ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1980, p. 753-760
0066-4804/80/11-0753/08$02.00/0

Vol. 18, No. 5

Identification of Tetracycline-Resistant R-Plasmids in *Streptococcus agalactiae* (Group B)

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In this report, 30 tetracycline-resistant clinical isolates of group B *Streptococcus* were examined to assess the extent to which tetracycline resistance is plasmid mediated. Of these, 27 showed no physical or genetic evidence of plasmid-mediated resistance; however, one conjugative and two small (3.5×10^6-dalton) multicopy non-self-transmissible tetracycline resistance plasmids were identified. The conjugative plasmid was transmissible to *Streptococcus faecalis* as well as to *Streptococcus agalactiae* (group B). The two nonconjugative plasmids were readily mobilized by a number of sex factors into these same two backgrounds and, in addition, readily transformed *Streptococcus sanguis* Challis to tetracycline resistance. Due to readily available sites for several site-specific endonucleases, these small, multicopy plasmids should prove useful as cloning vehicles in this host system.

Since 1957, there has been a significant increase in the incidence of tetracycline resistance among clinical isolates of species of *Streptococcus* (1, 2, 9, 15, 22, 28), and tetracycline (Tc) resistance is now commonly encountered. Surveys of *Streptococcus pyogenes* since 1974 (22) and *Streptococcus agalactiae* (group B) in 1976 (1, 9) found that 80% and 87.6% of the isolates, respectively, were resistant to high levels of tetracycline. However, only in a few cases has tetracycline resistance in *Streptococcus* been shown to be mediated by plasmids (4, 5, 8, 14, 29). These reports have all been confined to *Streptococcus faecalis* (group D), and no systematic analysis of the genetic basis of tetracycline resistance has been carried out.

In this report I extend my previous observations (11) on a plasmid transfer system to assess the extent to which tetracycline resistance determinants in group B *Streptococcus* are plasmid mediated. In the course of this work, 92.4% of 433 clinical isolates tested were resistant to tetracycline, and in the majority of cases we were unable to identify tetracycline resistance plasmids. Of 30 strains examined in detail, only 3 were found to contain tetracycline resistance plasmids. This is in contrast to the findings with gram-negative bacteria, whose tetracycline resistance is almost always plasmid associated. Of particular significance, two of the plasmids were small multicopy plasmids which readily transformed *Streptococcus sanguis* Challis. These properties render them attractive as vehicles for cloning in this system.

MATERIALS AND METHODS

Bacterial strains. *S. agalactiae* (group B) strains (Table 1) were identified from clinical samples by being weakly beta-hemolytic on sheep blood agar, positive in the CAMP (3) test, hippurate positive, and bile esculin negative, and were confirmed serologically by the method of Rantz and Randall (23).

Twenty-four strains were selected at random from among 433 independent clinical isolates collected over a 2-year period at the Duke University Medical Center. Six strains from Torrance, Calif. (1) (MV158, MV159, MV160, MV161, MV162, MV163), also represent independent isolates and are of several different serotypes. Other strains used are listed in Table 2. For clarity, subscripts following the strain designation will be used to differentiate the serotypes, e.g., JH2-2_D is a group D *Streptococcus*, and $MV158_B$ is a group B isolate.

Spontaneous mutants to rifampin resistance (*rif*) and streptomycin resistance (*str*) were obtained by plating broth cultures on solid media containing 100 μg and 1,000 μg of the antibiotic, respectively, per ml. Minimum inhibitory concentrations were determined on brain heart infusion agar (BHIA) plates containing doubling concentrations of antibiotic, by using a Steers multiple inoculator (27).

Media. Brain heart infusion broth (BHIB; Difco) was used for routine cultivation. BHIA was used as the solid medium. Five percent defibrinated sheep blood was added in some cases to test hemolytic characteristics of strains.

