ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1985, p. 439–444
0066-4804/85/040439-06$02.00/0

Vol. 27, No. 4

Evidence for a Disseminated Erythromycin Resistance Determinant Mediated by Tn*917*-Like Sequences Among Group D Streptococci Isolated from Pigs, Chickens, and Humans

LARRY D. ROLLINS,[1] LINDA N. LEE,[2] AND DONALD J. LEBLANC[2]*

Division of Therapeutic Drugs for Food Animals, Center for Veterinary Medicine, Food and Drug Administration, Rockville, Maryland 20857,[1] and Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Fort Detrick, Frederick, Maryland 21701[2]

Received 3 August 1984/Accepted 7 January 1985

A total of 199 streptococci isolated from feces of healthy chickens, pigs, and beef cattle and 26 human clinical isolates were tested for resistance to kanamycin, streptomycin, tetracycline, erythromycin, and lincomycin. Of 66 isolates resistant to these antibiotics, 12 transferred one or more resistance traits by conjugation in broth. Erythromycin resistance (Em^r) was transferred from 10 of the 12 successful donors. *Ava*I digests of plasmids isolated from Em^r transconjugants derived from two human, two chicken, and one pig isolate contained three fragments similar in size to those produced from Tn*3871*, an Em^r transposon. The three fragments from each of the five digests on Southern blots hybridized to radiolabeled Tn*3871*. Plasmid DNA from a transconjugant derived from a second pig isolate contained two of the three Tn*3871*-associated *Ava*I fragments. One of the *Ava*I fragments from each of the six plasmids hybridized with a radiolabeled probe containing a cloned *Ava*I fragment from Tn*3871* that contained the Em^r determinant. Transposition of the Em^r trait was demonstrated for the plasmids derived from one human and one pig isolate. We concluded that extensive DNA homology existed between plasmids from streptococcal strains obtained from two human patients, two chickens, and two pigs and the Em^r transposon Tn*3871*, which is very similar or identical to the well-characterized Em^r transposon Tn*917*. The detection of Tn*3871*-like sequences in streptococcal isolates from Arkansas, Illinois, North Carolina, and Washington, D.C. indicates wide dissemination of Em^r mediated by the same or closely related transposons.

Tn*917*, a 5.3-kilobase (kb) transposon which mediates resistance to macrolide-lincosamide-streptogramin B-type antibiotics (MLS^r), was originally identified on the nonconjugative plasmid pAD2 from *Streptococcus faecalis* DS16 (17). Plasmid pAD2 also mediates resistance to kanamycin (Km^r) and streptomycin (Sm^r) and is coresident with the conjugative plasmid pAD1, which mediates hemolysin-bacteriocin (Hly-Bcn) activity (18). Erythromycin serves as an inducer of transposition and resistance (17). Tn*917* contains four *Ava*I restriction endonuclease cleavage sites (3), two of which are within the transposon inverted repeats (D. B. Clewell, personal communication). Recent sequencing data have placed two of the *Ava*I restriction sites only eight base pairs from the outer boundary of the repeat sequences (14). Thus, any DNA molecule containing Tn*917* will contain three *Ava*I fragments that account for more than 99.9% of the transposon.

Banai and LeBlanc (1) used *Ava*I digestion patterns and Southern blot hybridization results to demonstrate the presence of a transposon, Tn*3871*, in the conjugative plasmid pJH1 (8), which is indistinguishable from Tn*917*. Plasmid pJH1 confers Km^r, Sm^r, neomycin resistance, tetracycline resistance (Tc^r), and MLS^r and is coresident with the Hly-Bcn plasmid pJH2 (8). These transposons were identified in strains isolated from a broad geographical range: strain DS16 (Tn*917*) was from Ann Arbor, Mich. (17), and strain JH1 (Tn*3871*) was from London, England (8). Because of this broad geographical distribution, this study was conducted to determine whether Em resistance (Em^r) mediated by Tn*3871*-like sequences, and by analogy, Tn*917*-like sequences, is more widely disseminated in group D streptococci from humans and farm animals. We screened isolates for multiple antibiotic resistance similar to that in strains JH1 and DS16 and then used *Ava*I digestion patterns and Southern blot hybridizations to identify DNA sequences very similar to or indistinguishable from Tn*3871*.

