The Macrolide Resistance Genes \textit{erm}(B) and \textit{mef}(E) Are Carried by Tn2010 in Dual-Gene \textit{Streptococcus pneumoniae} Isolates Belonging to Clonal Complex CC271

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The genetic elements carrying macrolide resistance genes in \textit{Streptococcus pneumoniae} isolates belonging to CC271 were investigated. The international clone Taiwan19F-14 was found to carry Tn2009, a Tn916-like transposon containing \textit{tet}(M) and \textit{mef}(E). The dual \textit{erm}(B) \textit{mef}(E) isolates carried Tn2010, which is similar to Tn2009 with the addition of a putative new transposon, the \textit{erm}(B) genetic element.

Macrolide resistance in \textit{Streptococcus pneumoniae} is mediated by one of two main mechanisms, (i) target modification due to a ribosomal methylase encoded by \textit{erm}(B), which confers high-level resistance to macrolides, lincosamides, and streptogramin B (MLS\textsubscript{B} phenotype), or (ii) an efflux transport system associated with the \textit{mef} gene, conferring resistance to 14- and 15-membered macrolides only (M phenotype) (10). \textit{erm}(B)-mediated erythromycin resistance is the most common mechanism in many areas of the world, including some European and Asian countries (14, 15, 17), whereas \textit{mef}, with its most common variants \textit{mef}(A) and \textit{mef}(E), is predominant in the United States (5), Canada (8), and the United Kingdom (1). In recent years, pneumococcal isolates carrying both macrolide resistance genes \textit{erm}(B) and \textit{mef} were observed with increasing frequency, particularly in some Asian countries, in South Africa, and in the United States (4, 9, 12). In the United States, an increase in the proportion of pediatric isolates carrying dual macrolide resistance genes was noted following the introduction of the conjugate pneumococcal vaccine (5). The dual-gene isolates were mainly of serotype 19F or 19A and were multidrug resistant, being resistant to penicillin and tetracycline resistance genes (1). These isolates included the Taiwan19F-14 type strain carrying \textit{mef} (13) and 12 isolates carrying both \textit{erm}(B) and \textit{mef}, selected among a large collection of strains obtained from different geographical areas in 1999 to 2003, mainly from community-acquired lower respiratory tract infections (4, 5). All of these isolates were multidrug resistant, being nonsusceptible to penicillin (MIC range, 2 to 8 \mu g/ml), resistant to high levels of erythromycin and clindamycin, and also resistant to tetracycline in all cases but one.

PN150 and PGX1416, carrying Tn2009 and Tn2010, respectively, were used as control strains in PCR assays (2). The two \textit{mef} variants, \textit{mef}(A) and \textit{mef}(E), \textit{erm}(B), and \textit{tet}(M) were detected by PCR (3, 14).

Analysis of the genetic elements in the isolates under study was performed by PCR mapping (2, 3). The sizes of the amplifications obtained were compared with those obtained with the control strains.

On the basis of the location of Tn2010 in PGX1416 (2), the insertions of the genetic elements were explored by two PCR assays. The left (L) junction was detected by primer SQ8 (5', CAAAGCTASTTTTATACATAG-3'), which anneals to the pneumococcal chromosome, and primer TN4 (3), which anneals to Tn916. The right (R) junction was detected by primer TN6, which anneals to Tn916 (3), and primer SQ7 (5', GTAA TACATTTCTTACAAACAG-3'), which anneals to the pneumococcal chromosome. In selected isolates, sequencing of both the L and R junctions was performed.

In the Taiwan19F-14 strain, erythromycin resistance was conferred by the \textit{mef}(E) variant of \textit{mef} and tetracycline resistance was conferred by \textit{tet}(M). By PCR mapping, the fragments obtained were of the same size as those obtained from control strain PN150, indicating that Taiwan19F-14 carries Tn2009, which encompasses \textit{mef}(E) and \textit{tet}(M).

In all of the 12 dual-gene \textit{S. pneumoniae} isolates, \textit{erm}(B), \textit{mef}(E), and \textit{tet}(M) were detected. The tetracycline susceptibility of strain GMRS06 (MIC, 0.25 \mu g/ml) is likely explained by the one-nucleotide insertion found in the \textit{tet}(M) coding
sequence, causing an early stop codon. By PCR mapping, the fragments obtained with these isolates were of the same size as those obtained with control strain PGX1416. This indicates a genetic organization corresponding to Tn2010, a composite transposon similar to Tn2009, with the addition of an \textit{erm}(B) genetic element integrated into an open reading frame (ORF) corresponding to the \textit{erm}(B) genetic element of Tn916 (2). The \textit{erm}(B) genetic element and the adjacent Tn916 regions are identical to sequences present in different multiresistant plasmids belonging to the \textit{inc}18 incompatibility group, such as pIP501 of \textit{Streptococcus agalactiae} (18) and pRE25 of \textit{Enterococcus faecalis} (16), and also to a region found in the Lactobacillus johnsonii chromosome that is designated the \textit{erm}(B) locus (accession no. DQ518904) (7). The last ORF of this region, designated \textit{\Delta}\textit{ao2}, is 100% identical to the C terminus of the \textit{\omega} protein, involved in copy number control of \textit{inc}18 plasmids (19). The

