patterns. Plasmid colocalization and cotransfer of *tet*(M) and *erm*(B) genes in porcine *E. faecalis* isolates indicates that antibiotic coselection and transferability could occur via this single genetic element. To our knowledge, this is the first report on plasmid colocalization and transferability of *erm*(B) and *tet*(M) genes in *E. faecalis* on a mobile genetic element of ~9 kb. Physical linkage between important antibiotic resistance determinants in enterococci is of interest for predicting potential transfer to other bacterial genera.

Enterococci are important nosocomial pathogens with intrinsic resistance to many classes of antibiotics such as cephalosporins, lincomycin, and clindamycin and low-level resistance to aminoglycosides *(24, 35)*. *Enterococcus faecalis* is intrinsically resistant to quinupristin-dalfopristin as a result of the presence of the *lsa* determinant, whose function remains to be explained *(40)*. In addition, enterococci have the capacity to easily acquire and express new resistance genes and can thus tolerate antibiotic selective pressure *(38)*. Antibiotic resistance among enterococci is not restricted to human hospitals. Large amounts of antibiotics are used in animal production for treatment and control of animal diseases. Consequently, the food animal microbial flora frequently carries antibiotic resistance to a range of antibiotics, including those classified as critically important for human therapy *(22)*. Previous studies have revealed that the use of avoparcin and gentamicin for growth promotion and therapy in food animals has led to the emergence of vancomycin- and gentamicin-resistant enterococci in animals and meat *(1, 19, 22)*.

Enterococci usually are found in large numbers in foods of animal origin *(21)*. Contamination of meat commonly occurs during slaughter of the animals, and resistant bacteria can be transmitted to humans through consumption and handling of contaminated food. Glycopeptide-resistant and streptogramin-resistant enterococci ingested by healthy volunteers with food in amounts similar to those present in meat sold in grocery stores can survive gastric passage, can multiply, and can be isolated in the feces for up to 14 days after ingestion *(43)*. Enterococci from food animals can transfer their antibiotic resistance genes, which are located on mobile genetic elements, to human intestinal enterococci *(29, 34)*. Mobile genetic elements and their contribution to the emergence of antibiotic-resistant *E. faecalis* have been recently reviewed *(23),* and both plasmids and transposons were cited as pivotal in the dissemination and persistence of antibiotic resistance in this species. Intestinal enterococci from animals may be more prone than food enterococci to transfer antibiotic resistance to human strains *(46)*. Thus, the presence of antibiotic-resistant enterococci in both animal fecal material and meat products may play a significant role in the dissemination of antibiotic resistance genes.

Despite the importance of mobile genetic elements, we have limited knowledge about the genetic content of specific elements in enterococci *(23),* and information about the molecular basis of antibiotic resistance in animal enterococci from Canada is scarce. A PCR-based typing method targeting replicon-specific plasmid DNA in enterococci and gram-positive bacteria was developed to determine 19 families of plasmids that are associated with very

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TABLE 1. *Selection criteria for the six representative multiresistant isolates of* E. faecalis *harboring* tet*(M) and* erm*(B) genes from the two predominant profiles for plasmid extraction and conjugation*

Antimicrobial resistance phenotype	Strain no.	Antimicrobial resistance genotype	Transposons	
			Tn*916*-Tn*1545* (*int* gene)	Tn*5397* like (*tdnX* gene)
BAC-M-KAN-STR-TET	817	*bcrR, bcrB, erm*(B), *aph(3′)-IIIa, linB, aadE, tet*(M)	−	−
	1,008	*erm*(B), *aph(3′)-IIIa, linB, aadE, tet*(M)	−	−
	1,153	*erm*(B), *tet*(M)	−	+
BAC-M-TET	729	*bcrR, bcrB, erm*(B), *tet*(M)	−	−
	748	*erm*(B), *tet*(M)	−	+
	1,252	*erm*(B), *msr*(C), *tet*(M)	−	−