MATERIALS AND METHODS

Bacterial strains. Twenty-six clinical isolates of group D streptococci were obtained from the Department of Microbiology, Walter Reed Army Medical Center, Washington, D.C. From a collection of more than 6,000 streptococci isolated from animal feces during a 3-year farm survey (Food and Drug Administration contract 223-77-7032), 199 streptococci (130 group D) from healthy broiler chickens, pigs, and beef cattle were selected for the study. The survey was conducted on randomly selected farms from the five or six states that produced the majority of the individual animal species (usually different states for each animal species). Sixty-five farms (30 pig, 20 chicken, and 15 beef cattle farms) were sampled in 1979, and 85 were tested in 1980. Those sampled in 1980 included the original set plus 10 additional chicken farms and 10 additional beef cattle farms; in 1981, specimens were collected from all farms except the 30 pig farms tested in 1979 and 1980. The survey also included pigs sampled at 10 randomly selected slaughter plants in 1979 and 1980.

Animal fecal samples were diluted and spread on Streptosel agar (BBL Microbiology Systems, Cockeysville, Md.) or Kenner fecal streptococcus agar (Difco Laboratories, Detroit, Mich.). Isolates were randomly selected from each plate by using a marked template and picking the isolate

* Corresponding author.

TABLE 1. Description of *S. faecalis* strains derived from other studies

Strain	Plasmid content	Relevant phenotypic properties[a]	Derivation	Reference
JH2-2	None	Fus^r, Rif^r (chromosomal)	Spontaneous mutant of JH2	8
OG1-SSp	None	Sm^r, Sp^r (chromosomal)	Spontaneous mutant of OG1	7
OG1-RF1	None	Fus^r, Rif^r (chromosomal)	Spontaneous mutant of OG1	5
DL76	pJH2	Hly-Bcn	Transconjugant of JH1 × JH2-2	10
DL77	pJH1	Km^r, Sm^r, MLS^r, Tc^r	Transconjugant of JH1 × JH2-2	10
DL315	pDL315	Em^r, Hly-Bcn^r	Transconjugant of JH1 × OG1-RF1	1

[a] Hly-Bcn, Production of hemolysin-bacteriocin.

nearest the marks. Isolates were chosen from this collection by first forming a subset that had erythromycin and lincomycin MICs of >128 μg/ml, streptomycin MICs of >512 μg/ml, and tetracycline MICs of ≥128 μg/ml. Isolates from each farm (20 maximum) and slaughter plant (30 maximum) that appeared in the subset were selected from the results of 31 additional phenotypic features. One representative with each set of reactions within the group of isolates collected from a farm or slaughter plant sampling was examined during this study. This resulted in the selection of 199 isolates for further study (93 from chickens, 72 from pigs, and 34 from beef cattle). Strains derived from other studies and used in this investigation are described in Table 1. Those derived from this study and used for plasmid analyses, DNA hybridization studies, and transposition experiments are described in Table 2.

Culture conditions. Stock cultures were maintained in fluid thioglycolate medium at ambient temperature (19). All cultures were grown in brain heart infusion (BHI) broth (Difco) or in BHI broth supplemented with additional (10 mM) D-glucose (exponential-phase cultures). Solid BHI contained 1.5% agar (Difco), and blood agar consisted of 5% defibrinated horse blood in Columbia blood agar base (Difco). All incubations took place at 37°C.

Susceptibility testing and conjugation experiments. Human and animal isolates were tested for resistance to antibiotics on solid BHI containing (in micrograms per milliliter): streptomycin, 2,000; kanamycin, 500; tetracycline, 10; or erythromycin plus lincomycin, 25 each. Isolates that were resistant to all of the antibiotics were used as donors in broth conjugation studies with the plasmid-free *S. faecalis* recipient strain JH2-2 as described previously (10). Solid BHI, used for counter-selecting donors, selecting antibiotic-resistant transconjugants, and testing recipient controls, contained 25 μg each of fusidic acid and rifampin per ml plus one of the following antibiotics (in micrograms per milliliter): streptomycin, 2,000; kanamycin, 500; tetracycline, 10; or a combination of erythromycin plus lincomycin, 25 each. The mating mixtures and donor and recipient controls were plated on BHI agar and BHI agar with fusidic acid and rifampin. Transconjugants were tested for resistance to antibiotics other than those used for selection by transferring them with sterile toothpicks to the same types of plates as those used for selecting transconjugants, except that streptomycin was used at 1,000 μg/ml.