Mating procedures. Fresh overnight cultures (1 ml) in BHIB were diluted and mixed at a ratio of 1 donor (0.05 ml) to 10 recipients (0.5 ml) or 1 donor (0.5 ml) to 1 recipient (0.5 ml) in a final volume of 5.0 ml of BHIB. One milliliter of this mixture was collected on a Millipore membrane filter (HAWP, 0.45-μm pore size, 13 mm), which was then placed on the surface of a BHIA plate and incubated at 37°C. After incubation, the cells on the filter were suspended in 1 ml of BHIB, and the mixture was plated on BHIA plates selective for transconjugants. The mating mixture was also plated on appropriate medium to determine the total number of donors and recipients pres-

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ent. Controls consisting of unmated donors and recipients were treated in the same way. Frequencies are expressed as the number of transconjugants per donor colony-forming unit. When appropriate, transconjugants were tested for unselected plasmid and chromosomal phenotypes by toothpick transfer. Drug concentrations used were as follows (in μg/ml): chloramphenicol (Cm), 10; erythromycin, 10; tetracycline, 2; rifampin, 100; streptomycin, 1,000; and kanamycin (Km), 1,000.

Tests for the effects of deoxyribonuclease (Worthington, 10 μg/ml) on transfer (11) and assays for the presence of possible transducing phage (11) were always negative.

Determination of type of resistance. To determine whether expression of tetracycline resistance was inducible or constitutive, a modification of the procedure of Hyder and Streitfeld (13) was used. Tetracycline was used as the inducer and as challenge drug at 0.5 and 2 μg/ml, respectively.

Transformation. Transformation of *S. sanguis* Challis ($V288_H$) by purified plasmid deoxyribonucleic acid (DNA) was accomplished according to the procedure of Lawson and Gooder (17). Cells were grown to competence in BHIB with 10% heat-inactivated equine serum before exposure to DNA. Cells were plated after 3 h of further incubation, using a double agar overlay (25).

Plasmid screening. Routine plasmid screening was performed as outlined by Macrina et al. (20), with minor modifications. Cultures were grown overnight in BHIB, diluted twofold, and grown with 5% glycine for 60 min in a final volume of 37.5 ml. Cells were harvested by centrifugation and washed with 0.2 M potassium acetate (pH 6.0) and TES buffer [50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 8.0)–5 mM ethylenediaminetetraacetic acid (EDTA)–50 mM NaCl]. Washed cells were suspended in 2.5 ml of 25% glucose in 100 mM Tris-hydrochloride (pH 8.0)–10 mM EDTA, followed by the addition of 0.75 ml of lysozyme (Worthington, 6 mg/ml in above buffer) for 1 h at 37°C. The cells were then treated with protease (Sigma; 0.75 ml of a 6-mg/ml solution in 100 mM Tris-hydrochloride [pH 8]–10 mM EDTA) for 1 h at 37°C. The cell suspension was then lysed with 50 μl of ribonuclease (Sigma; 10 mg/ml in 0.15 M NaCl) and 0.21 ml of 20% sodium dodecyl sulfate. If lysis did not occur within 15 min at room temperature, it could be enhanced by heating the lysates at 55°C for a few minutes before the addition of 1.05 ml of 5 M NaCl. The lysates were allowed to sit at 0°C overnight, and high-molecular-weight DNA was removed by centrifugation in a 60 Ti rotor at 30,000 rpm for 30 min at 4°C. The DNA in the supernatant was precipitated with 2 volumes of cold ethanol at −20°C overnight. The precipitate was collected by centrifugation and suspended in 20 mM Tris-hydrochloride–0.1 mM EDTA–50 mM NaCl, and samples were analyzed by agarose gel electrophoresis (11). To screen for small plasmids in $V288_H$ transformants, DNA could be analyzed directly after sodium dodecyl sulfate lysis with-

TABLE 1. *Tetracycline-resistant clinical isolates*

Strain	Resistance phenotype	Source/reference
$MV120_B$	Tc	Durham, N.C., 1977; this study
$MV158_B$	Tc Cm	Torrance, Calif., 1975 (1)
$MV160_B$	Tc Cm	Torrance, Calif. 1975 (1)
$MV163_B$	Tc Cm	Torrance, Calif., 1975 (1)
$MV206_B$	Tc	Durham, N.C., 1978; this study
$MV597_B$	Tc	Durham, N.C., 1978; this study

