ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1977, p. 925-932
Vol. 11, No. 6

Printed in U.S.A.

Drug Resistance in Group D Streptococci of Clinical and Nonclinical Origin: Prevalence, Transferability, and Plasmid Properties

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Received for publication 13 January 1977

Group D streptococci isolated from clinical specimens and from sewage were investigated with regard to resistance to tetracycline (Tc), erythromycin (Em), and chloramphenicol (Cm). The median values of the percentages of resistant strains from sewage were: for Tc, 14%; for Em, 2.8%; and for Cm, 0.1%. For the recent isolates of clinical origin, resistance percentages found were 58% for Tc, 12% for Em, and 14% for Cm, and, in comparison to clinical isolates from 1964, the incidence of drug resistance slightly increased. In strains of both sources, the drug resistance was often found to be transferable to another group D streptococcus, probably by conjugation. Two strains were able to transfer their Em resistance to a streptococcus strain of group B. No transfer of drug resistance to a group A streptococcus and *Escherichia coli* was observed. All beta-hemolytic streptococci were also bacteriocinogenic, and frequently these properties were found to be transferable. The function, size, and base composition of the plasmids of two drug-resistant *Streptococcus faecalis* strains were investigated; strain M439 harbors at least two conjugative plasmids: pRI401, molecular weight 30×10^6, coding for Tc resistance, and pRI402, molecular weight 41×10^6, coding for Em resistance. Strain M403 carries one single conjugative plasmid species, coding for Tc resistance. The molecular weight of this plasmid, pRI404, was 37×10^6. The guanine plus cytosine content of these plasmids was 35 to 36%.

In contrast to the abundance of information on the development of drug resistance in *Enterobacteriaceae* and staphylococci (12), the literature on drug resistance in group D streptococci is scarce. In the period of 1953 to 1968, the occurrence of drug-resistant isolates of group D streptococci increased dramatically (15, 28).

Several authors reported a high incidence of resistance to tetracycline (Tc), chloramphenicol (Cm), and, in a lesser degree, to erythromycin (Em) (14, 18, 20, 21, 24). All isolates of group D streptococci in these studies were of clinical origin. Because of the lack of information on the prevalence of drug resistance in indigenous enterococci from healthy people, we have compared the incidence of drug-resistant group D streptococci from clinical origin with that of group D streptococci in sewage from human origin.

Recently, it was shown that antibiotic resistance in group D streptococci is plasmid mediated (5, 6, 9, 14, 18) and that these plasmids either are self-transferable by conjugation (14) or can be mobilized by conjugative plasmids (9). In this study, the transferability of drug resistance in isolates from clinical and nonclinical origin was investigated, and the molecular properties of some resistance plasmids are reported.

MATERIALS AND METHODS

Bacterial strains. The strains frequently used in this study are listed in Table 1.

Media. Nutrient broth was prepared in this laboratory from fresh meat and contained, in addition, 0.5% NaCl, 1% peptone (Difco), and 0.064% Na_2CO_3, pH 7.5. Nutrient agar contained, in addition, 2% agar (BBL). Unless otherwise stated, these media were used throughout this study. Blood agar was nutrient agar supplemented with 7% horse blood. Burkwall and Hartman medium (BH medium) was used as described (3). Minimal inhibitory concentrations (MICs) were determined on DST medium (Oxoid) supplemented with 7% horse blood.

Determination of MICs. The MICs were determined by inoculating 1:100 diluted overnight cultures by means of a Steers replicator on media supplemented with the appropriate antibiotics. Plates were incubated overnight at 37°C. The antibiotics used were: Tc (Pfizer), Cm (Mycopharm), Em (Abbott), clindamycin (Upjohn), spiramycin (Societé Paris d'Expansion Chemique [SPECIA]), tylosine (Verafarm), lincomycin (Upjohn), oleandomycin (Pfizer), and factor S of virginiamycin (Recherche et Industrie Therapeutiques), pristinamycin Ia (SPECIA).