Transposition. To demonstrate transposition, the Hly-Bcn plasmid pJH2 was transferred to *S. faecalis* OG1-SSp (5) by conjugation with *S. faecalis* DL76 (10) with selection on blood agar containing 500 μg of spectinomycin per ml. A hemolytic, spectinomycin-resistant (Sp^r) transconjugant from this mating, strain DL1006, was then used as a recipient in matings with the human isolate, LDR46, and the animal isolate, LDR145. Transconjugants from both of these matings were selected on blood agar containing spectinomycin and erythromycin and were picked to blood agar containing spectinomycin plus kanamycin, tetracycline, or erythromycin. A hemolytic transconjugant that was Sp^r, Em^r, Km^r, and Tc^r (designated DL1035) from the LDR46 × DL1006 mating and a hemolytic transconjugant that was Sp^r, Em^r, and Km^r (designated DL1047) from the LDR145 × DL1006 mating were chosen as donors for further matings with strain JH2-2. After incubation for 18 h in BHI broth, cultures of strains DL1035 and DL1047 were diluted 100-fold in BHI broth containing 0.5 μg of erythromycin per ml and incubated for an additional 4 h. Each culture was mixed

TABLE 2. Description of transconjugants derived from this study

Derivation of transconjugants			Transconjugant designation	Resistance properties of transconjugants[a]
Donor	Origin	Recipient		
Field strains				
LDR2	Pig	JH2-2	LDR501	Km^r, Sm^r, Em^r, Tc^r
LDR20	Pig		LDR504	Km^r, Sm^r, Em^r, Tc^r
LDR42	Human		LDR505	Km^r, Sm^r, Em^r, Tc^r
			LDR506	Em^r
LDR46	Human		LDR507	Km^r, Sm^r, Em^r, Tc^r
LDR63	Human		LDR509	Km^r, Sm^r, Em^r, Tc^r
LDR75	Chicken		LDR511	Km^r
LDR145	Pig		LDR512	Km^r, Sm^r, Em^r, Tc^r
			LDR513	Em^r
			LDR514	Tc^r
LDR167	Chicken		LDR515	Km^r, Sm^r, Em^r, Tc^r
LDR175	Chicken		LDR517	Km^r, Sm^r, Em^r
			LDR518	Tc^r
LDR191	Chicken		LDR519	Tc^r
			LDR520	Em^r, Tc^r
			LDR521	Em^r
LDR227	Pig		LFR522	Tc^r
LDR259	Chicken		LDR524	Km^r, Sm^r, Em^r, Tc^r
			LDR523	Km^r, Sm^r, Em^r
			LDR525	Tc^r
Transposition expt				
DL76		OG1-SSp	DL1006	Sp^r, Hly-Bcn
LDR46		DL1006	DL1035	Km^r, Em^r, Tc^r, Sp^r, Hly-Bcn
LDR145		DL1006	DL1047	Km^r, Em^r, Sp^r, Hly-Bcn

[a] Hly-Bcn, Production of hemolysin-bacteriocin.

separately with a culture of strain JH2-2 and incubated for 3 h; samples were then spread on BHI agar plates, each containing erythromycin, fusidic acid, and rifampin. After incubation of the plates for 24 h, 17 plates, each containing 250 to 300 colonies from the DL1035 × JH2-2 mating, were replica plated onto BHI agar containing fusidic acid and rifampin plus either erythromycin or kanamycin. From the DL1047 × JH2-2 mating, 415 colonies that grew on the plates containing erythromycin, fusidic acid, and rifampin were picked to BHI agar containing fusidic acid and rifampin plus either erythromycin or kanamycin. Colonies from both matings that were Em^r but Km^s were further tested for Sm^s and Tc^s (DL1035 × JH2-2). Transconjugants that were Em^r but susceptible to the other antibiotics tested were selected for DNA isolation and restriction endonuclease analysis.

Plasmid isolation and purification. Purified plasmid DNA for restriction endonuclease analysis and hybridization experiments was isolated from 400-ml cultures as described previously (10). Radiolabeled DNA was obtained by growing cultures in the presence of [^{3}H]thymidine at 0.25 μCi/ml (New England Nuclear Corp., Boston, Mass.). DNA was precipitated with 0.05 volume of 3 M ammonium acetate and 2.5 volumes of 95% ethanol at −20°C. After precipitation, DNA was suspended in 10 mM Tris-HCl buffer (pH 8.0) and stored at 4°C. Plasmid DNA from transconjugants derived from the transposition experiments was isolated by a screening procedure described previously (9).

Restriction endonuclease digestion and agarose gel electrophoresis. Restriction endonucleases were obtained from Bethesda Research Laboratories, Gaithersburg, Md., and were used according to the instructions of the supplier. Reaction products were incubated for 1 to 4 h at 37°C, placed in a 70°C water bath for 5 min, and immediately transferred to ice water for 5 min. Reactions were terminated by the addition of stop mix (Bethesda Research Laboratories). Restriction fragments were separated by electrophoresis on horizontal 0.8% agarose slab gels in Tris-acetate buffer (12) at 24 V for 16 h. Gels were stained for 15 to 30 min in water containing 1 μg of ethidium bromide per ml. Gels were usually destained in water for 15 to 30 min and then viewed and photographed as described previously (10).