narrow or broad host ranges *(27)*. Acquired resistance to tetracyclines (TET), which is encoded by the *tet*(M) gene, is often present in enterococci from pigs carrying macrolide (M; erythromycin and tylosin are part of this class) resistance, conferred by the *erm*(B) gene *(17)*. Mobile genetic elements in enterococci that contain both of these genes are usually conjugative transposons with a size range of 25 to 65 kb, such as members of the Tn*916*-Tn*1545* family *(23)*. Recently, we reported on plasmid colocalization and transferability of *erm*(B) and *tet*(O) genes on a ~11-kb plasmid in *E. faecalis* isolates of poultry origin *(45)*. To our knowledge, plasmid colocalization and transferability of *erm*(B) and *tet*(M) genes on a mobile genetic element of ~ 9 kb have not been described in *E. faecalis*.

The aim of this study was to use abattoir pigs in Canada to characterize *E. faecalis* isolates carrying multiple resistances and study plasmid colocalization and transferability of both *erm*(B) and *tet*(M) genes.

MATERIALS AND METHODS

Sample collection and bacterial isolation. Samples were collected once each week from three porcine slaughterhouses located in Quebec, Canada, as previously described *(39)*. Pigs were randomly selected in each abattoir, for a total of 289 pigs representing 83 farms, and samples of cecal contents were collected from each animal. The fecal material was collected with a spatula from the cecum, which was incised with a scalpel. Samples were individually packaged in Whirl-Pak bags (Nasco, Modesto, CA) and assigned a unique identification number. The contents of the cecum were inoculated into enterococcosel broth (Fisher Scientific Canada, Nepean, Ontario, Canada) and incubated at 35°C for 24 h. Ten microliters of the mixture was then plated on enterococcosel agar and incubated at 35°C for 24 h. One to three suspected enterococci colonies (black halo) from each sample were randomly selected and subcultured for purity onto blood agar (tryptic soy agar plus 5% sheep blood; bioMérieux, Hazelwood, MO). From the presumptive enterococci colonies ($n = 701$), a subset of representative isolates ($n = 274$) were randomly selected to represent the 83 farms. These isolates were frozen at −20°C until further analysis.

Identification of *E. faecalis*. The 274 isolates were identified to species by PCR. The species-specific primer set for *ddl faecalis* (D-alanine–D-alanine ligase gene) was used for identification as previously described *(8)* with minor modifications. DNA extraction was performed with the Chelex 100 (Bio-Rad, Toronto, Ontario, Canada) ebullition method, in which many loopfuls of pure colonies were mixed with 10% Chelex and boiled for 20 min. The supernatant contained the DNA was then used in the PCRs. A 5-μl aliquot of DNA was added to 2.5 μl of 10× PCR buffer, 0.2 mM concentrations of the deoxynucleoside triphosphates (dNTPs), 2 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (GE Healthcare, Baie-D'Urfe, Quebec, Canada), and 200 mM concentrations of each primer in a total volume of 25 μl. DNA amplification reactions were carried out using a Whatman Biometra thermocycler (Montreal Biotech, Kirkland, Quebec, Canada) with the following conditions: denaturation for 10 min at 94°C, 30 cycles of 30 s at 94°C, 1 min at 62°C, and 1 min at 72°C, and a final extension of 5 min at 72°C. For visualization of PCR products, 5 μl of each amplicon was subjected to electrophoresis in a 1.7% agarose gel, which was stained with ethidium bromide. A TrackIt 100-bp DNA ladder (Invitrogen, Burlington, Ontario, Canada) was used as the marker. *E. faecalis* MA-58123 (Laboratoire de santé publique du Québec, Sainte-Anne-De-Bellevue, Quebec, Canada) was used as a positive control. Only one isolate of *E. faecalis* was kept for each cecum.

