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High-Level Tetracycline Resistance Resulting from TetM in Strains of *Neisseria* spp., *Kingella denitrificans*, and *Eikenella corrodens*

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Similar to *Neisseria gonorrhoeae*, tetracycline-resistant isolates of *N. meningitidis*, *Kingella denitrificans*, and *Eikenella corrodens* contained 25.2-megadalton plasmids carrying the TetM determinant. In contrast, tetracycline-resistant *N. subflava* biovar *perflava-N. sicca* and *N. mucosa* isolates carried the TetM determinant in the chromosome.

High-level tetracycline-resistant (Tc^r; MIC, ≥16 μg/ml) isolates of *Neisseria gonorrhoeae* have recently been described (9). Tc^r in these isolates was due to the presence of a 25.2-megadalton (MDa) plasmid which appears to have been created by insertion of the streptococcal TetM determinant into the 24.5-MDa conjugative plasmid indigenous to *N. gonorrhoeae* (9). Between August and December 1985, Tc^r *N. gonorrhoeae* isolates accounted for approximately 5% of all gonococcal isolates in DeKalb County, Georgia (8). During this period, high-level Tc^r (MIC, ≥16 μg/ml) oropharyngeal and urethral isolates of *N. meningitidis* and *Kingella denitrificans* were identified during routine screening of gram-negative, oxidase-positive organisms isolated on Thayer-Martin medium. As a result, we undertook two studies to determine the prevalence of high-level Tc^r in oropharyngeal isolates of *Neisseria* and related species.

To determine the frequency of high-level Tc^r in *N. meningitidis* and *K. denitrificans*, gram-negative, oxidase-positive organisms were isolated from oropharyngeal specimens inoculated on Thayer-Martin medium. Tc^r isolates were selected by their ability to grow on chocolate agar containing 10 μg of tetracycline per ml (8) and by measurement of the MIC by agar dilution susceptibility testing and confirmed by detection of the TetM determinant as described previously (9). Oropharyngeal specimens were obtained from 146 consecutive patients attending the DeKalb County Sexually Transmitted Diseases Clinic between February and April 1986. Gram-negative, oxidase-positive organisms were identified by acid production from glucose, maltose, fructose, sucrose, and lactose; production of polysaccharide; and nitrate reduction as described previously (6).

Of 146 patients, 23 (16%) were colonized with *N. meningitidis*, and another 23 (16%) were colonized with *K. denitrificans*. Of these 46 patients, 8 were colonized by Tc^r, gram-negative, oxidase-positive organisms, including 1 (4%) of the 23 *N. meningitidis* isolates and 7 (30%) of the 23 *K. denitrificans* isolates.

Because Tc^r isolates accounted for 16% of the *N. meningitidis* and *K. denitrificans* isolates, we undertook a second study to determine whether commensal *Neisseria* spp., not usually isolated on Thayer-Martin medium, were also Tc^r. In this study, oropharyngeal specimens were obtained from 22 patients attending the same clinic from July to August 1986. Specimens were inoculated onto LBV.SNR medium, which is selective for commensal *Neisseria* spp. (7), and onto Thayer-Martin medium to detect concurrent colonization with meningococci or *K. denitrificans*. A colony representative of each morphologic type from each specimen was inoculated onto chocolate medium containing 10 μg of tetracycline per ml to detect Tc^r isolates; Tc^r was confirmed by measurement of the tetracycline MIC. For those isolates which grew on tetracycline-containing medium, MICs were ≥16 μg of tetracycline per ml compared with MICs of ≤2.0 μg of tetracycline per ml for isolates which did not grow on tetracycline-containing medium. Isolates were identified as described above. Because biochemical tests could not differentiate between *N. subflava* biovar *perflava* and *N. sicca*, these isolates were identified as *N. perflava-N. sicca* in this study. Of the 22 patients, 9 (41%) were colonized by at least one Tc^r isolate of *N. perflava-N. sicca* or *N. mucosa* (Table 1). None of these patients were concurrently colonized by *N. meningitidis* or *K. denitrificans*. However, a single Tc^r strain of *Eikenella corrodens* was isolated; the identity of this isolate was confirmed by biochemical tests and by DNA hybridization with a reference strain (unpublished observations).