DNA blotting and hybridization. Fragments from restriction endonuclease digests of plasmid DNA were transferred from agarose gels to nitrocellulose paper by the method of Southern (16) as modified by LeBlanc and Lee (10). DNA to be used as a probe in hybridization reactions was labeled in vitro with ^{32}P by nick translation (13) as described previously (10).

Isolation of a DNA restriction endonuclease fragment containing Tn*3871*. A Tn*3871*-containing fragment of DNA was isolated from plasmid pDL315, which is composed of pJH2 with Tn*3871* inserted into the 1.4-kb *Eco*RI G fragment (1). This plasmid was digested with *Eco*RI and the fragments were separated by agarose gel electrophoresis. A fragment that migrated at a rate consistent with that for a size of 6.5 kb (the 1.4-kb *Eco*RI G fragment of pJH2 plus the 5.1-kb Tn*3871*) was extracted from the gel by electroelution (12) and subsequently radiolabeled with ^{32}P for use as a hybridization probe.

Cloning of the Em^r determinant of Tn*3871*. Plasmids pJH1 and pVA380-1 (11) were mixed and digested with *Ava*I and then ligated with T4 DNA ligase (Bethesda Research Laboratories). The restriction endonuclease digestion was conducted according to the instructions of the supplier, and the ligation reaction was performed by the method of Davis et al. (4). Competent cells of *Streptococcus sanguis* Challis were prepared for transformation by passing the strain six times in LCM broth (15) supplemented with 10 mM D-glucose. These passages were followed by serial dilutions from 10^{-1} to 10^{-9} in BHI broth supplemented with 10 mM D-glucose and 1% heat-inactivated horse serum and incubation for 18 to 20 h. A dilution tube with an optical density at 600 nm of 0.02 to 0.05 was selected for transformation. One μg of transforming DNA in 50 μl of sterile 0.1× SSC (1.0× SCC = 0.15 M NaCl plus 0.015 M sodium citrate) (12) was added to 450 μl of competent culture and incubated for 4 h under anaerobic conditions (GasPak; BBL). Portions of diluted and undiluted transformation cultures were spread on BHI agar containing 10 μg of erythromycin per ml.

TABLE 3. Antibiotic resistance of streptococci of human and animal origin

Origin	No. of isolates tested	% Resistant to[a]:				
		Sm^r	Km^r	(Em + Lm)^r	Tc^r	Sm^r, Km^r, Tc^r, Em^r, Lm^r[b]
Chickens	93	64	62	67	98	48
Pigs	72	57	36	79	93	22
Beef cattle	34	6	0	26	94	0
Humans	26	23	29	29	85	19

[a] Amount of antibiotics used in testing: streptomycin, 2,000 μg/ml; kanamycin, 500 μg/ml; erythromycin plus lincomycin, 25 μg/ml each; tetracycline, 10 μg/ml.

[b] Isolates were resistant to all of the antibiotics at the levels tested.

RESULTS

Resistance traits of donors. Twenty-six isolates of group D streptococci obtained from human patients and 199 streptococci from healthy farm animals were screened for Sm^r, Km^r, Tc^r, and resistance to a combination of erythromycin and lincomycin (Table 3). The occurrence of resistance was higher in isolates obtained from chickens and pigs than in those from beef cattle and humans. For all four groups the proportion that was Tc^r was highest, followed in descending order by the proportion resistant to erythromycin plus lincomycin and to aminoglycosides. None of the cattle isolates was Km^r, and only two were Sm^r. Sixty-six isolates (48% chicken, 22% pig, and 19% human) were resistant to all of the antibiotics and were chosen for further studies.

Conjugation experiments. Because preliminary testing by a plasmid screening procedure (9) indicated that the animal isolates usually contained multiple plasmids, the 66 strains that were resistant to all antibiotics tested were used as donors in conjugation experiments in an effort to separate plasmids responsible for antibiotic resistance. Of the 66 multiply resistant donors, 12 transferred one or more of their resistance traits to the plasmid-free recipient strain, *S. faecalis* JH2-2, during mixed incubation in broth culture (Table 2). The 12 successful donors, all group D streptococci, included three from humans, four from pigs, and five from chickens. Transconjugants that were Km^r, Sm^r, Em^r, and Tc^r were obtained with eight of the donors, and two produced transconjugants resistant to three of the antibiotics. Of the 12 donors, 10 transferred their Em^r.