TABLE 2. *Other bacterial strains*

Strain	Plasmid	Relevant markers[a]	Source/reference
Plasmid-containing strains			
$D25303_B$	pMV103	MLS, Tra^+	M. Dixon (7)
$MV154_B$	pIP501	MLS, Cm, Tra^+	T. Horod (12)
$MV176_B$	pMV176	MLS, Cm, Tra^+	B. Anthony (1)
$JH2\text{-}14_D$	pJH4	MLS, Sm, Km, Tra^+	A. Jacob (14)
$JH203_D$ (pAMβ1)	pAMβ1	MLS, Tra^+	This paper (DS5 × JH203)
$JH2\text{-}2_D$ (ERL1)	ERL1	MLS, Tra^+	This paper, (SM60-ERL1 × JH2-2)
$JH2\text{-}2_D$ (pAMα1)	pAMα1	Tc, Tra^-	This laboratory (DS 5 × JH2-2)
Recipient strains			
$MV157_B$	None	None	T. Horod
$MV762_B$	None	*rif str*	This paper (from MV157)
$JH2_D$	None	None	A. Jacob (14)
$JH2\text{-}2_D$	None	*rif fus*	A. Jacob (14)
$JH203_D$	None	*str*	This paper (from JH2)
$V288_H$	None	None; *S. sanguis* Challis	F. Macrina, NCTC 7868

[a] $Tra^{+/-}$ refers to the ability to mediate transfer. Sm, Plasmid streptomycin resistance; *str*, chromosomal streptomycin resistance; *fus*, fusidic acid resistance.

out further enrichment. Dye-buoyant density gradient centrifugation was carried out on plasmid-enriched fractions prepared as described above from 300 ml of culture. Fractions containing supercoiled plasmid DNA were pooled, the dye was extracted with *n*-butanol, and the samples were dialyzed against buffer (10 mM Tris-hydrochloride [pH 7.5]–50 mM NaCl–0.1 mM EDTA).

Restriction endonuclease analysis. Restriction enzymes *Hpa*I, *Bgl*II, and *Hin*dIII were purchased from Bethesda Research Laboratories and used according to recommended reaction conditions. *Eco*RI was a gift from P. Modrich.

Agarose gel electrophoresis. Plasmid DNAs were analyzed by electrophoresis on 0.7 or 1% agarose gels in Tris-borate buffer (10) at 8 V/cm for 3 h. Gels were stained in 0.5 μg of ethidium bromide per ml and photographed with a Polaroid MP-4 camera using type 665 film through Wrattan no. 23 plus ultraviolet filters.

RESULTS

The scheme used to screen the clinical isolates for potential tetracycline resistance plasmids is shown in Fig. 1. Two different recipient strains were used in every mating experiment. JH2-2_D is a widely used *S. faecalis* strain resistant to rifampin and fusidic acid. The other recipient was $MV762_B$, a plasmid-free pigmented group B strain made resistant to streptomycin (2,500 μg/ml) and rifampin (1,000 μg/ml). Rifampin was used as the marker selective for the recipient because the frequency of mutation to high-level resistance among most donor strains is low. Spontaneous mutation to tetracycline resistance among these recipient strains was not encountered.

The first step was to use tetracycline-resistant

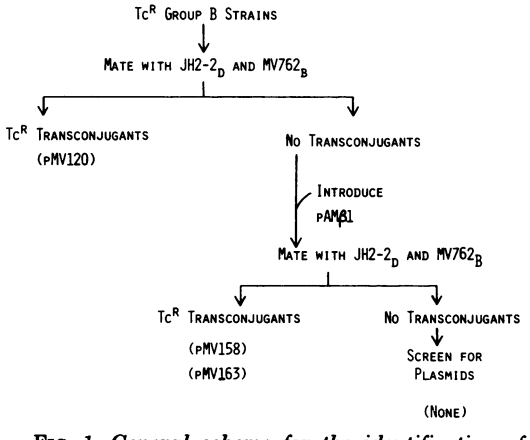

FIG. 1. *General scheme for the identification of tetracycline resistance plasmids. This figure shows the method used for detecting both conjugative and nonconjugative resistance factors present in clinical isolates. This is made possible by the ability of pAMβ1 to mobilize nonconjugative plasmids.*

isolates as potential donor strains in 18-h matings. In the cases where no transfer occurred, the conjugative plasmid pAMβ1 was introduced from $JH203_D$(pAMβ1) to test whether tetracycline resistance determinants could now be mobilized by the sex factor into a recipient strain.

Presumptive transconjugants obtained from each positive mating were analyzed for unselected chromosomal markers, including streptomycin resistance and pigmentation for $MV762_B$ and positive bile-esculin reaction and lack of hemolysis on sheep blood agar for JH2-2_D. This was important since the colonies sometimes observed on plates selective for transconjugants (tetracycline, 2 μg/ml; rifampin, 50 μg/ml) were Rif^r mutants of the donor strain being tested. If transfer or mobilization occurred, the parent strains as well as two transconjugants were tested for the presence of plasmid DNA.