Antibiotic susceptibility testing. PCR-confirmed isolates of *E. faecalis* were tested for MICs of 17 antibiotics (Table 1) according to the recommended Clinical and Laboratory Standards Institute guidelines (M31-A3 and M100-S20) *(11, 12)*. The broth microdilution method recommended by the National Antimicrobial Resistance Monitoring System (NARMS) (plates CMV1AGPF) was used with the ARIS Sensititre automatic system (Trek Diagnostic Systems, Cleveland, OH). Antibiotics tested (and their interpretation breakpoints) were as follows: bacitracin (BAC, ≥64 μg/ml), chloramphenicol (CHL, ≥32 μg/ml), ciprofloxacin (CIP, ≥4 μg/ml), daptomycin (DAP, >4 μg/ml), erythromycin (ERY, ≥8 μg/ml), flavomycin (FLA, ≥32 μg/ml), gentamicin (GEN, >500 μg/ml), kanamycin (KAN, ≥1,024 μg/ml), linezolid (LIZ, ≥8 μg/ml), nitrofurantoin (NIT, ≥128 μg/ml), penicillin (PEN, ≥16 μg/ml), streptomycin (STR, >1,000 μg/ml), tetracycline (TET, ≥16 μg/ml), tylosin (TYL, ≥32 μg/ml), and vancomycin (VAN, ≥32 μg/ml). Breakpoints from NARMS *(4)* were used for BAC, FLA, KAN (high level), and TYL. For DAP, only a susceptible breakpoint has been established. *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 and ATCC 51299 were used as quality controls. Multiresistance was defined as acquired nonsusceptibility to at least one agent in three or more antibiotic categories *(30)*.

Detection of antibiotic resistance genes. Detection of antibiotic resistance determinants was performed by the PCR method using primers and conditions previously described *(2, 5, 9, 17, 20, 25, 32, 36, 42, 44)* with minor modifications. The isolates were examined for the presence of the following genes: *bcrABD*

operon and its regulatory gene *bcrR,* which encode for both a bacitracin ABC transporter system and an overproduced undecaprenol kinase; *erm*(B), which encodes a ribosomal methylase that mediates macrolide resistance; *msr*(C), which encodes a macrolide efflux pump; *aph(3′)-IIIa* (O-phosphotransferase), *aadE, aadA* (both O-adenyltransferases), and *aac(6′)-Ie-aph(2″)-Ia* (bifunctional enzyme with 2″-phosphorylating and 6′-acetylating activities), which encode for aminoglycoside modifying enzymes; and *tet*(M), *tet*(O), and *tet*(S), which encode for TET-minocycline resistance via a ribosomal protection protein mechanism. The class 1 integron *qacEd1-Sul1* gene was determined as previously described *(33)*. A PCR assay for the detection of the *int* gene was also performed in *tet*(M)-positive isolates to identify the presence of members of the Tn*916*-Tn*1545*–like family transposons *(17)*. A 5-μl aliquot of DNA was added to 2.5 μl of 10× PCR buffer, 0.2 mM concentrations of dNTPs, 2 mM $MgCl_2$, 150 mM concentrations of each primer (500 mM for *aadE, aadA,* and *msr*(C)), and 1.25 U of *Taq* DNA polymerase (GE Healthcare) in a total volume of 25 μl. New primer constructions (5′ to 3′) were as follows: *bcrR* forward (F) tatagggttctcttgccgct and *bcrR* reverse (R) gttaccctaacatggagtcg (F: position 3,488, R: position 3,913; GenBank accession no. AY496968), *bcrA* F aatccgtcatgttggtagctgctct and *bcrA* R tattatgcacgagccggagcttct (F: position 2,492, R: position 2,805; GenBank accession no. AY496968), and *msr*(C) F cttaggggttgctcaggaaa and *msr*(C) R aacaaaatcgttcccggata (F: position 195, R: position 392; GenBank accession no. AJ243209). Positive and negative controls from collection of the Faculty of Veterinary Medicine (University of Montreal, Montreal, Quebec, Canada) were used in all PCRs. The identity of the gene products of at least one randomly selected isolate for each gene was verified by DNA sequencing on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Concord, Ontario, Canada).

Plasmid extraction and hybridization. Six representative multiresistant isolates of *E. faecalis* harboring *tet*(M) and *erm*(B) genes from the two predominant profiles were selected based on criteria (Table 1) for plasmid extraction and conjugation.