All of the donors listed in Table 2 that were isolated in the field exhibited a clumping reaction in response to pheromone produced by the recipient strain, JH2-2 (5). Clumping was observed during conjugation with 10 of the successful mating combinations and in only one of the donor and recipient mixtures that did not result in antibiotic resistance transfer.

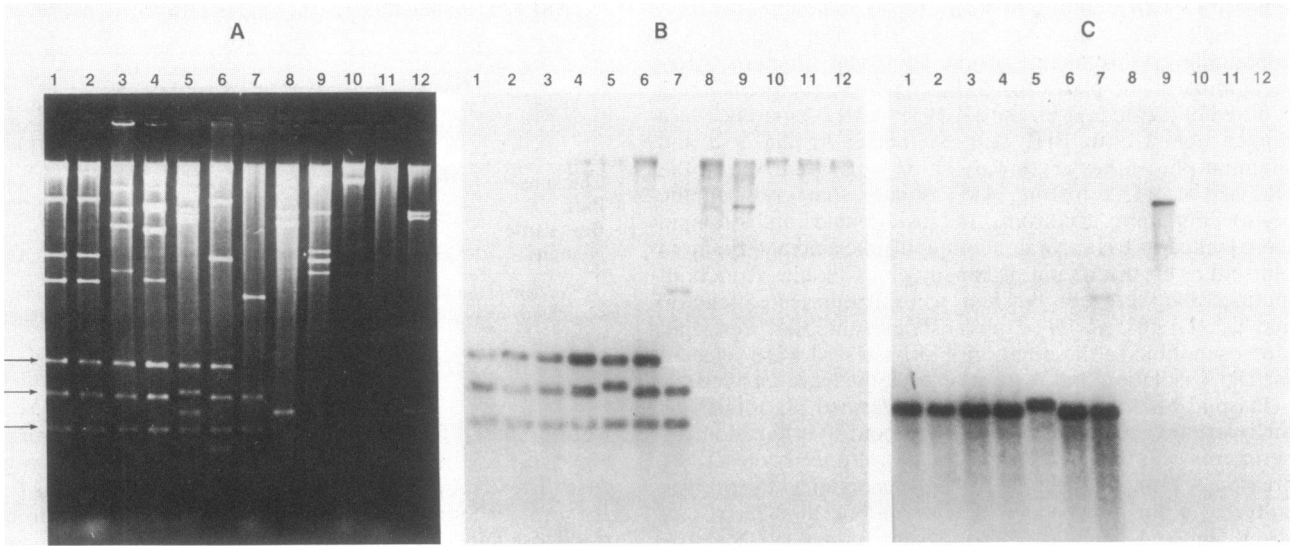

FIG. 1. Occurrence of Tn*3871*-like sequences and their Em^r determinant on plasmids among group D streptococci isolated from humans and animals. (A) Agarose gel electrophoresis of *Ava*I-digested plasmid DNA from strains LDR507 (lane 1), LDR505 (lane 2), DL77 (pJH1, lane 3), LDR515 (lane 4), LDR512 (lane 5), LDR520 (lane 6), LDR504 (lane 7), LDR514 (lane 8), LDR509 (lane 9), LDR517 (lane 10), LDR524 (lane 11), and DL76 (pJH2, lane 12). (B) Autoradiograph showing results of hybridization between the DNA from the gel in panel A, after transfer to nitrocellulose paper, and the ^{32}P labeled *Eco*RI G fragment of pJH2 containing a Tn*3871* insert from pJH1. (C) Autoradiograph showing results of hybridization between DNA from a gel prepared in the same manner as the one shown in panel A, after transfer to nitrocellulose paper, and the cloned *Ava*I fragment from pJH1 containing the Em^r determinant of Tn*3871*.

The two remaining successful donors, which did not clump during mating, formed clumps when exposed to a cell-free culture extract (6) of strain JH2-2, as did all of the other successful donors except one.