Cleared lysates were prepared from all Tc^r isolates and control strains (14). Isolates of *N. meningitidis*, *K. denitrificans*, and *E. corrodens* contained 25.2-MDa plasmids similar in size to those described in Tc^r *N. gonorrhoeae* isolates, and restriction analyses showed that these plasmids had restriction maps similar to those of previously characterized 25.2-MDa plasmids isolated from *N. gonorrhoeae* (data not shown) (9). In contrast, no plasmids were observed in the agarose gels of Tc^r isolates of *N. perflava-N. sicca* or *N. mucosa*. Southern blots were prepared and hybridized with each of two radiolabeled probes prepared by nick translation; these probes were the 5-kilobase *Hin*cII fragment from pJI3 which encodes the entire TetM determinant isolated from *Streptococcus agalactiae* (2, 9–13) and the 1.8-kilobase *Kpn*I-*Hin*dIII fragment containing 93% of the TetM structural gene (1). Both probes hybridized with the 25.2-MDa plasmids from the Tc^r isolates of *N. meningitidis*, *K. denitrificans*, and *E. corrodens*. However, the probes hybridized with the chromosomal DNA of the Tc^r *N. perflava-N. sicca* and *N. mucosa* isolates.

To confirm the high degree of plasmid relatedness, we hybridized (14) radiolabeled 25.2-MDa plasmid DNA iso-

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TABLE 1. Frequency of isolation of commensal *Neisseria* spp. harboring the TetM determinant in oropharyngeal specimens of 22 patients attending the DeKalb County Health Department Sexually Transmitted Diseases Clinic from July to August 1986

Species isolated	Total no. of patients colonized	No. of patients colonized with the following Tc^r *Neisseria* spp.:		*N. perflava-N. sicca* and *N. mucosa*[a]
		N. perflava-N. sicca	*N. mucosa*	
N. perflava-N. sicca only	7	3	0	0
N. perflava-N. sicca, *N. mucosa*	4	0	0	2
N. perflava-N. sicca, *N. mucosa*, *N. flava*	2	0	1	0
N. perflava-N. sicca, *N. mucosa*, *N. cinerea*	1	0	1	0
N. perflava-N. sicca, *N. cinerea*	5	1	0	0
N. perflava-N. sicca, *N. flava*	3	1	0	0

[a] At least one isolate of both *N. perflava-N. sicca* and *N. mucosa* was Tc^r.

lated from *N. gonorrhoeae* with unlabeled DNA from *N. meningitidis*, *K. denitrificans*, and *E. corrodens*. The plasmids from these strains had a substantial number of DNA sequences in common (60 to 100%) with the reference 25.2-MDa plasmid, suggesting that all of the 25.2-MDa plasmids descended from a common ancestral plasmid.

Previous studies suggested that the 25.2-MDa plasmids from *N. gonorrhoeae* were also highly related to the indigenous 24.5-MDa conjugative plasmids (9). To confirm that this was also the case with the 25.2-MDa plasmids from *N. meningitidis*, *K. denitrificans*, and *E. corrodens*, we hybridized radiolabeled 24.5-MDa plasmid DNA from *N. gonorrhoeae* with unlabeled DNA from *N. meningitidis*, *K. denitrificans*, and *E. corrodens*. Between 60 and 80% of DNA sequences were common to both the 24.5-MDa plasmid and the three 25.2-MDa plasmids. This supports the hypothesis that the 25.2-MDa plasmid was formed by insertion of the TetM determinant into the indigenous 24.5-MDa plasmid. Thus, as expected from the results described above and those presented previously (9), when the 24.5-MDa plasmid was used as a radiolabeled probe against Southern blots of the restricted 25.2-MDa plasmids, most of the restriction fragments hybridized with this probe. However, depending on the restriction enzymes used, the unique sequences which were specific to the TetM determinant could be identified and these fragments did not hybridize with the 24.5-MDa probe. In contrast, no DNA sequence homology was seen when the Tc^r isolates of *N. perflava-N. sicca* and *N. mucosa* were hybridized with the 24.5-MDa plasmid either by Southern blot or liquid DNA-DNA hybridization, suggesting that these strains do not carry DNA sequences related to the conjugative plasmid.

