

Characterization of Unusual Tetracycline-Resistant Gram-Positive Bacteria

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Tetracycline-resistant Tet M-negative isolates of *Actinomyces viscosus*, *Eubacterium lentum*, *Mobiluncus curtisii*, and *Mobiluncus mulieris* were screened with the Tet K, Tet L, and Tet O DNA probes. Ten (71%) of the resistant *Mobiluncus* strains hybridized with the Tet O probe, two of the three *E. lentum* strains hybridized with the Tet K probe, and the *A. viscosus* isolate hybridized with the Tet L probe.

Tetracycline is a broad-spectrum antibiotic which has been used for treatment of urogenital tract infections and periodontal disease (6, 9). In a recent study we identified tetracycline resistant (Tc^r) oral species of bacteria (20), and in a second study we identified Tc^r urogenital species (15). In both studies, Tet M was the only probe used and with the exception of the *Bacteroides* spp., *Eubacteria* spp., *Mobiluncus* spp., and some isolates of *Streptococcus agalactiae*, all other strains carried the Tet M determinant (15, 20). More recently, we extended our study on Tc^r urogenital flora and found that 45% of the Tc^r *Lactobacillus* spp. isolated hybridized with the Tet O probe, while the Tet K, Tet L, and Tet O probes hybridized with *Peptostreptococcus* spp. as well as other gram-positive cocci (14). Therefore, the present study was undertaken to determine whether the *Mobiluncus* and *E. lentum* isolates which were negative for the Tet M probe would hybridize with the Tet K, Tet L, or Tet O probe.

Dot blots were prepared and hybridized under stringent conditions at 42°C with 50% (vol/vol) formamide–0.1% (wt/vol) polyvinylpyrrolidone–0.1% (wt/vol) albumin–0.1% (wt/vol) Ficoll–0.1% (wt/vol) sodium dodecyl sulfate (SDS)–0.05 M monobasic sodium phosphate (pH 7.4)–0.005 M EDTA–0.76 M NaCl–boiled calf thymus DNA (100 µg/ml) overnight. The filters were then washed three times for 10 min at 52°C in 0.1% SDS–0.015 M NaCl–0.0015 M sodium citrate; this was followed by three 10-min washes at 52°C in 0.015 M NaCl–0.0015 M sodium citrate (14). Positive and negative controls were included in each set. Plasmids pAT102 (Tet K) (25), pVB11.15 (Tet L) (2), and pUOA4 (Tet O) (23) were used as probes. With this procedure, none of the probes cross-hybridized even though Tet K and Tet L share 69% homology (25). A total of 20 *Mobiluncus* strains were tested. This included six strains for which the MIC was ≤2 µg/ml and which were considered susceptible to tetracycline (Table 1). None of the susceptible strains hybridized with any of the three probes tested. Of the 14 strains for which the MIC was ≥4 µg/ml, 10 (71%) hybridized with the Tet O probe. The other four isolates (Table 1) were negative for the Tet O determinant. The MIC for three of these isolates (all *Mobiluncus curtisii*) was 32 µg/ml; the fourth isolate was *Mobiluncus mulieris* with an MIC of 8 µg/ml (Table 1). No

other probes hybridized with this group (Table 2). These results are similar to those observed with *Lactobacillus* spp., in which only a portion of the Tc^r strains hybridized with the Tet O probe (14).

Of the three Tc^r *Eubacterium lentum* strains, two hybridized with the Tet K probe, while the third did not hybridize with any of the probes (Table 2). A Tc^r isolate of *Actinomyces viscosus* which had been isolated earlier from a periodontal patient hybridized with the Tet L probe (Table 2).

Eight Tc^r *Mobiluncus* spp., the *A. viscosus* isolate, and the three isolates of *E. lentum* were grown, and their DNA was prepared and screened for plasmids by agarose gel electrophoresis as previously described (21). No plasmids were detected in any of the strains (data not shown). However, when Southern blots of *A. viscosus* were probed with the Tet L probe, the hybridizing band did not correspond to the chromosomal band but instead ran at a position below, suggesting the presence of a plasmid. The original Tet L determinant was located on a 5.4-kb non-self-transmissible plasmid, pMV158, which has a broad host range (2, 24). The *Mobiluncus* and *Eubacterium* species had hybridizing bands which ran with the chromosome, suggesting a chromosomal location for these determinants (data not shown). This has been the most common finding in our studies with the Tet M determinant (7, 15, 16, 18–20).

