International Clone of Neisseria meningitidis Serogroup A with Tetracycline Resistance Due to tet(B)

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Thirteen Neisseria meningitidis clinical isolates from Africa, Asia, and the United States for which the tetracycline MICs were elevated (≥8 μg/ml) were examined for 14 recognized resistance genes. Only the drug efflux mechanism encoded by tet(B) was detected. All isolates were in serogroup A, belonged to complex ST-5, and were closely related by pulsed-field gel electrophoresis analysis.

Neisseria meningitidis is a leading cause of bacterial meningitis and severe sepsis in the United States, in other industrialized countries of the Americas and Europe, and in developing parts of the world (16). It is also a major cause of periodic epidemics in sub-Saharan Africa and areas of the Middle East (16). Patients with invasive meningococcal infection must be treated with effective antibiotics because of the severity of meningococcaemia and meningitis, and close contacts should receive prophylactic treatment to prevent possible secondary cases. Penicillin has historically been the most effective antibiotic for therapy, but strains with reduced susceptibility to penicillin have been reported in Europe, South America, Asia, Australia, and the United States (3, 7, 14, 17, 18, 20). Resistance to antimicrobial agents that may be used for prophylaxis, but strains with reduced susceptibility to penicillin have been reported in Europe, South America, Asia, Australia, and the United States (3, 7, 14, 17, 18, 20). Resistance to antimicrobial agents that may be used for prophylaxis, but strains with reduced susceptibility to penicillin have been reported in Europe, South America, Asia, Australia, and the United States (3, 7, 14, 17, 18, 20). Resistance to antimicrobial agents that may be used for prophylaxis, but strains with reduced susceptibility to penicillin have been reported in Europe, South America, Asia, Australia, and the United States (3, 7, 14, 17, 18, 20).

A group of 441 N. meningitidis clinical isolates from 15 countries and 20 U.S. states recovered between 1917 and 2004 has been studied in order to develop susceptibility testing breakpoints for this species with a number of drugs, including tetracycline, minocycline, and doxycycline (with a subset of 124 isolates). MICs were determined by the NCCLS broth microdilution procedure with lysed horse blood-supplemented Mueller-Hinton broth incubated for 20 to 24 h at 35°C in 5% CO₂ (10). Streptococcus pneumoniae ATCC 49619 and Escherichia coli ATCC 25922 were used for quality control of the medium and antibiotics (11). Elevated tetracycline MICs (≥8 μg/ml) were noted for some strains, while most of the strains were inhibited by 2 μg/ml or less (modal value of 0.5 μg/ml) (Table 1). The mechanism responsible for the elevated tetracycline MICs was investigated in this study by PCR for the resistance genes with previously described primers for the tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), tet(K), tet(L), tet(M), tet(O), tet(Q), tet(P), tet(W), and tet(X) genes (1, 12, 13, 15). Universal primers RW01 and DG74, used to detect ribosomal DNA, were used to demonstrate adequate template DNA for amplification (8).

All 13 isolates for which the tetracycline MIC was 8 or 16 μg/ml produced a 664-bp PCR product consistent with tet(B) when matched with sequences in the GenBank database (http://www.ncbi.nlm.nih.gov) (2). However, unlike E. coli strains possessing tet(B), the meningococcal strains in this study were uniformly susceptible to minocycline (MIC ≤ 0.25 μg/ml) and the doxycycline MICs for them were slightly elevated (Table 1) (9).

DNA extracts from all 13 resistant meningococcal isolates were examined by multiple methods in an attempt to detect plasmids as the possible location of the tet(B) sequences. While

<table>
<thead>
<tr>
<th>MIC (μg/ml)</th>
<th>Tetracycline (n = 441)</th>
<th>Minocycline (n = 441)</th>
<th>Doxycycline (n = 124)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency of MIC</td>
<td>tet(B)</td>
<td>Frequency of MIC</td>
</tr>
<tr>
<td>0.06</td>
<td>10</td>
<td>NA*</td>
<td>20</td>
</tr>
<tr>
<td>0.12</td>
<td>85</td>
<td>NA</td>
<td>165</td>
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<tr>
<td>0.5</td>
<td>260</td>
<td>0/1</td>
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</tr>
<tr>
<td>1</td>
<td>57</td>
<td>0/10</td>
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<tr>
<td>2</td>
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<td>0/11</td>
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<td></td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>6/6</td>
<td></td>
</tr>
</tbody>
</table>

* NA, not available.
* Number of isolates with tet(B)/total.

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TABLE 1. Frequencies of MICs for 441 isolates of N. meningitidis and the presence or absence of tet(B)
these experiments did not reveal the presence of plasmids, it is not possible to say with certainty that the tet(B) sequences reside in the chromosome. However, Takahashi et al. (H. Takahashi, H. Watanabe, T. Kuroki, Y. Watanabe, and S. Yamai, Letter, Antimicrob. Agents Chemother. 46:4045-4046, 2002) found tet(B) to be chromosomally mediated in a single meningococcal isolate colonizing a patient in Japan. Additional experiments are under way to definitively characterize the location of the gene in our isolates.

All 13 tetracycline-resistant isolates belonged to serogroup A, clonal complex ST-5 (Deborah Talkington, personal communication), and were recovered from patients in five countries and four U.S. states between 1999 and 2002 (Table 2). Seven strains were previously determined to be in multilocus enzyme electrophoresis subgroup III (Talkington, personal communication), and all 13 isolates were either identical or closely related by pulsed-field gel electrophoresis (PFGE); i.e., they differed by no more than three bands (Fig. 1) (19). All 13 isolates were also resistant to sulfisoxazole and trimethoprim-sulfamethoxazole but were susceptible to penicillin, other relevant beta-lactams, and rifampin (data not shown).

Tetracycline resistance in this collection of meningococcal isolates from multiple continents was associated with the drug efflux mechanism encoded by tet(B). The presence of tet(B) was reported previously in a single strain (Takahashi et al., Letter). However, an earlier report indicated the presence of the ribosomal protection mechanism encoded by tet(M) in some meningococci (6). In the meningococcal isolates in this study, the tet(B) mechanism did not lead to elevated minocycline MICs. Possible shortcomings of this study include the fact that these strains were not collected consecutively or all derived from population-based surveillance studies. Thus, it is not possible to define the frequency of tetracycline resistance overall or even among all serogroup A isolates. Furthermore, we cannot determine when tetracycline resistance due to tet(B) first arose in meningococci or why it appears to be restricted to only one meningococcal serogroup. The isolate previously to contain tet(B) belonged to serogroup B (H. Takahashi, personal communication). Lastly, it is not clear why the efflux protein encoded by tet(B) in meningococci does not appear to be able to remove minocycline from the bacterial cells, as has been the case with tet(B)-mediated resistance in other organisms (e.g., E. coli) (9). However, this suggests that minocycline may continue to represent one option for prophylaxis of invasive meningococcal case contacts, even in those strains that contain the tet(B) determinant.

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**REFERENCES**