Restriction endonuclease analysis. Plasmids which contain Tn*3871*-like or Tn*917*-like sequences should contain three *Ava*I restriction fragments which migrate at the same rate in agarose gels as the three Tn*3871*-associated fragments from pJH1 (1). Plasmids were isolated from transconjugants that had received an Em^r trait from each of the donor strains which transferred this property (Table 2). *Ava*I digests of these plasmid preparations were compared with *Ava*I digests of pJH1 and pJH2 and plasmid DNA isolated from strain LDR514, a Tc^r transconjugant obtained by mating with the same donor strain, LDR145, that also transferred the Km^r, Sm^r, Em^r, and Tc^r phenotypes to another transconjugant, strain LDR512 (Fig. 1A). Three DNA fragments, indicated by the arrows to the left of Fig. 1, from LDR507, LDR505, LDR515, and LDR520 (lanes 1, 2, 4, and 6, respectively) migrated in an agarose gel at the same rates as three of the fragments from pJH1 (lane 3) which are associated with Tn*3871*. Plasmid DNA isolated from strain LDR512 (lane 5) contained two *Ava*I fragments that migrated at the same rates as two of the Tn*3871*-associated fragments, whereas a third fragment was slightly larger than the corresponding Tn*3871*-associated fragment. The two smallest transposon-associated *Ava*I fragments appeared to be present in plasmid DNA from strain LDR504 (lane 7). None of the Tn*3871*-associated *Ava*I fragments were observed in plasmids isolated from the Em^r transconjugants LDR509 (lane 9), LDR517 (lane 10), or LDR524 (lane 11), nor were they present in plasmid DNA from the Em^s transconjugant LDR514 (lane 8) or the Hly-Bcn plasmid pJH2 (lane 12).

DNA homology studies. A 6.5-kb *Eco*RI fragment from pDL315 consisting of the *Eco*RI G fragment of pJH2 plus the 5.1-kb Tn*3871* insert (1) was used as a hybridization probe with a Southern blot of the DNA from the gel (Fig. 1A). The results of this experiment, illustrated in the autoradiograph (Fig. 1B), showed that the three suspected Tn*3871*-specific *Ava*I fragments from plasmids isolated from strains LDR507 (lane 1), LDR505 (lane 2), LDR515 (lane 4), LDR512 (lane 5), and LDR520 (lane 6) all hybridized to the probe, as did the three Tn*3871*-specific *Ava*I fragments from pJH1 (lane 3). The two *Ava*I fragments from the plasmid obtained from strain LDR504 (lane 7) that migrated at the same rate as two of the Tn*3871*-associated fragments and a third *Ava*I fragment, larger than any of the Tn*3871*-associated fragments, also hybridized to the probe. One large fragment from the plasmid isolated from strain LDR509 (lane 9) also hybridized.

The cloned *Ava*I fragment of Tn*3871* that contains the Em^r determinant was used as a hybridization probe with a Southern blot of DNA from a gel prepared in the same manner as that shown in Fig. 1A. The autoradiograph (Fig. 1C) showed that the probe hybridized to the Tn*3871*-associated *Ava*I fragment that migrated to the middle position in lanes 1 through 6. This probe also hybridized to the corresponding middle fragment from plasmid DNA isolated from strain LDR504 (lane 7) and to a single fragment from the plasmid obtained from strain LDR509 (lane 9). In a separate experiment (data not shown), pVA380-1 hyridized to itself but not to any of the resistance plasmids shown in Fig. 1. These results indicate that the MLS^r determinant of Tn*3871* was located on one of the three *Ava*I fragments from each of the plasmids illustrated in lanes 1 through 7. This determinant was also present on the plasmid from strain LDR509 (lane 9) but in an *Ava*I fragment that did not correspond to any of the three Tn*3871*-specific *Ava*I fragments.

Evidence for transposition. The final matings used to demonstrate transposition (DL1035 × JH2-2 and DL1047 × JH2-2) resulted in transfer of Em^r at frequencies of 2.74×10^{-1} (per donor CFU) from strain DL1035 and 8.0×10^{-6} from strain DL1047. The frequency of transposition of the

Em^r determinant, obtained from the number of Em^r, Km^s and Sm^s, or Em^r, Km^s, Sm^s, and Tc^s transconjugants, was 0.02 and 5% for donors originally derived from strains LDR46 and LDR145, respectively.

Transposition of the Em^r trait from either donor was confirmed by isolation of plasmid DNA from the appropriate transconjugants, digestion with *Eco*RI, separation of the fragments by agarose gel electrophoresis, and comparison of the fragment patterns with that obtained from pJH2 treated in the same manner. Each plasmid examined had a fragment pattern identical to that from pJH2, with the exception of one fragment which was larger by approximately 5 kb, the size of Tn*3871* or Tn*917*. The Em^r transposon was inserted into either the *Eco*RI A fragment or the *Eco*RI D fragment of pJH2 in all plasmids examined. *Ava*I digests of the same plasmid preparations showed that the three fragments associated with Tn*3871* were present in the same manner as in the original donors, i.e., the middle fragment from the plasmid associated with strain LDR145 was slighly larger than the corresponding fragment from Tn*3871* or from the plasmid originally present in strain LDR46.