Extraction was performed using a Midiprep Kit (Qiagen, Valencia, CA) according to the manufacturer's specifications with a lysis step of 5 mg/ml lysozyme. Plasmid extracts were subjected to electrophoresis in a 1% agarose gel that was run at 110 V for 90 min and then stained with ethidium bromide. Probes for Southern hybridization were generated by substituting digoxigenin-labeled dNTPs (PCR Dig Probe Synthesis Kit, Roche Applied Science, Laval, Quebec, Canada) for standard dNTPs in the amplification reaction according to the manufacturer's instructions. DNA from plasmid extractions was transferred to positively charged nylon membranes with a vacuum blotter (model 785, Bio-Rad) and probed with the digoxigenin-labeled PCR products for the selected antibiotic resistance genes, *erm*(B) and *tet*(M). Prehybridizations and hybridizations were carried out at 70°C for 30 min and 18 h, respectively, in hybridization buffer with subsequent washings, as recommended by the manufacturer. The colorimetric method (nitroblue tetrazolium–5-bromocresyl-3-indolylphosphate substrate solution, Roche Applied Science) was then used to detect the presence of digoxigenin-labeled probes. PCR products were used as hybridization controls, and control digoxigenin-labeled DNA was used as a detection control. Purified DNA from *E. faecalis* JH2-2 (plasmid free) was used as a negative control for *erm*(B) and *tet(*M) genes in a dot blot assay (data not shown). Approximate size of the plasmids was determined using a supercoiled DNA ladder (Invitrogen) as a molecular weight marker.

Plasmid transferability. Filter mating experiments were performed as previously described *(28)*. The six *E. faecalis* isolates harboring *erm*(B) and *tet*(M) genes were used as donors, and *E. faecalis* JH2-2 (rif^R, fus^R, and *erm*(B) and *tet*(M) negative) was used as the recipient strain. The PCR assay, as described above, was performed to identify both genes in transconjugants. Plasmids from six donors and four transconjugants harboring *tet*(M) and *erm*(B) genes were extracted and hybridized as described above.

PCR for *rep* families and regions upstream of *tet*(M) gene. *rep* families were investigated because some plasmid families have been associated with a very narrow host range (present in one species) and others have a broader host range (present in more than one species) *(27)*. All donors and transconjugants were screened for *rep*-like sequences by PCR as previously described *(27)*. Sequenced PCR amplification products were used as positive controls for 14 *rep* families: rep_1, rep_2, rep_4, rep_6, rep_8, rep_9, rep_{11}, rep_{13}, rep_{14}, rep_{15}, rep_{16}, rep_{17}, rep_{18}, and rep_{Unique}. PCR detection and sequencing of the region upstream of the *tet*(M) gene was also performed on one donor and its transconjugant to further characterized the ~9-kb plasmid. A 5-μl aliquot of DNA was added to 1× PCR buffer, 0.2 mM concentrations of dNTPs, 2 mM $MgSO_4$, 200 mM concentrations of primer (5′-taattctgtaatcgctcactgtt-3′), and 1 U of Platinum *Taq* DNA polymerase high fidelity (Invitrogen) in a total volume of 25 μl. Sequencing was performed as described above.

RESULTS

Bacterial identification. A total of 701 colonies were purified from porcine ceca and identified as enterococci. Of those colonies, 274 randomly selected isolates were presumptively identified as *E. faecalis* by PCR assay. A total of 124 isolates were confirmed as *E. faecalis*.

Antibiotic resistance phenotypes. Percentages of antibiotic resistance based on MICs for *E. faecalis* (n = 124) from porcine are presented in Table 2. Low, intermediate, and high frequencies of antibiotic resistance were observed in isolates tested with specific antibiotics. Although all isolates were resistant to at least one antibiotic, all were susceptible to VAN, DAP, LIZ, NIT, and PEN. All isolates were resistant to TET, and resistance to M-TET was observed in 81.4% of isolates. A high level of resistance to TET and TYL (expressed as high MICs) was also observed. Numerous resistance phenotypes were noted in the *E. faecalis* isolates from swine (n = 23) (Table 3). The four following patterns were predominant: BAC-M-TET (n = 30), BAC-M-KAN-STR-TET (n = 18), M-TET (n = 15), and BAC-TET (n = 11).