Conjugative and nonconjugative tetracycline-resistant strains. By using the procedure outlined above, one conjugative (pMV120) and two nonconjugative (pMV158 and pMV163) plasmids were tentatively identified. Table 3 shows representative data for these strains as well as data representative of strains from which no transfer could be demonstrated. Twenty-four additional strains fell into the latter category (data not included). In every case, the sex factor pAMβ1 transferred at $\sim 4.2 \times 10^{-2}$ per donor colony-forming unit, and in two strains the presence of the sex factor was necessary for transfer of the two mobilizable plasmids.

Since a number of conjugative plasmids have been described in *S. faecalis* (6, 8, 14, 29), *S. agalactiae* (11, 12), and *S. pyogenes* (21), the question was posed whether sex factors other than pAMβ1 could mobilize these non-self-transmissible plasmids (NTPs). JH2-2_D(pAMα1) was included in the study at this point as a known NTP from *S. faecalis*. $MV158_B$, $MV163_B$, and JH2-2_D(pAMα1) derivatives were constructed containing the following sex factors: pAMβ1, pMV103, pIP501, pJH4, and ERL1. These conjugative plasmids (Table 2) all mediate erythromycin (macrolide)-lincosamide-streptogramin B (MLS) resistance with and without additional markers. In this way, sex factor transfer could be monitored independent of the NTP. The recipient was JH2-2_D [except that $JH203_D$ was used when the donor was a JH2-2_D(pAMα1) derivative]. These data are presented in Table 4. The significant results of this series of experiments were: (i) ERL1, originally described in *S. pyogenes* 13234, was demonstrated to be a sex factor and was able to mobilize the tetracycline resistance plasmids studied; (ii) all the sex factors were able to mobilize all the NTPs to JH2-2_D or $JH203_D$; (iii) pMV158 was mobilized best

TABLE 3. *Transfer of tetracycline resistance*[a]

Donor	Recipient	Tc transfer		Plasmid
		Without pAMβ1	With pAMβ1	
$MV120_B$	JH2-2	2×10^{-7}	Not applicable	pMV120
	MV762	2.5×10^{-7}	Not applicable	
$MV158_B$	JH2-2	$<1.0 \times 10^{-8}$	1.7×10^{-4}	pMV158
	MV762	$<3.3 \times 10^{-8}$	7.3×10^{-7}	
$MV160_B$	JH2-2	$<2.5 \times 10^{-8}$	$<1.4 \times 10^{-8}$	
	MV762	$<5.5 \times 10^{-9}$	$<1.0 \times 10^{-8}$	
$MV163_B$	JH2-2	$<1.0 \times 10^{-8}$	1.0×10^{-4}	pMV163
	MV762	$<1.0 \times 10^{-8}$	5.4×10^{-6}	
$MV206_B$	JH2-2	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$	
	MV762	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$	
$MV597_B$	JH2-2	$<1.0 \times 10^{-8}$	$<1.0 \times 10^{-8}$	
	MV762	$<5.2 \times 10^{-9}$	$<5.0 \times 10^{-9}$	

[a] Mating experiments were carried out by incubating donors and recipients together in a 1:1 ratio on filters for 18 h. pAMβ1 was introduced by mating from $JH203_D$(pAMβ1).

TABLE 4. *Transfer of Tet^r NTPs by different sex factors*[a]

Host	Sex factor	Transfer frequency		Relative transfer Tc/Em	Cotransfer ratio	
		Sex factor	NTP		Em Tc; Tc	Em Tc; Em
$MV158_B$	pAMβ1	1.5×10^{-1}	1.7×10^{-4}	0.046	98/100	0/100
	pMV103	6.0×10^{-2}	1.2×10^{-3}	0.020	100/100	0/100
	pIP501	1.1×10^{-2}	8.7×10^{-4}	0.079	100/100	0/100
	pJH4	3.6×10^{-3}	2.6×10^{-4}	0.072	50/50	0/50
	ERL1	1.0×10^{-4}	1.0×10^{-6}	0.010	—	—
$MV163_B$	pAMβ1	4.2×10^{-2}	1.0×10^{-4}	0.002	50/50	0/50
	pMV103	9.0×10^{-3}	4.7×10^{-5}	0.005	100/100	0/100
	pIP501	7.5×10^{-2}	4.2×10^{-3}	0.056	97/100	0/100
	pJH4	2.7×10^{-3}	1.5×10^{-5}	0.006	50/50	0/50
	ERL1	1.0×10^{-4}	6.0×10^{-7}	0.006	—	—
$JH2\text{-}2_D$(pAMα1)	pAMβ1	1.0×10^{-3}	6.4×10^{-5}	0.064	—	—
	pMV103	1.1×10^{-2}	3.7×10^{-4}	0.032	—	—
	pIP501	1.9×10^{-3}	7.3×10^{-4}	0.038	37/46	0/46
	ERL1	2.4×10^{-2}	2.8×10^{-4}	0.012	—	—

