Tetracycline Resistance and *tetM* in Pathogenic Urogenital Bacteria

MARILYN C. ROBERTS,^{1*} SHARON L. HILLIER,² JUDITH HALE,³ KING K. HOLMES,³ AND
GEORGE E. KENNY¹

Departments of Pathobiology,¹ Obstetrics and Gynecology,² and Medicine,³ University of Washington,
Seattle, Washington 98195

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Clinical isolates of *Gardnerella vaginalis*, *Streptococcus agalactiae*, *Bacteroides* spp., and *Mobiluncus* spp. were screened for resistance to tetracycline and for the presence of the streptococcal *tetM* determinant. The *S. agalactiae* and *G. vaginalis* strains contained DNA sequences homologous to the *tetM* determinant, while strains of the other two genera did not.

The *tetM* determinant, which codes for tetracycline resistance (Tc^r), was first described for streptococci and has been found in both oral and enteric species of streptococci (1, 3, 5, 6, 8). *tetM* in streptococci is often associated with a conjugative transposon, is usually located in the streptococcal chromosome, and can be found in multiple copies of a single organism (5, 6). A 5-kilobase (kb) *tetM* fragment cloned from a *Streptococcus agalactiae* strain has been characterized and is the prototype for *tetM* (2, 3). This determinant does not share DNA sequences with the other streptococcal Tc^r determinants *tetL* and *tetN* or a fourth unique streptococcal *tet* determinant tentatively named *tetO* (21), nor is it genetically related to the four enteric Tc^r determinants (classes A through D) previously described (3, 25). The *tetM* determinant does not code for an altered transport system but appears to affect the protein-synthesizing system owing to a ribosomally associated factor which can be removed by high salt concentrations (2). In contrast, the enteric Tc^r determinants are mediated by an energy-dependent efflux system (14).

We reported previously that high-level Tc^r strains of *Ureaplasma urealyticum* and *Mycoplasma hominis* contain DNA sequences which are homologous to the streptococcal *tetM* determinant (20, 21). More recently, we showed that *Neisseria gonorrhoeae* strains with high-level Tc^r also carry the *tetM* determinant, which is located on a 25.2-megadalton plasmid (16). Taylor (24) has data suggesting that the *tetM* determinant, isolated from *S. agalactiae*, may be distantly related to the plasmid-encoded Tc^r determinant of *Campylobacter jejuni*. However, recent data (P. Martin, P. Trieu-Cuot, and P. Courvalin, Abstr. Second ASM Conf. Streptococcal Genetics, abstr. no. 109, 1986) did not show homology between the Tc^r determinant of *C. jejuni* and a *tetM* determinant isolated from an *S. pneumoniae* strain. Others have shown that the *tetM* determinant can be transferred and stably integrated into the chromosome of *Bacillus anthracis* (B. Ivins, G. Knudson, and D. LeBlanc, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D81, p. 79).

We examined the correlation between Tc^r and the presence of *tetM* in 66 *Gardnerella vaginalis* strains (a facultative anaerobic, nonmotile, pleomorphic, gram-negative to gram-variable rod of uncertain taxonomic status) (7), 67 *Mobiluncus* strains (a gram-positive anaerobic bacterium with an unusual cell wall) (23), and 16 *Bacteroides* strains (a gram-negative anaerobe). The *G. vaginalis* and *Mobiluncus*

strains were isolated from the vaginas of women attending the Seattle Sexually Transmitted Disease Clinic, while the *Bacteroides* strains were isolated from patients at the University of Washington Hospital, Seattle. All of these bacteria have been associated with bacterial vaginosis (13, 19, 23), the most common type of vaginitis (17). We also included *S. agalactiae* strains, isolated from different anatomical sites, from a variety of services at the University of Washington Hospital. The *S. agalactiae* and *Bacteroides* isolates were screened by the Kirby-Bauer disk diffusion method (12), while MICs of tetracycline were determined by agar dilution for *Mobiluncus* strains (S. L. Hillier, S. Swanzy, and F. D. Schoenknecht, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1077, 1985) and *G. vaginalis*.