Antibiotic resistance genotypes. The two predominant multiresistant phenotypes were selected for evaluation by PCR and sequencing to examine the genes encoding antibiotic resistance (Table 4). The *erm*(B) and *tet*(M) genes were the most commonly observed resistance determinants in the phenotypic profile BAC-M-TET, and *erm*(B), *tet*(M), *aadE,* and *aph(3′)-IIIa* were the most commonly observed resistance genes in the BAC-M-KAN-STR-TET phenotypic profile. Among the 30 isolates harboring the predominant multiresistant phenotype BAC-M-TET, four antibiotic resistance genotypes were observed (Table 4). Six different antibiotic resistance genotypes were identified in the 18 isolates with the predominant phenotype BAC-M-KAN-STR-TET. Overall, 10

TABLE 2. *Antibiotic resistance in* E. faecalis *isolates from pigs based on MICs*

Antibiotic agent	Number of isolates at an MIC (μg/ml) of[a] :																% resistant isolates
	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	
Bacitracin							2	7	26 |	**57**	**19**	**13**					63.7
Chloramphenicol						4	105	2	**7** |	**6**							10.5
Ciprofloxacin			1	67	55 |		**1**										0.8
Daptomycin			8	91	25		||									0
Erythromycin			4	15	4			**101**									81.5
Flavomycin				121					|	**3**							2.4
Gentamicin											109	1 |	**2**	**5**	**7**		11.3
Kanamycin											81		1 |		**42**		33.9
Linezolid				4	120		||									0
Nitrofurantoin							94	20	2	8 |							0
Penicillin					14	105	5 |										0
Streptomycin													65 |	**3**	**15**	**41**	47.6
Tetracycline								**6** |	**8**	**110**							100
Tylosin				15	8				|	**101**							81.5
Vancomycin			3	93	26	2			|								0

[a] |, MIC breakpoint; ||, susceptible breakpoint. Numbers of resistant isolates are indicated in bold. The unshaded area includes the range of dilutions tested. The shaded area includes the numbers of isolates for which the MICs of the antibiotics were higher than the highest concentrations tested.

different antibiotic resistance genotypes were observed. Of the 47 isolates positive for *erm*(B) and *tet*(M) genes, 24 were positive for the Tn*916*-Tn*1545*–like integrase family gene. Resistance to BAC, M, STR, and KAN was not explained in 48, 1, 2, and 3 isolates, respectively. All other resistance genes conferring resistance to a particular antibiotic were found in all isolates exhibiting the corresponding phenotype. The class 1 integron *qacEd1-Sul1* gene was not found.

TABLE 3. *Phenotypic resistance profiles observed among* E. faecalis *isolates from pigs*

No. of isolates	Phenotypic resistance profile[a]
30	BAC, M, TET
18	BAC, M, KAN, STR, TET
15	M, TET
11	BAC, TET
7	BAC, M, STR, TET
6	M, STR, TET
5	BAC, CHL, M, KAN, STR, TET
4	M, GEN, KAN, STR, TET
4	BAC, M, GEN, KAN, STR, TET
3	STR, TET
3	BAC, STR, TET
3	BAC, CHL, TET
3	M, KAN, STR, TET
2	TET
2	BAC, CHL, M, GEN, KAN, STR, TET
1	M, FLA, LIZ, TET
1	BAC, GEN, KAN, STR, TET
1	BAC, M, FLA, TET
1	BAC, CHL, M, KAN, TET
1	BAC, M, GEN, KAN, TET
1	BAC, M, FLA, KAN, STR, TET
1	CHL, M, GEN, KAN, STR, TET
1	BAC, CHL, M, CIP, GEN, KAN, STR, TET

[a] BAC, bacitracin; M, macrolide; TET, tetracycline; KAN, kanamycin; STR, streptomycin; CHL, chloramphenicol; GEN, gentamicin; FLA, flavomycin; LIZ, linezolid; CIP, ciprofloxacin.