S. agalactiae and *S. dysgalactiae* isolated from cattle carry the Tet O determinant on a large conjugative plasmid of a size similar to that previously described for streptococci of human origin (1). Initially, the plasmid was difficult to visualize by ethidium bromide staining of agarose gels. However, the strains were able to transfer the Tet O plasmid to *Enterococcus faecalis* in mating experiments, where the plasmid was easier to visualize. The Tet O determinant is also associated with conjugative plasmids in *Campylobacter* species (22, 25). However, in *Streptococcus mutans*, Tet O appears to be associated with the chromosome (10). The Tet O determinant shares 76% of its DNA sequence with the Tet M determinant (22). However, to date, it has not been shown to be associated with a conjugative transposon, in contrast to the Tet M determinant (4, 17, 19).

Mobiluncus strains carrying the Tet O determinant were used as donors in mating experiments, by using the protocol developed for *Fusobacterium nucleatum* and *Peptostreptococcus* spp. (13, 19). We used an *E. faecalis* recipient that was chromosomally resistant to fusidic acid and rifampin, since it was successfully used in *F. nucleatum* matings (19). In addition, a tetracycline-susceptible (Tc^s), probe-negative

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TABLE 1. MICs for *Mobiluncus* species and hybridization with the Tet O probe

Tc MIC ($\mu\text{g/ml}$)	No. of strains			
	Probe positive		Probe negative	
	<i>M. curtisii</i>	<i>M. mulieris</i>	<i>M. curtisii</i>	<i>M. mulieris</i>
0.5	0	0	1	4
1	0	0	0	1
4	0	2	0	0
8	3	1	0	1
16	1	1	0	0
32	1	1	3	0

Mobiluncus isolate was made chromosomally resistant to streptomycin and used as a recipient. Mating mixtures were incubated for 18 and 60 h (19), but we were unable to detect transfer of the Tet O determinant in any experiments with either recipient. Because *Mobiluncus* strains are slow-growing and generally fastidious, we also tried transferring the Tet O determinant from an apparently plasmid-free *Lactobacillus* donor into *E. faecalis* and a Tc^s probe-negative *Lactobacillus* recipient. Again we were unable to detect transfer of the Tet O determinant. This is similar to our experience with a *Peptostreptococcus* donor carrying the Tet L, Tet M, and Tet O determinants (13). When this strain was used as a donor in matings with either *Peptostreptococcus* or *F. nucleatum* recipients, only Tet M was transferred. We do not know whether the inability to demonstrate transfer actually reflects the nonmobility of the Tet O determinant in these strains. Other explanations, such as incompatibility of the DNA, the frequency of transfer being below our detection limit, or conditions not being optimal for transfer, are also possible. However, it is clear that the Tet O determinants in *Mobiluncus*, *Lactobacillus*, and *Peptostreptococcus* isolates are not easily transferred in the laboratory. In contrast, we have demonstrated transfer of Tet O from *S. agalactiae* and *S. dysgalactiae* donors to *E. faecalis* recipients (1). The apparent difference in location (plasmid versus chromosome) of the Tet O determinant could be the reason for the difference in these two groups.

In this study, four unusual gram-positive bacterial species, *A. viscosus*, *E. lentum*, and *M. curtisii* and *M. mulieris*, have been shown to carry Tet L, Tet K, or Tet O determinants, respectively. In addition, four (29%) of the Tc^r *Mobiluncus* isolates and one Tc^r *Eubacterium* isolate did not hybridize with any of the probes, suggesting that they carry other uncharacterized Tet determinants. These results indicate that the Tet L, Tet K, and Tet O determinants have disseminated across a broader spectrum of bacterial species

TABLE 2. Distribution of Tet determinants

Organism	No. of strains hybridizing with probes for Tet determinant:				No. not hybridizing
	K	L	M	O	
<i>A. viscosus</i>	0	1	0	0	0
<i>E. lentum</i>	2	0	0	0	1
<i>M. curtisii</i>	0	0	0	5	4 ^a
<i>M. mulieris</i>	0	0	0	5	6 ^b

^a The MIC for one strain was 0.5 $\mu\text{g/ml}$; the MIC for the other three was 32 $\mu\text{g/ml}$.

^b The MICs were 0.5 $\mu\text{g/ml}$ for four strains, 1.0 $\mu\text{g/ml}$ for one strain, and 8 $\mu\text{g/ml}$ for one strain.

than has previously been supposed. However, Tet K and Tet L have only been found in gram-positive species to date (3, 4, 7, 14, 25), while both Tet M and Tet O have been found in gram-positive (1, 3, 4, 10, 14–16, 18, 25) and gram-negative (8, 9, 12, 14, 19, 20, 22, 23) bacteria.

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