TABLE 1. *Bacterial strains used*

Strain designation	Relevant properties	Origin
JH2	*Streptococcus faecalis*, susceptible to Tc, Cm, and Em, nonhemolytic	A. E. Jacob (14)
JH2-2	Mutant of JH2, resistant to fusidic acid and rifampin	A. E. Jacob (14)
M434	Mutant of JH2, resistant to streptomycin	This laboratory
M382	*Streptococcus agalactiae*, susceptible to Tc, Cm, and Em	Clinical isolate
M385	Mutant of M382, resistant to rifampin and fusidic acid	This laboratory
M519	Mutant of M382, resistant to streptomycin	This laboratory
M592	*Streptococcus pyogenes*, susceptible to Tc, Cm, and Em	Clinical isolate
M597	Mutant of M592, resistant to rifampin	This laboratory
SC181	*Escherichia coli* K-12, restrictionless	E. Lederberg
M665	Mutant of SC181, resistant to rifampin	This laboratory
M439	*S. faecalis*, resistant to Tc and Em, β-hemolytic	Clinical isolate
M403	*S. faecalis*, resistant to Tc, nonhemolytic	Clinical isolate
M440	*S. faecalis*, resistant to Tc and Em, nonhemolytic	Clinical isolate

Serological grouping and biotyping of streptococci. Group antigens were extracted with hot formamide according to the method described by Fuller (11). Ring precipitation tests were performed in special capillary tubes that have a thicker section where the reaction occurs (8). For recognition of the groups A through U, rabbit antisera prepared in this laboratory were used, with the exception of group D antiserum, which was obtained from Burroughs Wellcome.

The biochemical identification of group D streptococci was done according to the scheme of Facklam (10).

Isolation of streptococci from sewage. One-liter samples of sewage from influent reservoirs were homogenized, and 500 ml was centrifuged at 10,000 × *g* for 10 min. The pellet was suspended in 1 or 2 ml of nutrient broth. The concentrated suspensions and dilutions were plated on BH medium and BH plates containing the appropriate antibiotics (Tc, 8 μg/ml; Cm, 32 μg/ml; Em, 32 μg/ml). In an experiment to test the selectivity of BH medium for streptococci from sewage, all of 58 colonies tested were found to be enterococci, although the group D antigen could be demonstrated in 56 out of the 58 colonies grown on BH medium. Twenty-five belonged to biotype *Streptococcus faecalis*, 13 were of biotype *Streptococcus faecium*, and the other 20 did not belong to an established biotype. The biochemical reaction patterns of these 20 strains differed slightly from those of *S. faecalis* or *S. faecium*. Therefore, the selectivity of BH medium was satisfactory for our purpose.

Transfer of drug resistance and bacteriocin testing. Mating in liquid medium was done as described by Jacob and Hobbs (14). Matings on filters were done by filtering 1 ml of a mixture of equal volumes of overnight cultures of donor and recipient bacteria through a 0.45-μm-pore-size membrane filter (Sartorius). The filter was incubated overnight on a nutrient agar plate and then shaken with 1 ml of nutrient broth to release the bacteria from the filter. Mixtures of streptococcal matings were spread on plates containing the appropriate antibiotics: fusidic acid (LEO Pharmaceutical Products), 25 μg/ml; rifampin (Lepetit), 100 μg/ml; streptomycin (Mycopharm), 2 mg/ml; Em, 5 μg/ml; Tc, 5 μg/ml; and Cm, 25 μg/ml. When matings were done with *Escherichia coli* K-12 as a recipient, the following concentrations of antibiotics were used: rifampin, 50 μg/ml; Tc, 5 μg/ml; and Em, 500 μg/ml. Bacteriocin production was assayed as described by Dunny and Clewell (9), with strain JH2-2 as the indicator strain. Transfer frequencies are expressed as the number of drug-resistant recipients per total number of recipients.

DNA isolation and characterization. Cells were grown in 200 ml of nutrient broth at 37°C under vigorous agitation to an optical density of 0.5 at 600 nm. Deoxyribonucleic acid (DNA) was extracted as described by Clewell et al. (5) with some modifications. Cells were washed in 50 ml of TES buffer [0.01 M tris(hydroxymethyl)aminomethane, 0.001 M ethylenediaminetetraacetic acid (EDTA), 0.01 M NaCl, pH 8.1] at 0°C and resuspended in 5.6 ml of TES, containing 60% sucrose. Lysozyme (10 mg/ml in water), 0.50 ml, was added and the mixture was incubated for 10 min at 37°C. EDTA (0.2 M, pH 8.1), 2.40 ml, was added and the mixture was incubated for 10 min at 37°C. Subsequently, 1.12 ml of Pronase (5 mg/ml in TES, self-digested for 30 min at 37°C) and 10 ml 2% Sarkosyl [in 0.01 M tris(hydroxymethyl)aminomethane, 0.01 M NaCl, and 0.005 M EDTA, pH 8.1] were added, and the mixture was kept at room temperature for 15 min to complete lysis. Ethidium bromide and solid CsCl were added to 800 μg/ml and to 46.5% (wt/wt), respectively. Equilibrium centrifugation, purification, and electron microscopy of circular DNA have been described (30). Guanine plus cytosine (G+C) contents were calculated from the buoyant densities in CsCl as described by Schildkraut et al. (25).