[a] Strains containing tetracycline-resistant NTPs and one of several MLS-resistant sex factors were used as donors in 6-h filter matings with $JH2\text{-}2_D$ in a donor-to-recipient ratio of 1:10. Transfer frequencies of both the sex factor (plates supplemented with erythromycin, 10 μg/ml, and rifampin, 50 μg/ml, or with erythromycin, 10 μg/ml, and streptomycin, 1,000 μg/ml) and NTP (plates supplemented with tetracycline, 2 μg/ml, and rifampin, 50 μg/ml, or with tetracycline, 2 μg/ml, and streptomycin, 1,000 μg/ml) were determined independently. From each set of selection conditions, 50 or 100 colonies were picked to confirm the recipient phenotype and test cotransfer of the other plasmid. Em Tc; Tc and Em Tc; Em are the number resistant to both erythromycin and tetracycline when initial selection was made on tetracycline or on erythromycin, respectively. —, Not tested.

while pMV163 mobilization was poorest overall; (iv) pIP501 was most efficient at mobilizing the NTPs; (v) NTP and sex factor transfer was highly asymmetric, consistent with the relative transfer frequencies; (vi) the NTPs were donated much less often (0.2 to 7.9%) than the sex over a 450-fold range of sex factor transfer frequencies.

One important genetic criterion for determining whether a resistance is plasmid associated is demonstration of multiple rounds of transfer. First-round transconjugants $JH2\text{-}2_D$(pMV120)

and JH2-2_D harboring the NTPs with and without pIP501 were used as donors in matings with $JH203_D$ (Table 5). JH2-2_D(pMV158) and JH2-2_D(pMV163) did not behave as donors unless pIP501 was present in the same cell. This agrees with the initial observation that pMV158 and pMV163 cannot be transferred unless a sex factor is present in the same host background. Transfer has been shown to occur readily from group B strains to group B and group D recipients. Group D hosts are also able to donate plasmids back to group B.

A single transconjugant which had the following characteristics was found when JH2-2_D(pMV158 pAMβ1) was used as the donor. It was tetracycline resistant (indicating the presence of pMV158) and erythromycin (MLS) susceptible, but was still able to retransfer pMV158 at a high frequency. Analysis of plasmid DNA isolated from this transconjugant demonstrated the presence of two plasmids. One was identical with pMV158; the larger plasmid was almost the size of pAMβ1. *Hin*dIII digestion of the larger plasmid was identical with that of pAMβ1 except that fragment 2 was missing and a new band, 1,000 base pairs smaller, appeared (data not shown). This is consistent with the data of Weisblum et al. (30), who identified this band as carrying the MLS gene by using Southern hybridizations (26).

Characteristics of transfer. Transfer required that donors and recipients be held in close proximity on a filter, since no transfer was detected during mixed growth in broth culture. In 6-h mating experiments, transfer was resistant to deoxyribonuclease (10 μg/ml in the presence of 10 mM $MgSO_4$), and phage have never been detected in any culture tested to date. Furthermore, some function of the sex factor is involved in transfer of the NTPs, further suggesting that transformation and transduction are not responsible for the transfer that does take place.