Eighty percent of the *S. agalactiae* strains, isolated from patients of the University of Washington Hospital, were Tc^r by the Kirby-Bauer criteria (12). No differences in antibiotic susceptibility were noted among strains isolated from different anatomical sites or from different types of patients. Fifty Tc^r *S. agalactiae* strains were selected, 25 from the urogenital tract and the rest from other anatomical sites. Of the 16 *Bacteroides* strains, 10 were Tc^r, of 67 *Mobiluncus* strains, 12 were Tc^r (MICs ≥ 16 μ g/ml), and of 66 strains of *G. vaginalis*, 41 were Tc^r (MICs ≥ 16 μ g/ml). McCarthy et al. (13) reported that the MICs of tetracycline for 49% of the *G. vaginalis* strains examined were ≥ 16 μ g/ml. We found that 20 of 39 (51%) of the randomly chosen *G. vaginalis* strains isolated in 1978 and 21 of 27 (78%) of the randomly chosen strains isolated in 1984 were Tc^r. The percentage of Tc^r strains isolated in 1978 is very similar to that reported by McCarthy et al., while the percentage of Tc^r strains isolated in 1984 is significantly larger. Similar increases in Tc^r frequency in the same population during this period were noted for *M. hominis* strains (11).

All *Bacteroides*, *Mobiluncus*, and *S. agalactiae* strains were grown on 5% sheep blood agar-heart infusion base plates; chocolate plates were used for *G. vaginalis* strains. The *Bacteroides* and *Mobiluncus* strains were grown under anaerobic conditions. The *S. agalactiae* and *G. vaginalis* strains were grown under 3% CO₂. Bacterial colonies were removed from the plates and suspended in 0.5 ml of saline, 25 μ l was spotted onto nitrocellulose, and the colonies were lysed as previously described (21). A radiolabeled probe prepared by nick translation was made from pJ13, a plasmid composed of the *Escherichia coli* vector pACYC177 (4) and a 5-kb *HincII* fragment from *S. agalactiae* that encodes *tetM* (3, 9). A total of 17 (34%) of the Tc^r *S. agalactiae* strains, 41

* Corresponding author.

TABLE 1. Correlation of tetracycline resistance and *tetM* in *M. hominis*, *U. urealyticum*, *G. vaginalis*, and *S. agalactiae* strains

Strain	No. of Tc ^s (MIC, <16 μg/ml) strains	No. of Tc ^r (MIC, ≥16 μg/ml) strains	No. of Tc ^r strains which hybridize with <i>tetM</i> probe
<i>M. hominis</i> , 1966–1984 (<i>n</i> = 85)	36	49	49
<i>U. urealyticum</i> , 1970–1984 (<i>n</i> = 72)	58	14	14
<i>G. vaginalis</i> , 1978 (<i>n</i> = 39)	19	20	20
1984 (<i>n</i> = 27)	6	21	21
<i>S. agalactiae</i> , 1984 (<i>n</i> = 50)	0 ^a	50	17
<i>Mobiluncus</i> spp., 1982–1985 (<i>n</i> = 67)	55	12	0
<i>Bacteroides</i> spp., 1984 (<i>n</i> = 16)	6	10	0

^a No Tc^s strains were tested.

(100%) of the Tc^r *G. vaginalis* strains, and none of the Tc^r *Bacteroides* or *Mobiluncus* strains hybridized with the *tetM* probe in the dot blot assay (Table 1). No strains hybridized with the vector pACYC177 when it was used as a probe.

Ten of the Tc^r *Mobiluncus* strains, five of the Tc^r *Bacteroides* strains, and ten Tc^r and three Tc^s *G. vaginalis* strains were chosen for further study. Each was grown in 1 liter of soy peptone-fresh yeast dialysate broth without serum (10). Whole-cell DNA was prepared as previously described (23). DNA was cleaved with *Eco*RI and electrophoresed on agarose gels, and Southern blots were prepared and hybridized with pJ13 as previously described (18, 19, 22). Again, no hybridization was evident with either anaerobe or the Tc^s *G. vaginalis* strains, but the Tc^r *G. vaginalis* strains hybridized with the probe. The apparent molecular weight of the hybridizing fragments varied with the strain, suggesting that the strains were not identical.

Cleared lysates were prepared from selected Tc^r and Tc^s *G. vaginalis* strains (18). No plasmids were evident from the agarose gels, and blots made with these Tc^r lysates showed hybridization with the chromosomal band, suggesting that *tetM* was in the chromosome, as it appears to be in streptococci, *M. hominis*, and *U. urealyticum* (5, 18, 19).