Plasmid extraction, hybridization, and transferability. The ~9-kb plasmid carrying *erm*(B) and *tet*(M) genes was first discovered in *E. faecalis* strain 748 (Fig. 1). Among the isolates positive for *erm*(B) and *tet*(M) genes but negative for the Tn*916*-Tn*1545*–like integrase family gene, five other strains representing different combinations of antibiotic resistant genes were selected for plasmid extractions based on criteria found in Table 1. In these strains, hybridization results revealed that *tet*(M) and *erm*(B) were both colocalized on a low-molecular-weight plasmid of ~9 kb. Filter mating experiments revealed that four of those strains had the capacity to transfer both genes to a model recipient strain, *E. faecalis* JH2-2. Colocalization and cotransfer of both *tet*(M) and *erm*(B) on genetic elements of the same size range was also demonstrated in those strains and their respective four transconjugants (data not shown).

***rep* families and analysis of the region upstream of the *tet*(M) gene.** *rep* families were investigated to further characterize the ~9-kb plasmid host range. Families rep_{11}, rep_{15}, and rep_{16} were detected in strain 748 and its transconjugant, and rep_1 was detected in strain 1153 and its transconjugant. rep_9 was the only plasmid family that was common to all strains and their transconjugants carrying the ~9-kb plasmid. A nonspecific PCR product was obtained using the defined primers for the rep_1 plasmid family. The nonspecific replicons from one donor and one transconjugant were sequenced, which revealed 94% identity with regions corresponding to a DNA segment between open reading frames (ORFs) 14 and 15 of Tn*916* and Tn*916*-like

TABLE 4. *Genotypic profiles of the two predominant multiresistant phenotypes of* E. faecalis *isolates*

Predominant multiresistant phenotype	No. of isolates	Multiresistant genotypic profile
BAC-M-KAN-STR-TET	13	*erm*(B), *aph(3′)-IIIa, aadE, tet*(M)
	1	*erm*(B), *tet*(M)
	1	*erm*(B), *aadE, tet*(M)
	1	*bcrR, bcrB, erm*(B), *tet*(M)
	1	*bcrR, erm*(B), *aph(3′)-IIIa, aadE, tet*(M)
	1	*bcrR, bcrB, erm*(B), *aph(3′)-IIIa, aadE, tet*(M)
BAC-M-TET	19	*erm*(B), *tet*(M)
	5	*erm*(B), *msr*(C), *tet*(M)
	5	*bcrR, bcrB, erm*(B), *tet*(M)
	1	*tet*(M), *tet*(O)

transposons found in different *Streptococcus* species (such as GenBank no. FR671418), in *E. faecalis* (GenBank no. EFU09422), and in *Enterococcus faecium* (GenBank no. HM243623). PCR products for the region upstream of the *tet*(M) gene in one donor and one transconjugant were sequenced, which revealed 76% identity with different regions of the Tn*916* transposon, mainly the ORF13 protein gene, of different species of streptococci. The same identity was observed with regions of the Tn*916*-like transposon found in enterococci.

DISCUSSION

In this study, all *E. faecalis* isolates tested were resistant to at least one antibiotic class, and numerous resistant phenotypes were identified. A high level of resistance to individual antibiotic classes also was found. The percentages of resistant isolates were generally significant and higher than or occasionally comparable to those previously reported for enterococci recovered from pig farms and slaughterhouses *(2, 16, 31)*. Various combinations of antibiotic resistance genes were detected in isolates of the two predominant multiresistant phenotypes. Phenotypes conferring resistance to macrolide, tetracycline, kanamycin, and streptomycin were mostly associated with *erm*(B), *tet*(M), *aph(3′)-IIIa,* and *aadE,* respectively.

