Clonal complex 271 (CC271) is one of the emerging multidrug-resistant clones of *Streptococcus pneumoniae*, spreading in many areas of the globe (3) and including serotype 19F and the nonvaccine serotype 19A (4). CC271 pneumococci often carry dual macrolide resistance genes *erm*(B) and *mef*(E), determining a macrolide resistance phenotype (MLS$_\beta$) identical to that conferred by *erm*(B) alone (5).

We examined a serotype 19F *S. pneumoniae* isolate (S43) belonging to CC271 (sequence type 1428), obtained in Hungary in 2003 from an infant with meningitis. S43 was multidrug resistant, showing resistance to penicillin (MIC = 4 µg/ml), erythromycin (MIC > 256 µg/ml), clindamycin (MIC > 256 µg/ml), and tetracycline (MIC = 16 µg/ml) and carrying *erm*(B), *mef*(E), and *tet*(M).

We have previously demonstrated that CC271 dual-gene isolates carried the composite transposon Tn2010, a Tn916 family transposon including *erm*(B), *mef*(E), and *tet*(M) (1).

To verify the presence of Tn2010 in *S. pneumoniae* S43, PCR mapping was performed (1). The presence of a Tn916-like transposon including *tet*(M) and harboring *mef*(E) in a mega element integrated in *orf6* (1) was confirmed. However, the *erm*(B) genetic element described in Tn2010 was not found. Since *erm*(B) can be carried by Tn917 (6), we investigated the presence of Tn917 and its physical linkage with the Tn916-like transposon in S43. Three sets of primer pairs were designed on the Tn917 sequence (GenBank accession no. M11180), as follows: Tn7 (5'-GCAGGTGTAT TTCTTATCTATGG-3') and EB2 (1); O29 (5'-TCATCAACACGTGATTCACAAGG-3') and Tn9 (5'-AGCTTTCTTCTCC ATCTTGAC-3'); TN8 (5'-ACACGATTCCAAACGAA CAG-3') and TN11 (5'-CCTTAACCTATGTTCAGG-3'). Amplicons of the expected size were obtained with primer pairs O29/TN9 and TN8/TN11, but not with TN7/EB2 due to a 182-bp deletion from positions 240 to 421 of Tn917 sequence including primer TN7. This deletion in Tn917 had been already described in isolates from Japan (7). By using the primer pair EB2 and SG5 (1), an amplicon was obtained showing a physical linkage between Tn917 and mega. Sequence analysis of a 1,053-bp fragment (GenBank accession no. FJ208941) showed that the left end of Tn917 was inserted in *orf9* of Tn916 in proximity to the insertion of mega in *orf6* of Tn916 (Fig. 1). Tn917 was inserted in Tn916 at the identical site in which it is inserted to form Tn3872 (6). These findings confirmed the identification in *S. pneumoniae* S43 of a composite transposon of ca. 28.5 kb that was designated Tn2010, based on a new modular combination of Tn916, Tn917, and mega. The left and right junctions of Tn2010 in S43 were identical to those described for Tn2010 in CC271 isolates (2).

In a previous study, we characterized three dual-gene isolates from Italy belonging to CC15 (1). Although these isolates contained Tn916, Tn917, and mega, they did not carry the newly described Tn2010 but, rather, carried the composite transposon Tn3872, with Tn917 inserted in Tn916, while mega was independently inserted in spr0166, a common mega insertion site (1).

These findings indicate that in order to correctly trace the spreading of the antibiotic resistance determinants, it is
necessary to define the physical linkage between the resistance elements identified and the boundaries of the exogenous elements in the chromosome.

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