Detection of tet(M), tet(O) and tet(S) in tetracycline/minocycline-resistant Streptococcus pyogenes bacteraemia isolates

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Sir,

Streptococcus pyogenes is the most common cause of bacterial pharyngitis and causes scarlet fever, impetigo and erysipelas. S. pyogenes may also give rise to severe invasive manifestations such as sepsis, necrotizing fasciitis and streptococcal toxic shock syndrome.

Tetracycline/minocycline resistance is often encoded by the tet(M) gene in Gram-positive bacteria, and more rarely by the tet(O), tet(Q), tet(S), tet(T) and tet(W) genes, which all encode ribosomal protection proteins.1 Tetracycline resistance (TcR) alone is often encoded by the efflux genes tet(K) and tet(L).1

Ninety-two S. pyogenes isolates were received at the Department of Clinical Microbiology at Hvidovre Hospital, Denmark, during 1990–1999. Susceptibility to erythromycin, ciprofloxacin, penicillin, minocycline and tetracycline was assayed by Etest (AB Biodisk, Solna, Sweden). Thirty-one (33.7%) strains were resistant to tetracycline. This was surprising because tetracycline is not usually employed to treat S. pyogenes infections in Denmark. Only one isolate from the first bacteraemic episode from each patient was included in this study, except in one case where two isolates from one patient were obtained from two episodes of bacteraemia 2 years apart.2

The 31 (TcR) S. pyogenes isolates were investigated for the presence of tet(K), tet(L), tet(M), tet(O), tet(Q), tet(S), tet(T) and tet(W) genes by PCR. Only one isolate was resistant to erythromycin; it was also investigated for the presence of erm(A), erm(B) and mef(A) by PCR. None of the isolates was resistant to ciprofloxacin or penicillin. All S. pyogenes isolates were T typed with 24 monovalent antisera from the Statens Serum Institut. The 31 S. pyogenes isolates were typed by PFGE using Smal. Transfer of tet(M) (two isolates), tet(O) (one isolate) and tet(S) (three isolates) was studied by filter mating to Enterococcus faecalis recipient JH2-2.

The nucleotide sequences of the amplification products of tet(S) were determined by cycle sequencing for three isolates.

In all isolates, the tetracycline and minocycline resistance was encoded by a single gene, tet(M), tet(O) or tet(S) (Table 1). tet(K), tet(L), tet(Q), tet(T) and tet(W) were not detected.

Table 1. Distribution of the tet(O), 1erm(A), 1tet(M) and 1tet(S) genes, T types and PFGE types among Danish tetracycline-resistant Streptococcus pyogenes isolates

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T typea</th>
<th>PFGE typeb</th>
<th>No. of isolatesc</th>
</tr>
</thead>
<tbody>
<tr>
<td>tet(O), 1erm(A)</td>
<td>28</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>tet(M)</td>
<td>4</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>tet(M)</td>
<td>11</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>tet(M)</td>
<td>11</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>tet(M)</td>
<td>3, 13, B3264</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>tet(M)</td>
<td>3, 13, B3264</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>tet(M)</td>
<td>3, 13, B3264</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>tet(M)</td>
<td>NT</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>tet(M)</td>
<td>NT</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>tet(M)</td>
<td>NT</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>tet(S)</td>
<td>3, 13, B3264</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>tet(S)</td>
<td>NT</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

aOne strain was resistant to erythromycin encoded by 1erm(A).

bNT, non-typeable.

cPFGE types are similar within three bands.

*dNumber of isolates with the combinations of genotype, T type and PFGE type.

Tet(S) was detected in seven of the 31 isolates. This result was confirmed by sequencing of the 622 bp PCR product, which was identical to GenBank sequence L09756.

To our knowledge, tet(S) has not been reported previously in S. pyogenes isolates, although it has been detected in Listeria spp., Enterococcus spp. and Lactococcus lactis.3,4 It was not possible to transfer tet(S) into E. faecalis from any of the three S. pyogenes isolates tested. All seven tet(S) isolates were collected in 1998 or 1999, and had the same PFGE type, indicating a clonal relationship and vertical spread of the tet(S) gene, but analysis of patient data did not indicate any epidemiological relationship.

Tet(M) was detected in 23 of the 31 TcR S. pyogenes isolates. Ten of the tet(M)-positive strains were T non-typeable and had the same PFGE type (type 3), which indicates a clonal relationship of these strains. Two of the tet(M) PFGE type 3 strains were obtained from the same patient, but with a 2 year interval (1993 and 1995). The patient was an intravenous drug user who had a chronic ulcer on the leg; it is possible that he carried the strain during the entire period. Three other PFGE type 3 isolates were obtained from intravenous drug users in 1993; it is not known whether any of these subjects were related socially to the possible carrier.

The remaining 13 tet(M)-positive isolates had eight different PFGE types and three different T types, indicating a horizontal transfer of the tet(M) gene. One tet(M)-positive strain with the most common PFGE type and T type (PFGE type 3/T type NT), and another tet(M) strain with PFGE type 2 and T type 3, 13, B3264 were used as donors in filter mating. It was possible to transfer tet(M) from the two tet(M) S. pyogenes isolates into an E. faecalis recipient (transfer frequency was 6 × 10^{-6} and 1 × 10^{-8} transconjugants/recipient, respectively). It has been hypothesized that the transposon carrying the tet(M) gene, as typified by Tn916, was the original Gram-positive

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It is suggested that over time other antibiotic resistance genes were inserted directly into this family of transposons, creating larger units carrying two to four different antibiotic resistance genes.\textsuperscript{1}

tet(O) was present in a single isolate from 1999; this isolate was also resistant to erythromycin encoded by \textit{erm}(A). The combination of \textit{erm}(A) and tet(O) has recently been detected in other macrolide/tetracycline resistant \textit{S. pyogenes} isolates.\textsuperscript{6} It was not possible to transfer the tet(O) gene into an \textit{E. faecalis} recipient in our study.

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References