DISCUSSION

The results presented here provide evidence for the presence of a DNA sequence mediating Em^r which is similar to or indistinguishable from Tn*3871* on 5 of 12 transmissible plasmids from group D streptococci of human and animal origins. This conclusion is based on the presence in each of the five plasmids of three *Ava*I restriction fragments with sizes characteristic of Tn*3871*-derived *Ava*I fragments, homology of these three fragments to a radiolabeled Tn*3871* probe, and the demonstration of transposition from two of the plasmids. A summary of the geographical distribution of the streptococcal strains exhibiting Em^r mediated by the Tn*3871*-like sequences (Table 4) suggests that this transposon, or one very similar to it, is widely disseminated in nature. These Tn*3871*-like DNA sequences were present in streptococcal isolates from a pig in Illinois, a chicken in North Carolina, a chicken in Arkansas, and two human patients in a hospital in Washington, D.C. In addition, an Em^r determinant with homology to that of Tn*3871* was also present in a strain isolated from a pig in Nebraska and a human patient from the same hospital in Washington, D.C.

TABLE 4. Distribution of Tn*3871*-like DNA sequences among group D streptococci of human and animal origin

Tn3871-like DNA sequences present	Strain	Source of isolate	Geographical origin	Yr isolated
Total[a]	LDR42	Human (urine)	Washington, D.C.	1982
	LDR46[b]	Human (urine)	Washington, D.C.	1982
	LDR145[b]	Pig (feces)	Illinois	1979
	LDR167	Chicken (feces)	North Carolina	1980
	LDR191	Chicken (feces)	Arkansas	1980
Partial[c]	LDR63	Human (urine)	Washington, D.C.	1982
	LDR20	Pig (feces)	Nebraska	1979

[a] Plasmid DNA contained three *Ava*I fragments corresponding in size to three *Ava*I-derived fragments from Tn*3871* with homology to a Tn*3871* probe.
[b] Transposition was demonstrated.
[c] Plasmid DNA contained one or more *Ava*I fragments with homology to Tn*3871* and to the cloned MLS^r determinant from the transposon but was missing one or more fragments characteristic of *Ava*I-digested Tn*3871*.

The significance of these findings should be viewed in light of the history of the animal isolates examined. Samples were collected from randomly selected, healthy animals on farms from the states producing the majority of the particular animal species. Streptococcal isolates were picked from antibiotic-free plates which contained a medium selective for streptococci. This degree of randomization in the original collection of more than 6,000 animal isolates may indicate that Tn*3871*-like sequences are quite prevalent in streptococci present in the intestinal flora of animals. The full extent of dissemination of Tn*3871*-like sequences within animal populations can be determined only by testing additional isolates for their presence. It was not very difficult to find Tn*3871*-like sequences among the 26 human isolates examined.

The histories of the human and animal isolates are similar in that both came from backgrounds of intense antibiotic use. Antibiotic usage in hospitals is more intense than it is in the community at large, and antibiotics are commonly used on pig and chicken farms at low levels for extended periods to improve animal production. Since transposon-mediated MLS^r is present on plasmids that mediate resistance to other antibiotics, e.g., kanamycin, streptomycin, and tetracycline, it would not be necessary to use a macrolide-lincosamide-streptogramin B-type antibiotic to select for this transposon. Lincomycin and virginiamycin (a defined mixture of streptogramin A- and B-type components) are fed to chickens and pigs. Tylosin, which selects for MLS^r (2), is also commonly used in pig feed.

In addition to Em^r, multiple antibiotic resistance, i.e., Km^r, Sm^r, and Tc^r, was used as a selective criterion for this study because Tn*917* and Tn*3871* had been shown to occur in natural isolates in conjunction with some [pAD2(Tn*917*)(18)] or all [pJH1(Tn*3871*)(8)] of these traits. This criterion added a degree of stringency to the selection of strains for study. Indeed, three of the donor strains studied provided Tn*3871*-like sequences on plasmids from transconjugants that were Km^s, Sm^s, and Tc^s (data not shown). These results indicate that the Em^r determinant is likely to be present alone or with fewer other resistance determinants in nature.