RESULTS

Incidence of drug-resistant group D streptococci from sewage. Samples of influent of two small sewage treatment plants were investigated with regard to the incidence of drug-resistant group D streptococci. The influents originated from the villages Groenekan and Maartensdijk, which have about 900 and 1,400 inhabitants, respectively. According to the lo-

cal authorities for the maintenance of waterworks, no sewage from animal husbandry farms or industries is discharged into these sewage systems. On 6 days, between March 1976 and June 1976, samples of each of the two installations were collected. The total viable numbers of group D streptococci and the percentages of organisms resistant to Tc, Cm, and Em were determined. The results are summarized in Table 2. The fraction of drug-resistant group D streptococci varied greatly among the various samples. We have no explanation for this variation. Tc resistance was most frequently encountered (median values for the two plants, 13 and 15%), followed by Em resistance with median values of 2.7 and 2.9%. For Cm, resistance median values of 0.1% were found at both plants.

Transferability of drug resistance of strains isolated from sewage. The transferability of drug resistance markers of group D streptococci from sewage samples was investigated. Five colonies grown on BH plates containing Tc, Em, or Cm, respectively, were purified, and the resistance pattern and transferability of the resistance markers were investigated. The results are shown in Table 3. Only one of the 15 strains investigated was found to be beta-hemolytic. The strains selected on Em plates were Tc resistant as well, and three of the five strains selected on Cm resistance plates were also resistant to Em and Tc. Eight strains were able to transfer one or more resistance determinants to *S. faecalis* JH2-2 with a frequency higher than the detection level of 10^{-8} per recipient.

Drug resistance in group D streptococci of clinical origin. Two groups of human clinical isolates of group D streptococci were investigated. One group consisted of a collection of 44 strains isolated in 1964, and the other group included 106 strains isolated in the period between 1974 and 1976. All strains were obtained from hospitals in the Netherlands. The main sources of isolation were urine (40%), mouth and throat (20%), blood (14%), and wounds (8%). The MICs of Tc, Em, Cm, and clindamycin were determined, and the results are presented graphically in Fig. 1. The percentage of Tc-resistant strains (having an MIC >4 μg/ml) was high for both groups of D streptococci: 37 and 58%, respectively. Em, Cm, and clindamycin-resistant strains (having MICs of >16, >16, >8 μg/ml, respectively) were encountered less frequently. Also, for these drugs there was a tendency toward increasing prevalence during the period between 1964 and 1976. The Cm-resistant strains were also found to be resistant to Tc, and the Em-resistant strains were also resistant to Tc and clindamycin.

Transferability of drug resistance of clinical isolates. Thirty-six drug-resistant clinical isolates were mated with the recipient strain JH2-2. Matings were performed in liquid medium and on filters. Twenty-two of thirty-six tested were able to transfer one or more of their resistance markers to JH2-2. The results are summarized in Table 4. The transfer frequencies of filter matings ranged from 10^{-4} to the detection level of 10^{-8} for the different strains tested. In liquid medium, the transfer frequency was generally 10 to 100 times lower than that of matings on filters. About half of the strains were able to transfer the Tc resistance determinant. Cm and Em resistance was transferable in all strains tested. The three Em-resistant donor strains were also resistant

TABLE 3. *Transferability of drug resistance of group D streptococci isolated from sewage to S. faecalis JH2-2*[a]

No. of strains	Biotype	Resistance pattern	Markers transferable[b]
1	*S. faecalis*	Tc	
1	*S. faecium*	Tc	
3	–[c]	Tc	
3	*S. faecalis*	Em, Tc	Em, Tc
1	*S. faecium*	Em, Tc	Tc
1	*S. faecium*	Em, Tc	
2	*S. faecalis*	Cm, Em, Tc	Cm, Em, Tc
2	*S. faecalis*	Cm	Cm
1	*S. faecalis*	Cm, Em, Tc	

[a] All matings were performed on filters.
[b] Detection level of transfer in the mating experiments was 10^{-8} per recipient.
[c] –, Not belonging to an established biotype.

