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Characterization of the Tet M Determinants in Urogenital and Respiratory Bacteria

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Tetracycline-resistant *Fusobacterium nucleatum*, *Haemophilus ducreyi*, *Mycoplasma hominis*, *Peptostreptococcus* spp., *Ureaplasma urealyticum*, and *Veillonella parvula* had DNA sequences which showed homology throughout the length of the Tet M transposon, Tn*916*. In contrast, *Gardnerella vaginalis*, commensal *Neisseria* spp., and the 25.2-megadalton plasmid family lacked the complete transposon.

Tn*916* is a conjugative transposon originally identified on the chromosome of *Enterococcus faecalis* (6). It is the prototype of a closely related family of transposons that carry the Tet M determinant either alone or as part of a multiple drug resistance determinant (3, 5, 6, 26). Over the last few years, the Tet M determinant has been identified in the chromosome of a variety of clinical strains including *Clostridium difficile* (8), *Fusobacterium nucleatum* (23), commensal *Neisseria* spp. (11, 23), *Mycoplasma hominis* (22), *Streptococcus* spp. (3, 6), *Peptostreptococcus* spp. (23), *Ureaplasma urealyticum* (18), and *Veillonella parvula* (23). It has also been found on conjugative plasmids in *Eikenella corrodens* (11), *Haemophilus ducreyi* (15), *Kingella denitrificans* (11), *Neisseria gonorrhoeae* (13), and *Neisseria meningitidis* (11). Hachler et al. (8) have shown that tetracycline-resistant *C. difficile* has homology along the entire length of the transposon Tn*916* and that these strains are able to transfer tetracycline resistance to other strains of *C. difficile*.

A number of different strains within each species carry the Tet M determinant (11, 13, 18, 22, 23). Based on this, the assumption has been that the Tet M determinant was introduced into each species from an outside source and then spread among the different strains by conjugation, rather than being introduced from an outside source multiple times (22).

To examine whether the various species carry the complete Tet M transposon and thus could possibly act as donors, I chose for study three *F. nucleatum*, three *Gardnerella vaginalis*, two *M. hominis*, four *Neisseria perflava/sicca*, three *Neisseria mucosa*, four *Peptostreptococcus* spp., three *U. urealyticum*, and one *V. parvula* strains that have previously been shown to carry the Tet M determinant in the chromosome (11, 17, 22, 23) and one each of *H. ducreyi*, *N. gonorrhoeae*, *K. denitrificans*, and *E. corrodens*, all carrying the Tet M determinant on conjugative plasmids (11, 13, 15). Plasmid pAM120 carrying the complete Tn*916* transposon and *E. faecalis* DS160 were used as controls (5, 6).

Plasmid pAM120 and *E. faecalis* DNA were treated with restriction enzyme *Hin*cII, while the other DNAs were treated with the restriction enzymes *Hin*cII and *Pst*I. Southern blots were prepared and hybridized at 42°C under stringent conditions of 50% (vol/vol) formamide with the complete Tn*916* transposon. Under these conditions, sequences which share 76% homology do not hybridize (27). A variety of hybridization patterns were observed (Fig. 1).

G. vaginalis, commensal *Neisseria* spp., and those strains carrying the 25.2-megadalton (MDa) plasmid showed a single hybridizing band with Tn*916* (Fig. 1). When these blots were compared with blots probed with the 1.8-kilobase (kb) *Kpn*I-*Hin*dIII fragment, which carries 95% of the structural Tet M gene (1), they were identical (data not shown), suggesting that the single band in these strains represents the structural Tet M gene. This hypothesis is supported by my recent work with a cloned 4.9-kb fragment isolated from the 25.2-MDa plasmid. This fragment encodes for the Tet M gene. It has a restriction pattern which is identical to that previously described for the 4.9-kb Tet M fragment cloned from *U. urealyticum* (1; unpublished data) and gives the same hybridization pattern as the natural plasmid when either Tn*916* or the 1.8-kb *Kpn*I-*Hin*dIII probe is used. Therefore, I assume that the single hybridizing bands in the commensal *Neisseria* and *G. vaginalis* species are like that in *N. gonorrhoeae* and represent the structural Tet M gene.

