Genetic Basis of Tetracycline Resistance in *Moraxella* (*Branhamella*) *catarrhalis*

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Two high-level-tetracycline-resistant *Moraxella* (*Branhamella*) *catarrhalis* strains were shown to carry DNA sequences which hybridized with the Tet B probe. The determinant appeared to be located in the chromosome and was nontransferable.

Recently the first tetracycline-resistant (Tc^r) *Moraxella* (*Branhamella*) *catarrhalis* strains (MIC, 16 µg/ml) isolated in the United States were reported (3). However, the mechanism of tetracycline resistance was not studied. In the past, *M. catarrhalis* isolates have been shown to carry BRO-type β-lactamases, which appear to be unique to *Moraxella* species, rather than the more widespread TEM-type β-lactamases (5, 24). Therefore, it was of interest to examine whether the Tc^r *M. catarrhalis* isolates carried one of the 14 previously described Tet determinants (7). Since *M. catarrhalis* is a gram-negative species, we chose to screen the strains with the four exclusively gram-negative Tet A-Tet D determinants (12, 13) and the Tet M determinant, which has been found in both gram-positive and gram-negative species (8, 9, 14, 15, 17, 19, 21, 22). Both Tc^r strains, Bc-300 and Bc-301, and a tetracycline-susceptible (Tc^s) strain, NRL 30018 (20), were grown in supplemented GC broth (Difco Laboratories, Detroit, Mich.), and cleared lysates were prepared (15). Dot blots were made with the cleared lysates and hybridized under previously described stringent conditions (18) with one of the following whole-plasmid probes: pSL18 (Tet A), pRT11 (Tet B), pBR322 (Tet C), pSL106 (Tet D) (7, 11-13), or pUW-JKB1 (Tet M) (4). Positive controls were included with each set. The Tc^r *M. catarrhalis* strains hybridized only with the Tet B probe, while susceptible NRL 30018 was negative with all probes tested.

These Tc^r *M. catarrhalis* isolates have been shown to carry conjugative BRO-1 β-lactamases, which are associated with the chromosome (24). Plasmid DNA from *M. catarrhalis* is difficult to prepare (1); therefore, we examined two different methods of lysis. Using the standard 30-ml lysing protocol (15), we observed that both strains had plasmid bands that ran above the chromosome with an approximate size of 23 megadaltons, while with the Birnboim and Doly procedure (2) additional plasmid bands running below the chromosomal band were seen. However, not all preparations showed the plasmids, and they were lost under storage conditions. Southern blots were prepared and probed with the 1.27-kilobase *Hinc*II Tet B fragment from plasmid pRT11 (10). This probe includes the structural gene but not the gene encoding the repressor protein. The Tet B probe hybridized with the chromosome of both strains rather than with either plasmid band (data not shown). However, since the plasmids were so fragile, it was impossible to rule out the possibility

that Tet B was located on a fragile plasmid on the basis of Southern blot data alone.

To compare the two strains, DNA from each was digested with *Bam*HI, *Eco*RI, or *Pst*I, and Southern blots were prepared and hybridized with the 1.27-kilobase probe. A single hybridizing band was observed with each enzyme, and the hybridizing bands were identical for the two strains (data not shown).

The Tet B determinant is the most common Tet determinant found among enteric species (12) and was until recently the sole basis for transferable tetracycline resistance among various *Haemophilus* spp. (10, 11, 17). Most *Haemophilus* spp. carry the Tet B determinant on large conjugative R plasmids which are found only within the genus (7, 11). A few nontransferable Tc^r determinants in *Haemophilus* spp. have also been described (11). Two of these strains, one *Haemophilus influenzae* and one *Haemophilus parainfluenzae*, were shown to carry the Tet B determinant in their chromosome (11).