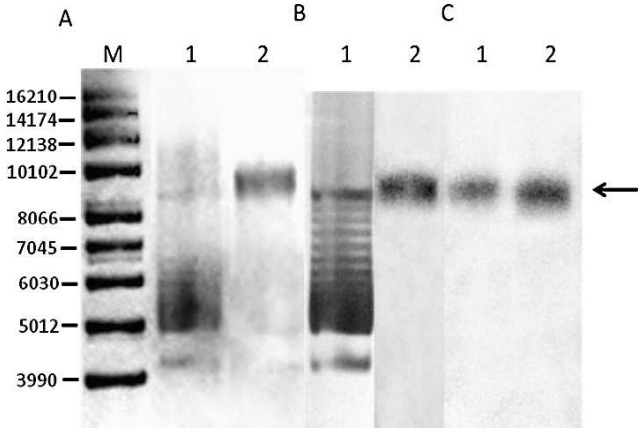

FIGURE 1. *Profiling and hybridization of undigested plasmid DNA of the multiresistant* E. faecalis *strain 748 and one of its transconjugants. (A) Agarose gel of* E. faecalis *strain 748 and transconjugant plasmids. (B) Southern blot of plasmids probed with* erm*(B). (C) Southern blot of plasmids probed with* tet*(M). Lane M, a supercoiled DNA ladder (Invitrogen) used as a molecular weight marker; lane 1,* E. faecalis *strain 748 plasmids; lane 2, transconjugant plasmids. Base pairs are indicated on the left. Black arrow indicates the ~9-kb plasmid.*

Overall, *erm*(B) and *tet*(M) were the most common resistance genes. Mobile genetic elements have been associated with resistance to macrolide and tetracycline in enterococci *(23)*. In the present study, many *E. faecalis* isolates positive for *erm*(B) and *tet*(M) genes were negative for the Tn*916*-Tn*1545*–like integrase family gene. Because these two genes can be colocalized on transposons of this family, isolates negative for this family of genes were selected for further analysis. Another criterion for isolate selection was the presence or absence of the *tdnX* transposase gene of the Tn*5397*-like transposon because a new composite structure (Tn*1116* of ca. 50 kb) has been identified and apparently resulted from the insertion of *erm*(B)-containing DNA, of likely enterococcal origin, into the *tet*(M) gene of the Tn*5397*-like transposon *(6)*. The six selected *E. faecalis* strains harbored a low-molecular-weight plasmid of ~9 kb with both the *tet*(M) and *erm*(B) genes, indicating that our strains do not harbor this high-molecular-weight composite structure. Also, four of those strains had the capacity to transfer both genes to a model recipient strain *E. faecalis* JH2-2. The rep_9 family was detected in these strains and their respective transconjugants; thus, we hypothesized that the ~9-kb plasmid is part of the rep_9 plasmid family. This family has been associated with a narrow host range because it has been reported in only one species *(27)*. However, this range characterization is dubious because the rep_9 family usually contains pheromone-responsive plasmids in which the lowest molecular weight plasmid observed has been 36.7 kb (pAM373) *(15)*. Thus, the association between the ~9-kb plasmid of the present study and the *rep* family of plasmids requires further investigation. Known genetic elements having *tet*(M) and *erm*(B) genes are usually 25 to 65 kb *(23)*. Colocalization of both genes was observed in a *Streptococcus pyogenes* strain on DNA elements ranging from 21 to more than 50 kb *(6)*. To our knowledge, the present article is the first report of *tet*(M) and *erm*(B) genes on the same low-molecular-weight plasmid of ~9 kb in *E. faecalis*.