TABLE 2. *Prevalence of drug resistance in group D streptococci isolated from two sewage treatment plants*

Plant	Total viable group D streptococci per ml of influent[a]	Organisms resistant (%) to[a]		
		Tc	Em	Cm
Groenekan	1.7×10^3 (4.2×10^2 – 9.0×10^3)	15 (0.6–24)	2.9 (0.37–5.3)	0.1 (0.003–0.6)
Maartensdijk	4.0×10^3 (3.5×10^2 – 7.2×10^3)	13 (6–55)	2.7 (0.20–2.9)	0.1 (0.01–2)

[a] Median values of six samples taken on different days are presented; values in parentheses are the highest and the lowest observed.

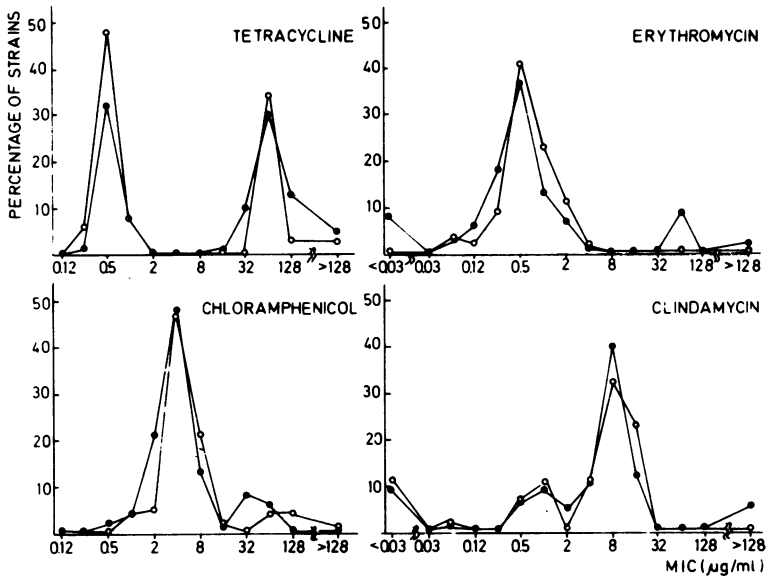

FIG. 1. *Distribution of MICs of four antibiotics. All strains were of clinical origin. Symbols: ●, Strains (106) isolated in the period 1974 to 1976; ○, strains (44) isolated in the year 1964.*

TABLE 4. *Transferability of drug resistance of group D streptococci from clinical sources to S. faecalis JH2-2*

No. of strains	Biotype	Hemolysis	Resistance pattern	Markers transferable
9	*S. faecalis*	γ	Tc	
1	*S. faecium*	γ	Tc	
4	*S. faecalis*	β	Tc	
6	*S. faecalis*	γ	Tc	Tc
7	*S. faecalis*	β	Tc	Tc, β
2	*S. bovis*	γ	Tc	Tc
2	*S. faecalis*	γ	Tc, Em	Tc, Em
1	*S. faecalis*	β	Tc, Em	Tc, Em, β
2	*S. faecalis*	β	Tc, Cm	Cm, β
2	*S. faecalis*	β	Tc, Cm	Tc, Cm, β

to clindamycin, lincomycin, oleandomycin, spiramycin, tylosine (MICs >50 μg/ml), factor S of virginiamycin (MIC >128 μg/ml), and pristinamycin Ia (MIC >32 μg/ml). Em-resistant transconjugants from the matings with the three Em resistance donor strains were also coresistant to all these antibiotics.

One strain transferred its Tc and Cm resistance as one linkage group. In the other double-resistant donor strains, no linkage between the Tc, and the Cm or Em, resistance determinants was observed.

Sixteen of the thirty-six donor strains used were beta-hemolytic, and all these strains were bacteriocinogenic, whereas no bacteriocin production could be demonstrated in the other 20 non-beta-hemolytic strains. In 10 strains, the property of hemolysin production was frequently cotransferred with one of the drug resistance determinants. All hemolytic transconjugants tested acquired the bacteriocinogenic property as well. These observations are in agreement with the view that the hemolysin of group D streptococci is identical to a bacteriocin (1, 2).