The *tetM* determinant is known to be associated with a conjugative transposon in some strains (5, 8). We examined 17 Tc^r *tetM*-containing *S. agalactiae* strains for the ability to

transfer Tc^r in cell-to-cell filter mating by using a laboratory *S. faecalis* strain chromosomally resistant to fusidic acid and rifampin. Two of the strains were able to transfer Tc^r and the *tetM* determinant, as measured by dot blot hybridization, to the laboratory *S. faecalis* strain. Similar experiments were performed with three different Tc^r *G. vaginalis* strains as the donors and an *S. faecalis* strain as the recipient. None of these matings resulted in transfer of the Tc^r determinant.

The 5-kb *Hinc*II fragment was subcloned from a 19-kb fragment, which does not include the entire resistance region and represents no more than 26% of the original cloned fragment (3, 9). This fragment contains both internal *Sst*I and *Hind*III sites (Fig. 1). All the subclones obtained which mediated Tc^r retained the *Hind*III and *Sst*I sites of the *Hinc*II fragment (J. M. Inamine, Ph.D. thesis, Duke University, Durham, N.C., 1982). The 3.3-kb *Hinc*II fragment of pJ12 (on the left side of the 5-kb fragment) is thought to contain normal streptococcal group B cellular DNA found in both Tc^r and Tc^s strains, while the resistance region extends to the right of the 5-kb fragment (Inamine, Ph.D. thesis). Therefore, it was of interest to determine whether the flanking DNA sequences were present in some of the Tc^r urogenital bacteria. For these experiments, we used pJ12.14, which does not confer Tc^r and contains all the fragments of pJ12 to the right of the 5-kb region (thought to be part of the resistant determinant) and less than 1 kb of the left end of pJ12 (known to be present in normal streptococcal group B cellular DNA [3, 9; Inamine, Ph.D. thesis]) (Fig. 1). Dot blot assays were performed with Tc^r *tetM*-containing strains of the urogenital bacteria *M. hominis*, *G. vaginalis*, *U. urealyticum*, and *N. gonorrhoeae* (Table 2) and a matched number of Tc^s strains. Two-thirds of the *M. hominis* and *N. gonorrhoeae* strains reacted with the probe, suggesting that most but not all *tetM*-containing Tc^r strains contain sequences represented on the resistance region which are not directly related to Tc^r. DNA homology with the flanking region was found for one of the two *G. vaginalis* strains and for both *U. urealyticum* strains (Table 2). None of the susceptible strains hybridized with the probe.

The data suggest that the *tetM* determinant is very widely distributed within the urogenital flora and is able to cross into cell-wall-free bacteria, bacteria of uncertain taxonomic status, and classical gram-negative (*Neisseria* spp.) and gram-positive bacteria. The mechanism of gene transfer into these various species is not clear. We have been able to transfer *tetM* in the laboratory between *N. gonorrhoeae* strains by both conjugation and transformation (16). It has also been moved from streptococci into the chromosome of *Bacillus anthracis* by mating under laboratory conditions (Ivins et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1986). How far this determinant will spread is unknown, but one could predict that the number of Tc^r species will continue to increase with time. The presence of the *tetM* determinant should be considered whenever a new Tc^r species is exam-

FIG. 1. Maps of cloned insert DNA from pJ12 and pJ13 (both Tc^r) and pJ12.14 (Tc^s), showing restriction endonuclease cleavage sites (adapted with permission from reference 8 and from Inamine, Ph.D. thesis).

TABLE 2. Presence of flanking regions in Tc^r *tetM*-containing bacteria

Strain	No. of strains tested	No. of strains hybridizing with pJ12.14 ^a
<i>M. hominis</i>	9	6
<i>G. vaginalis</i>	2	1
<i>U. urealyticum</i>	2	2
<i>N. gonorrhoeae</i>	25	16

^a Hybridization was done by dot blot assay.

ined. However, neither Tc^r *Mobiluncus* strains nor Tc^r *Bacteroides* strains carried the *tetM* determinant, although both species are frequently isolated along with genital mycoplasmas and *Gardnerella* spp. The presence of different Tc^r determinants in these two species may be due to earlier introduction of these uncharacterized determinants into the species; these other Tc^r determinants may fit *Mobiluncus* and *Bacteroides* physiology better or provide higher MICs. It is also possible that physical or biochemical factors restrict the spread and maintenance of *tetM* into *Mobiluncus* and *Bacteroides* spp. As we learn more about the Tc^r determinants in the urogenital bacteria, we hope to be able to distinguish between the various possibilities.

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