Because the data indicate that the 25.2-MDa plasmids from the four different bacterial species are highly related to each other and to the indigenous 24.5-MDa conjugative plasmid, the most logical sequence of events in the distribution of the 25.2-MDa plasmid is that the ancestral plasmid was formed in *N. gonorrhoeae* by insertion of the TetM determinant into the 24.5-MDa plasmid and subsequently spread to *N. meningitidis*, *K. denitrificans*, and *E. corrodens*. The 24.5-MDa conjugative plasmid has been detected only in *N. gonorrhoeae*. In previous studies, it could not be transferred or maintained in *N. meningitidis* and was generally not transferred into other *Neisseria* spp. (4). In contrast, the 25.2-MDa plasmids from the various species are conjugative and can be transferred into a variety of *Neisseria* spp. (12). This suggests that the host range of the 25.2-MDa plasmid differs from that of the parental 24.5-MDa plasmid. Whether these changes are due directly to the TetM determinant or indirectly to insertion of the TetM determinant into the indigenous 24.5-MDa gonococcal plasmid is not clear.

The Tc^r isolates of *N. perflava-N. sicca* and *N. mucosa* possessed a chromosomal TetM determinant, suggesting that either the 25.2-MDa plasmid is not stable in these species or they have acquired the TetM determinant from some other bacterial species. The host range of the 25.2-MDa plasmids has recently been examined (12), and the data suggest that the 25.2-MDa plasmids can be introduced and maintained in *N. subflava-N. sicca* and *N. mucosa* strains under laboratory conditions. This observation supports the hypothesis that the commensal *Neisseria* isolates may have acquired the TetM determinant from other bacterial species and not from *N. gonorrhoeae*. Southern hybridization studies of some of the *N. perflava-N. sicca* strains showed that the TetM determinant was located on different-size restriction fragments, indicating that the strains possessing this determinant are not identical (data not shown).

Although many patients were concurrently colonized by other *Neisseria* spp., only isolates of *N. perflava-N. sicca* and *N. mucosa* were Tc^r and possessed the TetM determinant. We do not know whether chromosomal TetM carriage is specific to these species or results from differences in the colonization of the oropharynx by commensal *Neisseria* spp. Isolates of *N. perflava-N. sicca* and *N. mucosa* may colonize the oropharynx in high numbers compared with other *Neisseria* spp. (7). Thus, the failure to detect the TetM determinant in other *Neisseria* spp. may have resulted from the relative paucity of cells of these other species in the oropharynx rather than their inability to harbor the TetM determinant.

The patients who participated in this study stated that they had not had receptive oral sex, further supporting the theory that the Tc^r commensal *Neisseria* spp. may have acquired the TetM determinant from other bacterial species. Thus, the TetM-containing commensal *Neisseria* spp. may not be limited to the population of sexually active patients but may be spread nonsexually between persons. The widespread therapeutic use of tetracycline may select for a variety of TetM-containing bacterial species which may be a silent reservoir for the TetM determinant. A review of the interviews with seven of the nine patients colonized by strains possessing the TetM determinant revealed that three patients had been treated with tetracycline within 1 month of the study; four patients had received no tetracycline therapy in this period. Although tetracycline therapy undoubtedly selected for Tc^r strains in some patients, the spread of these strains has occurred in the absence of direct tetracycline selective pressure in others. We predict that the number of Tc^r species possessing the TetM determinant will continue to increase. It is impossible to estimate the indirect impact that the 25.2-MDa conjugative plasmids will have on the microbiologic ecology of mucosal surfaces in patients treated with tetracycline. It may be possible for strains possessing the 25.2-MDa plasmid to acquire additional antibiotic resistance genes. Dillon et al. (3) reported the presence of the gono-

coccal 4.4-MDa β-lactamase and conjugative plasmids in the meningococcus in 1983. Recently, Ikeda et al. (5) successfully transferred the 4.4-MDa β-lactamase plasmid from *N. gonorrhoeae* to strains of *N. meningitidis*; the transconjugants did not inherit the conjugative plasmid. With the introduction of the 25.2-MDa plasmid into strains of *N. meningitidis*, the possibility exists that meningococci will be able to acquire gonococcal β-lactamase plasmids, thus increasing the potential for transfer of both plasmids both within and between species.

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