It was of interest to determine whether tetracycline resistance was transferable in these *M. catarrhalis* isolates. We chose the previously characterized *M. catarrhalis* NRL 30018, which had been selected for chromosomal resistance to streptomycin, as a recipient (20), while the donors were susceptible to streptomycin. Both donors have previously been shown to transfer ampicillin resistance (Amp^r) by conjugation (3), and transfer of Amp^r was used as a positive control. We used standard filter matings to look for transfer of tetracycline or ampicillin resistance (16, 20). Transconjugants were selected on supplemented GC agar plates (Difco) which contained either 250 µg of streptomycin per ml and 10 µg of tetracycline per ml or 250 µg of streptomycin per ml and 10 µg of ampicillin per ml. We found that the frequency of Amp^r transfer to NRL 30018 was similar (10⁻⁶ to 10⁻⁷) to the frequency previously reported with other recipient strains (3). In contrast, we were unable to detect transfer of the Tet B determinant for either donor. Attempts to transfer the Tet B determinant to two additional *M. catarrhalis* recipients, strains L-65 and L-58 (24), by both conjugation and transformation with total DNA were also unsuccessful, although both methods readily transferred the Amp^r determinant. These results are similar to the results with the two *Haemophilus* spp. strains (11), which carried the Tet B determinant in the chromosome, and support but do not prove a chromosomal location for the Tet B determinant in the *M. catarrhalis* strains.

To examine whether Tet B gene expression in *M. catarrh-*

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FIG. 1. Induction experiments with Tc^r *M. catarrhalis* Bc-301 and R222 (Tet B)-containing *E. coli* D1-7. *M. catarrhalis* was incubated at a turbidity of 0.1 to 0.5 U of A_{530} in supplemented brain heart infusion broth with 0.5 μ g of tetracycline per ml for 30 min before being challenged with 10 μ g/ml. Higher challenge levels inhibited the Tc^r strains. The *E. coli* strains were incubated with 1 μ g/ml and challenged with 100 μ g/ml as previously described (10). Bc-301 and D1-7 were grown in the presence (Δ) or absence ($\circ$) of a subinhibitory concentration of tetracycline and a control culture without tetracycline ($\square$).

alis was inducible, as found in enteric bacteria, or constitutive, as seen in members of the genus *Haemophilus*, induction studies were done as previously described (10). Both Tc^r strains, Bc-300 and Bc-301, were tested, while the Tc^s strain NRL 30018, *Escherichia coli* D1-7 carrying an inducible Tet B on plasmid R222, and *E. coli* M603 carrying the constitutive *Haemophilus* Tet B gene in the chromosome were used as controls (10). Growth curves for the *E. coli* controls were similar to those previously published (10), with the constitutive-Tet B strain showing little difference between the induced and uninduced cultures, while the inducible-Tet B strain had a lower rate of growth in the culture not pretreated with tetracycline compared with the culture treated with tetracycline. The Tc^s NRL 30018 did not grow in the presence of Tc (data not shown). Bc-300 and Bc-301 had similar growth curves, with the induced and uninduced cultures growing at similar rates, suggesting that the Tet B determinant was constitutive in the Tc^r *M. catarrhalis* strains. Results with Bc-301 and *E. coli* containing R222 are illustrated in Fig. 1.

In this study, we have shown that two Tc^r *M. catarrhalis* strains carry nontransferable Tet B determinants. The data

are consistent with a chromosomal location and represent the first description of the Tet B determinant in *M. catarrhalis* and the first time an antibiotic resistance determinant found in other gram-negative genera has been associated with *M. catarrhalis*. Three reports of Tc^r *M. catarrhalis* strains, from China (25), the Netherlands (6), and Spain (23), have been described in the literature. These strains may also be resistant to tetracycline because of the presence of the Tet B determinant.

The source of the Tet B determinant in the Tc^r *M. catarrhalis* strains is not known. Nevertheless, since *M. catarrhalis* appears to carry a constitutive Tet B, it is tempting to speculate that *M. catarrhalis* received the determinant from a *Haemophilus* species, especially since these two species can be found in the same host environment. The constitutive Tet B in *Haemophilus* spp. is due to the presence of an inactive repressor protein (10). Therefore, more definitive proof will require the sequencing of the Tet B repressor gene from both species to determine whether both the *M. catarrhalis* Tet B and the *Haemophilus* Tet B carry the same defect.

The data demonstrate that the gene pool found in gram-negative bacteria is continuing to expand into new genera of gram-negative bacteria and indicate that continued surveillance of susceptibility to antibiotics is needed.

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