The results of this study clearly indicate a common origin of the Em^r trait found in several strains of group D streptococci isolated from human and animal sources, namely, a Tn*3871*-like transposon or the resistance determinant from it. The stringent conditions used to choose the strains and the fact that the animal isolates were not originally selected on antibiotic-containing media suggest that DNA sequences similar to or indistinguishable from Tn*3871* or the Em^r determinant from the transposon may be even more prevalent in animal strains than is indicated here. Previous reports of closely related Em^r transposons in human isolates of *S. faecalis* from Michigan (Tn*917*) and England (Tn*3871*) and the strains from Washington, D.C. described in this communication suggest a wide distribution of very similar or indistinguishable transposons among human isolates as well. The inducibility of Tn*917* transposition by erythromycin (17) and tylosin (G. Dunny, personal communication) and the extensive use of these antibiotics in hospitals and in animal feeds may contribute to the dissemination of the Em^r phenotype. Indeed, the distribution of Tn*917*, Tn*3871*, and other closely related transposons may extend beyond the streptococci, since a considerable relationship between Tn*917* and Tn*551* (from *Staphylococcus aureus*) has been demonstrated (14). The obvious implications are that humans and animals have reservoirs of a mobile Em^r trait that are available to each other through various food and environmental chains

and direct contact. This resistance can be further expanded by plasmid transfer, transposition, and selective antibiotic pressure.

ACKNOWLEDGMENT

We thank M. Banai for providing the purified *Eco*RI G fragment of pJH2 containing a Tn*3871* insert from pJH1.

LITERATURE CITED

1. **Banai, M., and D. J. LeBlanc.** 1984. *Streptococcus faecalis* R plasmid pJH1 contains an erythromycin resistance transposon (Tn*3871*) similar to transposon Tn*917*. J. Bacteriol. **158:** 1172–1174.
2. **Christie, P. J., J. N. Davidson, R. P. Novick, and G. M. Dunny.** 1983. Effects of tylosin feeding on the antibiotic resistance of selected gram-positive bacteria in pigs. Am. J. Vet. Res. **44:** 126–128.
3. **Clewell, D. B., P. K. Tomich, M. C. Gawron-Burke, A. E. Franke, Y. Yagi, and F. Y. An.** 1982. Mapping of *Streptococcus faecalis* plasmids pAD1 and pAD2 and studies relating to transposition of Tn*917*. J. Bacteriol. **152:**1220–1230.
4. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Dunny, G. M., B. L. Brown, and D. B. Clewell.** 1978. Induced cell aggregation and mating in *Streptococcus faecalis*: evidence for a bacterial sex pheromone. Proc. Natl. Acad. Sci. U.S.A. **75:**3479–3483.
6. **Dunny, G. M., R. Craig, R. Carron, and D. B. Clewell.** 1979. Plasmid transfer in *Streptococcus faecalis*. Production of multiple sex pheromones by recipients. Plasmid **4:**454–465.
7. **Franke, A. E., and D. B. Clewell.** 1981. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J. Bacteriol. **145:**494–502.
8. **Jacob, A. E., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117:**360–372.
9. **LeBlanc, D. J., and L. N. Lee.** 1979. Rapid screening procedure for detection of plasmids in streptococci. J. Bacteriol. **140:** 1112–1115.
10. **LeBlanc, D. J., and L. N. Lee.** 1982. Characterization of two tetracycline resistance determinants in *Streptococcus faecalis* JH1. J. Bacteriol. **150:**835–843.
11. **Macrina, F. L., P. H. Wood, and K. R. Jones.** 1980. Genetic transformation of *Streptococcus sanguis* (Challis) with cryptic plasmids from *Streptococcus ferus*. Infect. Immun. **28:**692–699.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Maniatis, T., A. Jeffrey, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage lambda. Proc. Natl. Acad. Sci. U.S.A. **72:**1184–1188.
14. **Perkins, J. B., and P. J. Youngman.** 1984. A physical and functional analysis of Tn*917*, a *Streptococcus* transposon in the Tn*3* family that functions in *Bacillus*. Plasmid **12:**119–138.
15. **Rogosa, M.** 1969. Type strains of *Lactobacillus* species, p. 16. *In* Report of The Taxonomic Subcommittee on Lactobacilli and Closely Related Organisms, a subcommittee of the International Committee on Nomenclature of Bacteria of the International Association of Microbiological Societies. American Type Culture Collection, Rockville, Md.
16. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
17. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1980. Properties of erythromycin-inducible transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **141:**1366–1374.
18. **Tomich, P. K., F. Y. An, S. P. Damle, and D. B. Clewell.** 1979. Plasmid-related transmissibility of multiple drug resistance in *Streptococcus faecalis* subsp. *zymogenes* strain DS16. Antimicrob. Agents Chemother. **15:**828–830.
19. **Wittenberger, C. L., A. J. Beaman, and L. N. Lee.** 1978. Tween 80 effect on glucosyltransferase synthesis by *Streptococcus salivarius*. J. Bacteriol. **133:**231–239.