Resistance characteristics. Stable strains containing Tc^r plasmids of low (pMV120, pIP614, pJHl) and high (pMV158, pMV163, pAMα1) copy number (see following section) exhibited levels of resistance 10 to 30 times greater than the host background. The degree of enhanced resistance does not correlate with the copy number, suggesting that a single copy of the gene is sufficient to saturate all sites in the cell, assuming that there is a single type of tetracycline resistance. We also asked whether plasmid-mediated tetracycline resistance and chromosomal resistances could be distinguished on the basis of their pattern of expression. Single and multicopy streptococcal plasmids were tested in the original host as well as JH2-2_D and $MV762_B$ host backgrounds. In all cases, tetracycline resistance was shown to be constitutively expressed. As a control for inducible expression of tetracycline resistance, *Escherichia coli* containing pSC101 was tested and showed growth typical of an inducible resistance under the same set of conditions used to test the streptococcal strains.

Attempts to cure plasmid-containing strains of tetracycline resistance by growth at 42°C or growth in the presence of acridine orange (0.5 to 10 μg/ml) were negative. Tests on over 1,000 colonies of each strain were carried out using parental strains. Failure to cure is not uncommon in the case of some multicopy plasmids.

Plasmids. Lysates of parental strains and transconjugants from a number of matings were enriched for plasmid DNA and analyzed by agarose gel electrophoresis. Two forms of pMV158 and pMV163 were present, corresponding to co-

TABLE 5. *Retransfer of NTPs*[a]

Donor	Recipient	Transfer frequency	
		Sex factor	NTP
$MV158_B$(pIP501)	JH2-2_D	3.0×10^{-3}	1.5×10^{-5}
JH2-2_D(pMV158)	$JH203_D$	—	$<3.1 \times 10^{-9}$
JH2-2_D(pMV158 pIP501)	$JH203_D$	4.2×10^{-4}	6.0×10^{-6}
$JH203_D$(pMV158 pIP501)	$MV762_B$	1.6×10^{-3}	1.9×10^{-6}
$MV163_B$(pIP501)	JH2-2_D	1.2×10^{-3}	1.2×10^{-5}
JH2-2_D(pMV163)	$JH203_D$	—	$<4.5 \times 10^{-6}$
JH2-2_D(pMV163 pIP501)	$JH203_D$	4.0×10^{-4}	1.6×10^{-6}
$JH203_D$(pMV163 pIP501)	$MV762_B$	8.4×10^{-4}	1.7×10^{-6}
$MV120_B$	JH2-2	6.0×10^{-7}	—
JH2-2_D(pMV120)	$JH203_D$	2.5×10^{-7}	—
JH2-2_D(pMV120)	$MV762_B$	4.0×10^{-7}	—

[a]Donors and recipients were incubated together on filters at a ratio of one donor to one recipient for 18 h. On retest, no transconjugants carried donor chromosomal markers. —, Not applicable.

valently closed circular and open circular molecules. In all cases, the plasmids that were recovered corresponded to the resistance phenotype of the strain. Plasmids from transconjugants from different rounds of transfer appeared identical in mobility to plasmid DNA isolated from the parental strains. Figure 2 shows examples of plasmids recovered from parental strains and transconjugants of different phenotypes.

Accurate measure of molecular weights can be obtained from linearized DNA or the sum of the size of fragments generated by site-specific endonucleases. Such digests are shown in Fig. 3. The sizes of the plasmids are 45,000 base pairs for pMV120, 5,200 base pairs for pMV158, and 5,400 base pairs for pMV163, calculated from this and other gels. Based on recovery of satellite DNA from cesium chloride-ethidium bromide gradients, pMV120 exists in three to five copies, whereas pMV158 and pMV153 have greater than 50 copies per chromosome.

The strains that showed no genetic evidence for the presence of plasmids were also examined for the presence of plasmid DNA. Several lysis procedures (11, 20) which routinely yielded plasmid from known strains were consistently negative even when cells were labeled with [^{3}H] thymidine and DNA was centrifuged in cesium chloride-ethidium bromide gradients.

Transformation. We sought to use transformation as further confirmation that the tetracycline resistance phenotype is plasmid associated. *S. sanguis* ($V288_H$) was used as the recipient in attempts to transform plasmid DNA from $MV158_B$, $MV163_B$, and JH2-2_D(pAMα1). Plasmid DNA was mixed with competent cells at 37°C, and transformation was allowed to proceed for 3 h. No tetracycline-resistant colonies were observed in control cultures or when pAMα1 plasmid DNA was used. Plasmids pMV158 and pMV163 were able to effect transformation of $V288_H$ at frequencies of about 4×10^{-5} transformants per recipient per μg. The $V288_H$ host strain is completely inhibited by 0.1 μg of tetracycline per ml. However, pMV158 and pMV163 transformants required minimum inhibitory concentrations of 2.5 μg/ml. The presence of these plasmids had no effect on colony morphology or sugar fermentation pattern of $V288_H$.