In a recent study, an association was found between plasmid size and mobility *(41)*. Classification of plasmids

in terms of mobility revealed that conjugative plasmids distribute around an average of 100 kb, whereas mobilizable plasmids have a mean peak at 5 kb and a broad, flat secondary peak at around 150 kb. The smallest putatively conjugative plasmid found in proteobacteria was 21.8 kb (pCRY from *Yersinia pestis*). This plasmid was highly homologous to conjugative plasmids. Thus, the small size of the plasmid in the present study suggests that it may be nonconjugative and would need mobilization by a coresident conjugative element *(10)*. In a previous study, a plasmid of 8 kb (pSES20) isolated from a *Staphylococcus lentus* strain, harbored part of a Tn*917*-like transposon that included the left terminal repeat, a gene almost identical to *erm*(B), and its regulatory region in addition to the internal direct repeat *(47)*. In our study, the nonspecific replicons from one donor and one transconjugant resulted in 94% identity with regions from the Tn*916* and Tn*916*-like transposons found in streptococci and enterococci, respectively. A PCR product of the region upstream of the *tet*(M) gene was 76% identical to different regions of the Tn*916* transposon of streptococci and regions of the Tn*916*-like transposon of enterococci. Therefore, it is tempting to speculate that the ~9-kb plasmid found in the present study is composed of parts of other elements, such as the Tn*916* or Tn*916*-like transposons. The possibility of dissemination through the food chain of this low-molecular-weight plasmid associated with macrolide and tetracycline resistance in enterococci is of concern and may add to the burden of already widespread genetic elements related to Tn*916*-Tn*1545*, Tn*5397*, CW*459*TetM/Tn*5801*, and CTn*6000* among enterococci from different sources *(23)*. The use of macrolide antibiotics could maintain tetracycline-resistant microorganisms in porcine operations via the coselection process and vice versa.

Although a large proportion of isolates were susceptible to gentamicin, kanamycin, and streptomycin, high-level resistance to aminoglycosides was detected in a significant percentage of isolates, in accordance with previous reports *(19, 26)*. The prevalence of high-level resistance to kanamycin and streptomycin was much higher than high-level resistance to gentamicin. Because aminoglycosides are antibiotics of choice for treating enterococcal infections, in combination with cell-wall inhibiting antibiotics *(14)*, the possibility of dissemination through the food chain of genes conferring a high level of resistance to aminoglycosides in enterococci is of much concern. Streptomycin has been used in combination with penicillin in drinking water in Canadian pork production facilities *(3)*. Gentamicin is used for pigs in the United States *(19)*. However, this antibiotic is voluntarily used less frequently in Canada because of the long withdrawal period required. Kanamycin is not listed for use as an antibiotic in pigs in Canada *(3)*. The broad-spectrum bifunctional enzyme AAC(6′)-Ie-APH(2″)-Ia can modify all 2-deoxystreptamine aminoglycosides, such as gentamicin, tobramycin, amikacin, and kanamycin *(13)*, and the *aph(3′)-IIIa* gene, which is most commonly associated with high-level kanamycin resistance, was the most prevalent in the present and other studies *(2)*. Acquired bacitracin resistance in *E. faecalis* can be mediated by an ABC transporter (BcrAB) and a novel regulatory protein, BcrR *(32)*. *bcrR* and *bcrB* were the only bacitracin resistance genes detected in the present study. In most bacitracin-resistant isolates, no *bcr* genes were found. Our results likely suggest the presence of other bacitracin resistance mechanisms. As expected, vancomycin resistance was not found, which is consistent with previous findings in the United States *(7, 37)* because avoparcin has not been approved for use in North America. However, dissemination of vancomycin-resistant *E. faecium* strains among pigs has recently been reported in three Michigan counties *(18)*.

Our data provide further evidence of the frequent occurrence of antibiotic-resistant enterococci in abattoir pigs. Vancomycin-resistant *E. faecalis* isolates were not found, but a significant percentage of *E. faecalis* isolates had a high level of resistance to aminoglycosides. For the first time, we provide evidence of a new plasmid associated with colocalization and cotransfer of *tet*(M) and *erm*(B) genes in porcine *E. faecalis* isolates, indicating that antibiotic coselection and transferability could occur via this single genetic element. Results of this study indicate that enterococcal strains in healthy pigs at slaughter could be a reservoir for aminoglycosides (high level), tetracycline, and macrolide resistance genes.

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