Host range of transfer of R-factors in group D streptococci. To learn whether the transfer of drug resistance of group D streptococci is limited to recipients of this serological group, 11 strains were selected that transferred one or more of their resistance markers to JH2-2 with a frequency $>10^{-7}$. These strains were used as donors in filter matings with the following recipients: a group A streptococcus (M597), a group B streptococcus (M385), and a restrictionless *E. coli* (M665).

No transfer of drug resistance to the recipients M597 and M665 was observed. In two matings with the group B streptococcus, M385, transfer frequencies were above the detection level of about 10^{-8} (see Table 5). The Tc- and Em-resistant donor strains M439 and M440 transferred their Em resistance to M385 with frequencies of 5.10^{-8} and 3.10^{-5}, respectively. Once transferred to M385, the Em resistance was transferred more efficiently to another derivative of this group B strain (M519), as is shown in Table 6.

Mechanism of transfer and plasmid properties of strains M439 and M403. Strains M439 and M403 were chosen for further study. Strain M439 is beta-hemolytic and is resistant to Tc and Em. The kinetics of transfer on filters of the drug resistance determinants and the hemolysin character are shown in Fig. 2. The Tc and Em resistance determinants are transferred independently, and 2 h after the beginning of the mating, no significant further increase of transfer was observed. We never observed hemolytic Tc-resistant transconjugants or hemolytic transconjugants that were Em susceptible. Tc- or Em-resistant transconjugants were able to transmit their drug resistance further to strain M434, a streptomycin-resistant derivative of JH2. Once introduced in JH2-2, the hemolysin character was always cotransferred with the Em resistance determinant on further transfer to M434 and, therefore, these two properties seemed to be linked in the transconjugants.

We have been unable to demonstrate any plaque-forming activity on JH2-2 in cell-free filtrates of cultures of M439. Furthermore, when cell-free filtrates of M439 were used instead of bacterial cultures, no transfer of Tc or Em resistance to JH2-2 was observed. Therefore, transduction is not likely to be the mechanism of drug resistance transfer. Also, transformation is highly improbable, because the presence of $MgCl_2$ (0.01 M) and deoxyribonuclease I (300 μg/ml) did not significantly affect the rate of transfer of Tc and Em resistance from M439 to JH2-2.

TABLE 5. *Transferability of drug resistance determinants of group D streptococci to strain S. agalactiae M385*

No. of strains tested	Resistance pattern	Hemolysin production	Transferability of resistance		
			Tc	Em	Cm
3	Tc	+	−		
2	Tc	−	−		
1	Tc, Em	+	−	+	
1	Tc, Em	−	−	+	−
3	Tc, Cm	+	−		
1	Tc, Cm	−	−		−

TABLE 6. *Transfer frequencies of Em resistance of M439 and M440 between streptococci of groups D and B*

Donor strain	Group	Recipient	
		JH2-2 (group D)	M519 (group B)
M439	D	1×10^{-4}	5×10^{-8}
M385 (439 EM)[a]	B	3×10^{-4}	2×10^{-3}
M440	D	1×10^{-2}	3×10^{-5}
M385 (440 EM)[b]	B	3×10^{-5}	2×10^{-3}

[a] Em-resistant transconjugant of the mating M439 × M385.

[b] Em-resistant transconjugant of the mating M440 × M385.

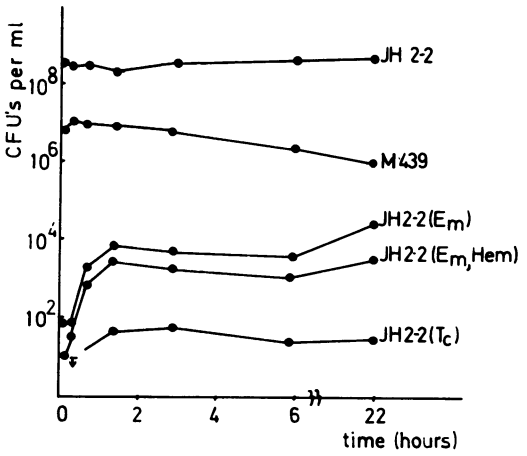

FIG. 2. *Kinetics of transfer of the drug resistance determinants Tc and Em and the hemolysin (Hem) character of strain M439 to strain JH2-2. Donor and recipients were mixed at time zero. The mating was performed in nutrient broth at 37°C.* ↧, *Below the level of detection.*

To confirm the plasmid nature of the drug resistance of M439, covalently closed circular (CCC) DNA was extracted from this strain and several JH2-2 transconjugants. Jacob and Hobbs (14) showed that strain JH2-2 does not contain any detectable plasmid DNA, and therefore this strain was used as host throughout these studies. It should be noted that in an attempt to find a plasmidless strain of *S. faecalis*, five drug-susceptible, nonhemolytic clinical isolates were examined for the presence of CCC DNA. All five strains, however, contained CCC DNA and, therefore, plasmids seem to be common in this bacterial biotype.