![transposase region](http://aac.asm.org/)

FIG. 1. Organization of the \textit{erm}(B) genetic element of Tn2010. The two distinct regions composing the element [the transposase region and the \textit{erm}(B)-\textit{\Delta}\textit{ao2} region] are shown below the element, with the respective indications of the origin of identical sequences (microorganism name or plasmid designation). Arrows correspond to ORFs, with the direction of transcription indicated by the arrowheads. The striped bar corresponds to Tn916 sequences at the insertion of the \textit{erm}(B) element (orf20). The 8-bp direct repeats (DR) flanking the element are boxed. The positions of the putative imperfect IRs (\textit{IRL}, \textit{IRR}1, and \textit{IRR}2) and their corresponding sequences (in italics) are indicated. The positions of the 27-bp perfect IRs are indicated by the black bars.

### TABLE 1. Characteristics of the \textit{S. pneumoniae} isolates used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Country of origin</th>
<th>Year(s) isolated</th>
<th>Serotype</th>
<th>Resistance pattern</th>
<th>Presence of resistance gene:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>\textit{mef}(E) \textit{erm}(B) \textit{tet}(M)</td>
</tr>
<tr>
<td>Taiwan\textsuperscript{a} 199-14</td>
<td>Taiwan</td>
<td>1997</td>
<td>19F</td>
<td>Pen' Ery' Tet'</td>
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</tr>
<tr>
<td>GRMS01</td>
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<td>1999-2000\textsuperscript{a}</td>
<td>19F</td>
<td>Pen' Ery' \textit{Cl'} Tet'</td>
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</tr>
<tr>
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<td>United States</td>
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<td>Pen' Ery' \textit{Cl'} Tet'</td>
<td>+ + +</td>
</tr>
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<td>Pen' Ery' \textit{Cl'} Tet'</td>
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<td>China</td>
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<td>Pen' Ery' \textit{Cl'} Tet'</td>
<td>+ + +</td>
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<td>19F</td>
<td>Pen' Ery' \textit{Cl'} Tet'</td>
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<td>Pen' Ery' \textit{Cl'} Tet'</td>
<td>+ + +</td>
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<tr>
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<td>Unknown</td>
<td>19F</td>
<td>Pen' Ery' \textit{Cl'} Tet'</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Pen', penicillin resistance; Ery', erythromycin resistance; \textit{Cl'}, clindamycin resistance; Tet', tetracycline resistance.

\textsuperscript{b} The gene sequence contains a frameshift mutation (see text).

\textsuperscript{c} Control strain.

\textsuperscript{d} Winter season.

\textit{erm} \textit{mef} (B) genetic element of Tn916 \textit{erm}(B) genetic element (7). The last ORF of this region, designated \textit{\Delta}\textit{ao2}, is 100% identical to the C terminus of the \textit{\omega} protein, involved in copy number control of \textit{inc}18 plasmids (19). The

![transposase region](http://aac.asm.org/)

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**erm(B)-Δω2 region** is flanked by perfect 27-bp inverted repeats (IRs). Putative imperfect IRs can be identified, two at both ends of the transposase region (*IR1* and *IRR1*) and an additional one at the R end of the *erm(B)-Δω2* region (*IRR2*). The sequences of *IRR1* and *IRR2* partly overlap the sequences of the 27-bp IRs (Fig. 1). On the basis of these observations, it could be hypothesized that the *erm(B)* genetic element is a putative transposon derived from the assembly of a transposase region from *L. reuteri*, with an erythromycin resistance region of likely plasmid origin. Consistent with the *erm(B)* genetic element arriving by transposition was the identification of 8-bp direct repeats flanking the element, likely representing target duplication (Fig. 1).

The analysis of the chromosomal location showed that both Tn2009 in Taiwan19F-14 and Tn2010 in the dual-gene isolates were inserted at the same site, previously identified in strain PGX1416, inside an ORF corresponding to spr1764 of R6 (2). This finding supports the hypothesis that *erm(B)*-*mef(E)* dual-gene isolates belonging to CC271 have evolved from multidrug-resistant Taiwan19F-14 by acquisition of the *erm(B)* genetic element (4, 9). Although the *erm(B)* genetic element does not appear to be transferable by conjugation among pneumococci, it cannot be excluded that CC271 isolates have acquired this element from other species of streptococci or other gram-positive bacteria by conjugation.

The selective advantage of acquiring *erm(B)* on top of *mef(E)* consists of gaining not only high-level erythromycin resistance but also an increased number of antimicrobial classes to which the bacteria are resistant, including lincosamides and streptogramins.

**Nucleotide sequence accession number.** The sequence of the *erm(B)* element in PGX1416 has been assigned GenBank accession no. EF592165.

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**REFERENCES**