DNA from $V288_H$ and $V288_H$ transformants was extracted and analyzed by electrophoresis on 0.7% agarose gels. $V288_H$ gave only a band of chromosomal DNA,whereas transformants contained a plasmid which comigrated with purified plasmid DNA isolated from the parent strain (Fig. 4).

DISCUSSION

Among the *Enterobacteriaceae*, the majority of tetracycline resistance determinants among clinical isolates are plasmid mediated. To assess the extent to which tetracycline resistance in

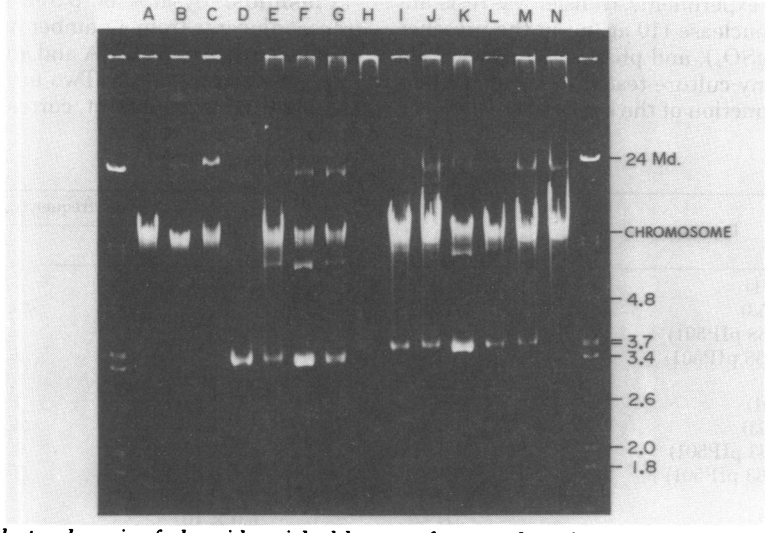

FIG. 2. *Gel electrophoresis of plasmid-enriched lysates of parental strains and some transconjugants. In the outside lanes of the gel are covalently closed marker plasmids from V517 (20) plus R6K. The other tracks contain, from left to right: (A) $MV120_B$; (B) JH2-2_D(pMV120); (C) $MV762_B$(pMV120); (D) $MV158_B$; (E) $MV158_B$(pIP501); (F) JH2-2_D(pMV158 pIP501); (G) $JH203_D$(pMV158 pIP501); (H) $JH203_D$(pMV158); (I) $MV163_B$; (J) MV163(pIP501); (K) JH2-2_D(pMV163 pIP501); (L) $MV762_B$(pMV163); (M) $MV762_B$(pMV163 pIP501); (N) $MV762_B$(pIP501). The broad band is chromosomal DNA.*

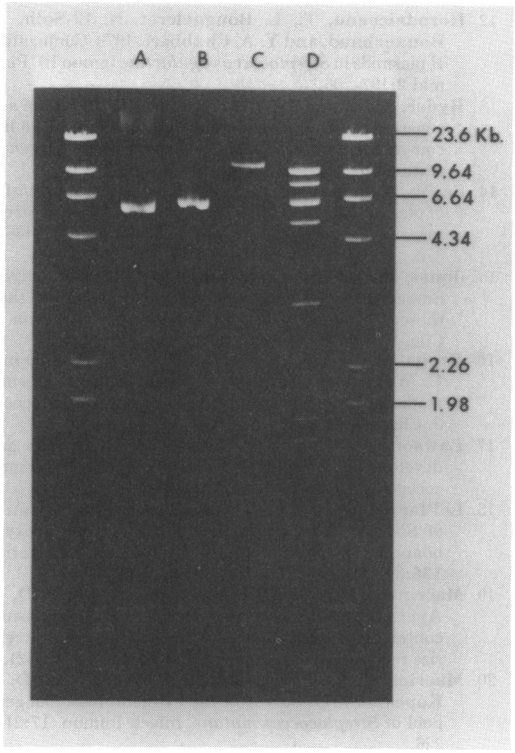

FIG. 3. *Gel electrophoresis of plasmid DNA after endonuclease treatment. The tracks on the gel contain, on the outside, a HindIII digest of λ DNA used as molecular weight marker; (A) pMV158 digested with HindIII; (B) pMV163 digested with EcoRI; (C) pAMα1 digested with BglII; and (D) pMV120 digested with HpaI.*