Table 7 shows that strain M439 contains circular molecules with two different contour lengths. In the Tc-resistant transconjugant M510, a species with a mean contour length of 14.5 μm was found, whereas, in the Em-resistant transconjugants M507 and M505, molecules of the larger size class with contour lengths of 19.8 and 18.5 μm, respectively, were mainly found. Occasionally, smaller molecules were observed. The buoyant densities of all plasmid preparations amounted to 1.694 to 1.695. The corresponding guanine plus cytosine (G+C) values of 35 to 36% are close to the G+C values

TABLE 7. *Molecular properties of plasmids in strains M439, M403, and their transconjugants*

Strain	Phenotypic[a] properties	Plasmid sizes[b] observed (μm)	Mol wt	Designation of plasmid	Buoyant density in CsCl	G+C content (%)[c]
M439	Tc, Em, β	14.5 ± 0.7 (*n* = 20)	29.8 × 10⁶		1.6949	35.3
		19.9 ± 0.5 (*n* = 13)	41.0 × 10⁶			
M510[c]	Tc	14.5 ± 0.2 (*n* = 23)	29.8 × 10⁶	pRI401	1.6958	36.5
		6.2 (*n* = 1)	12.8 × 10⁶			
M507[d]	Em	19.8 ± 0.3 (*n* = 21)	40.8 × 10⁶	pRI402	1.6948	35.5
		1.32 (*n* = 1)	2.7 × 10⁶			
		0.50 (*n* = 1)	1.0 × 10⁶			
M505[d]	Em, β	18.5 ± 0.3 (*n* = 21)	38.1 × 10⁶	pRI403	1.6939	36.1
		16.70 (*n* = 1)	34.4 × 10⁶			
		0.76 (*n* = 1)	1.6 × 10⁶			
M403	Tc	17.8 ± 0.5 (*n* = 12)	36.7 × 10⁶	pRI404	1.6939	34.6
M410[e]	Tc	17.0 ± 0.4 (*n* = 13)	35.0 × 10⁶	pRI 404	1.6947	35.4

[a] β, β-hemolytic.
[b] Size and standard deviation; numbers in parentheses show how many molecules were measured.
[c] The G+C content was derived from the buoyant density in CsCl.
[d] Transconjugants of the mating M439 × JH2-2.
[e] Transconjugant of the mating M403 × JH2-2.

of 34.5 and 36.5% as found by Courvalin et al. (6) for Tc and Em resistance plasmids in *S. faecalis* and close to the G+C values of 35 to 38% reported for chromosomal DNA of *S. faecalis* (6, 25).

It is concluded that M439 harbors a Tc resistance plasmid (molecular weight, 30 × 10⁶), designated pRI401, and an Em resistance plasmid (molecular weight, 41 × 10⁶), designated pRI402. It is not clear from our experiments whether M439 also carries the plasmid designated pRI403, which codes for hemolysin production plus Em resistance, as found in the transconjugant M505 and is about the same size as pRI402. Royer-Pokora and Goebel (23) found that hemolysin plasmids from transconjugants originating from the same *E. coli* donor strain may differ considerably in their nucleotide sequences, although they have the same physical sizes. In analogy to these findings, pRI403 may be thought to be formed by a recombination between plasmids present in strain M439 or by translocation of the hemolysin determinant to pRI402.

The nonhemolytic strain M403 was exceptional among the Tc-monoresistant isolates, because the frequency of transfer was high, 10^{-3} to 10^{-4}, whereas in all other Tc-resistant isolates no higher values than 10^{-6} to 10^{-7} were observed. When the same techniques as described above were used, no indication could be found that transformation or transduction was the mechanism of transfer. Strain M403 harbors a plasmid species, homogenous in size, with a molecular weight of 36.8 × 10⁶ and a G+C content of 35%. The transconjugant M410 carried a plasmid with identical molecular properties (Table 7). This plasmid is designated pRI404.