To verify the results of these experiments, I examined other Southern blots with plasmid pJI2.14 (3). This plasmid carries the right side of a Tet M transposon from *Streptococcus agalactiae* B109 but does not carry the structural Tet M gene and does not confer tetracycline resistance (3). The strains which showed multiple hybridizing bands with Tn*916* (*E. faecalis*, *H. ducreyi*, *F. nucleatum*, *M. hominis*, *Peptostreptococcus* spp., *U. urealyticum*, and *V. parvula*) hybridized with the pJI2.14 probe (Fig. 2). This suggests that regions outside and distant from the structural Tet M gene are present in these strains. I examined three *F. nucleatum* and one *Peptostreptococcus* sp. which hybridized with pJI2.14 as donors in transfer experiments and found that all four are able to transfer Tet M to other members of their species by conjugation. This indicates that they carry functional conjugative transposons (M. C. Roberts and J. Lansciardi, manuscript in preparation). In contrast, when *M. hominis* strains were used as donors, no transfer of the Tet M was observed, suggesting that they do not carry functional conjugative transposons (19). Those strains which had a single hybridizing band with the Tn*916* probe did not hybridize with pJI2.14. This suggests that they do not have the complete transposon, since these distal sequences are not present.

DNA-DNA hybridization studies with the complete Tn*916* as the radiolabeled probe were done against some of these strains. I found that the strains carrying the 25.2-MDa Tet M plasmid and the commensal *Neisseria* spp. had only between 35 and 40% homology with the Tn*916* sequences (data not

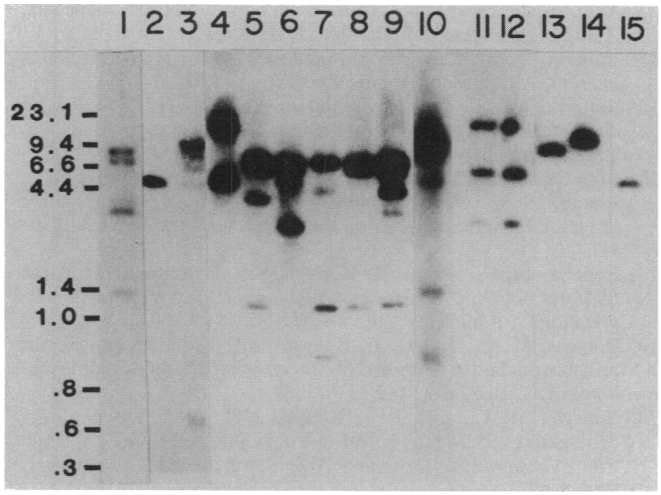

FIG. 1. Southern blot of a 1.0% agarose gel probed with ^{32}P-radiolabeled 17.7-kb fragment carrying the complete Tn*916* transposon. Lane 1, *Hin*cII-restricted DNA from *E. faecalis* DS160. All other DNAs were restricted with *Hin*cII and *Pst*I. Lanes: 2, *N. gonorrhoeae*; 3, *H. ducreyi*; 4, *M. hominis*; 5, *Peptostreptococcus* spp.; 6, *Peptostreptococcus anaerobius*; 7 and 8, *Peptococcus asaccharolyticus*; 9, *V. parvula*; 10, *U. urealyticum*; 11 and 12, *F. nucleatum*; 13 and 14, *N. perflava/sicca*; 15, *G. vaginalis*. Numbers on the side represent sizes of the lambda *Hin*dIII and φX174 *Hae*III fragments in kilobases.