Streptococcus is plasmid mediated, we devised a scheme to identify both conjugative and NTP tetracycline resistance plasmids. Previous to this study, the only examples of transmissible tetracycline resistance in *Streptococcus* have been a few examples in *S. faecalis* (group D) (8, 14, 29) and transfer of chromosomal resistance in *Streptococcus pneumoniae* (24). Among 30 tetracycline-resistant clinical isolates of *S. agalactiae* examined in this study, 27 could not be shown to harbor tetracycline resistance plasmids by physical or genetic criteria. However, two distinct NTPs and one large conjugative plasmid were identified.

The sex factor, pMV120, has a molecular weight of 30×10^6 and is transferred at low frequency ($\sim 2 \times 10^{-7}$ per donor colony-forming unit) to both group D and group B recipient strains. The two NTPs, pMV158 and pMV163, were identified by taking advantage of the mobilization properties of sex factors. An observation related to these mobilization studies concerned the ability of a number of MLS-resistant sex factors to mobilize pMV158 and pMV163. Numerous attempts were made to introduce other sex factors, e.g., pAMγ1 (8) and pEBC15 (16), into group B streptococcal backgrounds without success. The basis of this difference in host range of different plasmids is not understood.

The ability of pMV158 and pMV163 to transform *S. sanguis* indicates that plasmid-mediated tetracycline resistance can be expressed in this strain. Previous attempts by others to transform this strain to tetracycline resistance have not

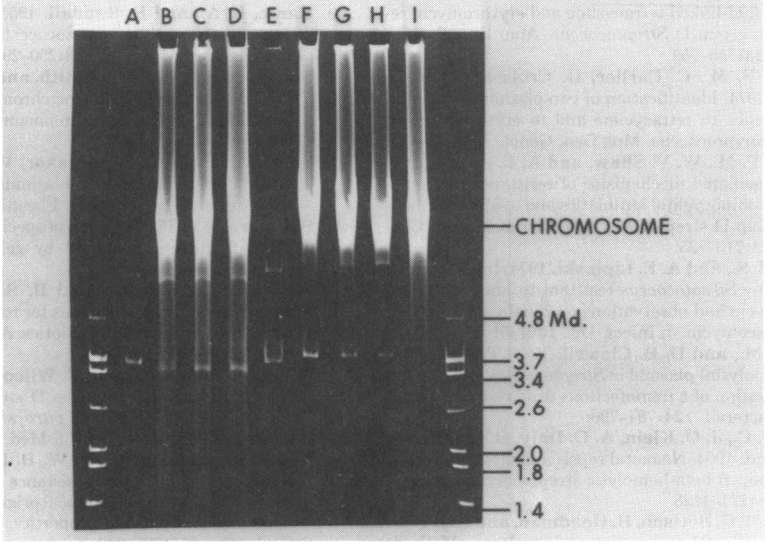

FIG. 4. *Gel electrophoresis of lysates of tetracycline-resistant transformants. The outside tracks represent V517 plasmids as markers. (A) pMV158; (B–D) pMV158 transformants; (E) pMV163; (F–H) pMV163 transformants; (I) V288. Slight discrepancies can be seen in the migration of purified plasmid DNA and plasmid DNA in lysates. These differences are not real and may reflect salt or viscosity differences.*

been successful (18; D. LeBlanc and F. Macrina, personal communications). I have confirmed this finding with one such plasmid, indicating that successful transformation to tetracycline resistance is plasmid dependent. These two plasmids have several advantages for use as cloning vehicles in *S. sanguis*. They are small (~3.5×10^6 daltons) and contain a readily selectable marker; they exist in multiple copies per chromosome; and each contains a number of readily available sites for cloning DNA fragments generated by site-specific endonucleases (V. Burdett, manuscript in preparation). Such a vector system would be exceedingly useful for study of genetics of polysaccharide biosynthetic pathways as well as fine-structure genetic analysis of drug resistance determinants in this system. For these reasons I am testing the potential of this system.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-15619 from the National Institute of Allergy and Infectious Disease.

The excellent technical help of Jennifer A. Teunissen is gratefully acknowledged.

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