DISCUSSION

The results of the MIC determinations of the group D streptococci isolates of clinical origin show a tendency to increasing antibiotic resistance during the period between 1964 and 1976. The present incidence of Tc-resistant strains, about 50%, is as high as the values of 40 to 70% reported by other authors for group D streptococci (18, 20, 21, 24, 27, 28). However, our observations on resistance to Cm and Em suggest that the incidence of resistance to these drugs (14 and 12%, respectively) is lower than that reported by Toala et al. (28) and Schaal and Rütten (24), who found resistance percentages of around 50%.

The incidence of drug resistance in group D streptococci from sewage of human origin differed highly from sample to sample. We have no explanation for this variation. Although the data of the clinical isolates and isolates from sewage are not fully comparable because of the different methods used, the median values of the percentages of drug-resistant organisms in sewage suggest that drug resistance among the indigenous group D streptococcal flora in healthy people is less common than that found in clinical isolates. Nevertheless, an incidence of 14 and 2.8% for Tc and Em resistance, respectively (Table 2), points to a widespread occurrence. This seems hard to explain by selective pressure solely due to human therapeutic use of these antibiotics.

The frequently observed transferability to JH2-2 of drug resistance of group D streptococci from clinical and nonclinical origin suggests that drug resistance is often plasmid mediated. The experiments with strains M439 and M403 confirm the extrachromosomal nature of drug resistance and indicate that the mechanism of transfer is likely to be conjugation. Conjugal transfer of R-factors in *S. faecalis* has been observed previously (9, 14). In the isolates of clinical origin, we frequently observed cotransfer of hemolysin and bacteriocin production with one of the drug resistance determinants. These observations are consistent with the view that bacteriocin and hemolysin production are linked genetically and mediated by plasmids (9, 13, 19, 29) and that some particular bacteriocins and hemolysins of *S. faecalis* are identical substances (1, 2) or probably coded by the same gene (13).

In this study we have not been able to transfer plasmids of *S. faecalis* to a group A streptococcus strain and to *E. coli* K-12. However, the Em resistance of the strains M439 and M440 was transferable to the group B streptococcus M385. In spite of this, Em resistance in group B streptococci is not frequently encountered. In a pilot study, none of 197 strains of clinical origin tested was found to be Em-resistant, whereas 15% were resistant to Tc (B. van Klingeren, unpublished data).

The molecular sizes of the plasmids in M439 and M403 differ from those found for drug resistance plasmids in other streptococci (4, 5, 6, 14, 17). The conjugative Em resistance plasmids studied here exhibit coresistance to macrolides other than Em and to lincomycin, clindamycin, factor S of virginiamycin, and pristinamycin Ia. With respect to this coresistance, these plasmids are similar to the Em resistance plasmids β (31) and Ero (6, 7) found in *S. faecalis*. Furthermore, pRI402 is expressed constitutively in JH2-2 (J.D.A. van Embden, unpublished observations), similar to the behavior of plasmid β. Yagi et al. (31) showed at least 95% base sequence homology between the plasmid β and a plasmid of the same size (17×10^6 daltons) found in a streptococcus strain of group A. This finding suggests that plasmid exchange between group A and group D streptococci might have taken place. Our results indicate that pRI402 and other conjugative Em resistance plasmids are not easily transferable to the group A strain M597.

In the Netherlands and in other European countries, macrolide antibiotics and virginiamycin (which is partly composed of factor S) are used as feed additives in animal husbandry. Potentially, this might result in a selection of macrolide-resistant organisms. Preliminary studies (J.D.A. van Embden, unpublished observations) indicate that virginiamycin can select in vitro group D streptococci carrying plasmids that code for macrolide resistance. In vivo, such a selection might result in a buildup of a reservoir of macrolide-resistant group D and group B streptococci that are indigenous to animals (16, 26). These organisms could be transferred to humans by consumption of contaminated meat or animal handling (22). Considering the therapeutic value of the macrolide antibiotics lincomycin and clindamycin in human therapy and the transferable, plasmid-borne nature of resistance to these drugs, investigations on the consequences of the use of macrolides and virginiamycin as feed additives are desirable.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of M. M. M. Meyers-van Nesselrooy, N. Soedirman, and L. J. van Wijngaarden.

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