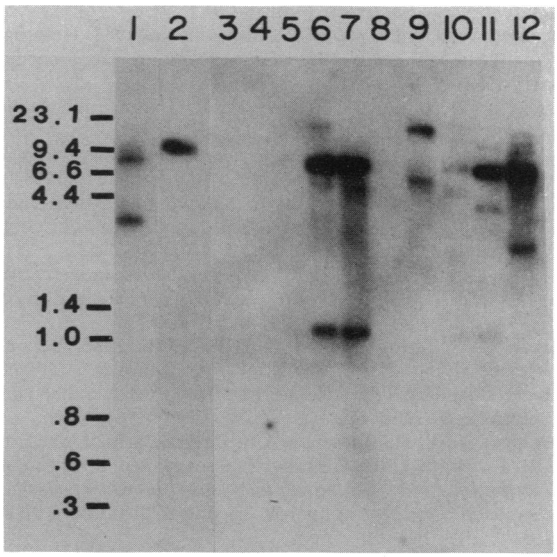

FIG. 2. Southern blot of a 1.0% agarose gel probed with ^{32}P-radiolabeled pJI2.14 carrying the transposon-specific sequences to the right of the structural TetM determinant. Lane 1, *Hin*cII-restricted DNA from *E. faecalis* DS160. All other DNAs were restricted with *Hin*cII and *Pst*I. Lanes: 2, *H. ducreyi*; 3, *N. gonorrhoeae*; 4, *N. perflava/sicca*; 5, *N. mucosa*; 6, *U. urealyticum*; 7, *Peptostreptococcus* spp.; 8, *G. vaginalis*; 9, *M. hominis*; 10, *V. parvula*; 11, *Peptostreptococcus asaccharolyticus*; and 12, *Peptostreptococcus anaerobius*. Numbers on the side represent sizes of the lambda *Hin*dIII and φX174 *Hae*III fragments in kilobases.

shown). This strongly suggests that these strains carry only part of the transposon. The *G. vaginalis* strains are currently being tested.

On the basis of this information, I hypothesized that these strains would not have a functional conjugative transposon and should not be able to transfer the Tet M determinant. To test this hypothesis, I used eight different strains of commensal *Neisseria* species and six different strains of *G. vaginalis*, all showing no homology with the pJI2.14 probe, as donors in matings. None were able to transfer the Tet M determinant.

Why only part of the Tet M transposon is present in some of the species is unclear, especially since under laboratory conditions the entire Tn*916* can be moved to a variety of species (10, 19). However, similar situations have previously been described in *N. gonorrhoeae* with the TEM β-lactamase plasmids (4, 16). In these plasmids, only 40% of the Tn*A* transposon sequences are found, but the incomplete transposon can give rise to a functional transposon when linked to the left part of Tn*A*, suggesting that the ancestral gene was a complete transposon (7). Related β-lactamase plasmids which carry the entire Tn*A* transposon can be found in *H. ducreyi* strains (2, 4, 12). This correlates with the data presented here on the Tet M determinant, which suggests that the *H. ducreyi* plasmid carries most of if not the entire Tet M transposon. A second plasmid family found in both commensal *Neisseria* species and *E. corrodens* has recently been described which is genetically related to the RSF1010 plasmid (14, 24–26). These plasmids code for TEM β-lactamase and streptomycin and sulfonamide resistance. This plasmid family is thought to have been created by the transposition of Tn*A* onto RSF1010 (25). However, the natural plasmids do not carry the entire Tn*A*, while those created in the laboratory do have the complete transposon (9). Thus, the presence of an incomplete Tet M transposon in commensal *Neisseria* spp. and the 25.2-MDa plasmid family follows a pattern previously seen with the transposon Tn*A*.

Those species which carry only part of the Tet M transposon in their chromosome are not likely to be able to transfer the Tet M determinant. Thus, the hypothesis that once the Tet M determinant was introduced into a species, transfer among the strains was the most likely explanation for the dissemination within the species is not reasonable for these organisms. The alternative of multiple introduction of the Tet M determinant into the commensal *Neisseria* species and *G. vaginalis* now seems more plausible. In contrast, even though *N. gonorrhoeae* carries an incomplete Tet M transposon, this transposon can be transferred between strains and species by conjugation of the plasmid it resides on (20, 21). Therefore, the hypothesis that the 25.2-MDa plasmid was created once and then transferred to other strains and species is still reasonable. That hypothesis also works for both *F. nucleatum* and *Peptostreptococcus* spp. because I have shown that they carry functional conjugative transposons which can be transferred to different strains. Thus, transfer among different strains may still account for the dissemination of the Tet M determinant within some, but not all, of the species examined in this study.

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