ICESpy009, a Conjugative Genetic Element Carrying mef(E) in Streptococcus pyogenes

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Efflux-mediated macrolide resistance due to mef(E) and mel, carried by the mega element, is common in Streptococcus pneumoniae, for which it was originally characterized, but it is rare in Streptococcus pyogenes. In S. pyogenes, mega was previously found to be enclosed in Tn2009, a composite genetic element of the Tn916 family containing tet(M) and conferring erythromycin and tetracycline resistance. In this study, S. pyogenes isolates containing mef(E), apparently not associated with other resistance determinants, were examined to characterize the genetic context of mega. By whole-genome sequencing of one isolate, MB56Spy009, we identified a novel composite integrative and conjugative element (ICE) carrying mega, designated ICESpy009, belonging to the ICESa2603 family. ICESpy009 was 55 kb long, contained 61 putative open reading frames (ORFs), and was found to be integrated into hylA, a novel integration site for the ICESa2603 family. The modular organization of the ICE was similar to that of members of the ICESa2603 family carried by different streptococcal species. In addition, a novel cluster of accessory resistance genes was found inside a region that encloses mega. PCR mapping targeting ICESpy009 revealed the presence of a similar ICE in five other isolates under study. While in three isolates the integration site was the same as that of ICESpy009, in two isolates the ICE was integrated into rplL, the typical integration site of the ICESa2603 family. ICESpy009 was able to transfer macrolide resistance by conjugation to both S. pyogenes and S. pneumoniae, showing the first evidence of the transferability of mega from S. pyogenes.

In streptococci, resistance to 14- and 15-membered macrolides without resistance to lincomamides or streptogramins (M phenotype) is conferred by a two-component drug efflux system (a major facilitator superfamily efflux pump and an ATP-binding cassette transporter) encoded by two adjacent cotranscribed genes, mef and msr(D)/mel (1). Different subclasses of mef genes have been identified, including mef(A), originally described for Streptococcus pyogenes (2), and mef(E) (3) and mef(I) (4), both originally described for Streptococcus pneumoniae. Each mef subclass was found to be associated specifically with a different genetic element. In S. pyogenes, mef(A) was carried by Tn1207.1, a defective transposon that was found to be integrated into the prophages Φ1207.3 (5), Φ10394.4 (6), and Φm461 (7). Similarly, in S. pneumoniae, mef(A) was carried by Tn1207.1, which was found to be integrated into the chromosome at celB (8); mef(E) was carried by the megal element (1), and mef(I) was found to be associated with catQ in the IQ element (4).

In S. pyogenes, mef(A) is by far the most common mef subclass: among a global collection of 1,175 mef-positive S. pyogenes clinical isolates, 95.4% carried mef(A), and only 4.6% carried a different mef subclass (9). Each of the other mef subclasses, corresponding to mef(E), mef(I), and the mosaic variant mef(A/E), was specifically associated with a composite genetic element including other resistance genes (10). mef(E), carried by mega, was included in Tn2009, a composite transposon of the Tn916 family containing tet(M) (11). mef(I) was linked to catQ in the IQ-like element and was found to be carried by integrative and conjugative elements (ICEs) (12) belonging to the Tn5253 family, such as ICESpy005IQ and ICESpy0291Q (13). The mef(A/E) mosaic variant was found to be associated with tet(O) in a genetic element similar to prophage Φm461 (7).

The genetic elements reported above appear to be nonconjugative, with the exception of ICESpy005IQ, whose conjugative transfer was obtained from S. pyogenes to streptococcal recipient strains (10, 13). A lysogenic transfer can be hypothesized for the mef(A/E) variant carried by the Φm461-like element, on the basis of the transferability of mef(A) carried by Φm461 reported by Giovanetti et al. (14), but experimental evidence is missing.

In a large global collection of S. pyogenes isolates (9), we identified isolates in which mef(E) and mel were apparently not associated with other resistance genes. In the present study, we characterized the genetic context of mega in these isolates and used whole-genome sequencing (WGS) to identify a novel composite ICE enclosing mega, namely, ICESpy009, that was able to transfer macrolide resistance by conjugation.

(Preliminary data from this study were presented at the 22nd European Congress of Clinical Microbiology and Infectious Diseases, London, United Kingdom, 31 March to 3 April 2012 [15].)
MATERIALS AND METHODS

Bacterial strains. For this study, 12 *S. pyogenes* isolates carrying *mef(E)* and showing susceptibility to tetracycline were selected from a global collection of clinical isolates obtained between 1999 and 2005 (9). Exclusion of tetracycline-resistant isolates was directed at ruling out the presence of *tet(M)*-carrying Tn2009-like elements, including mef, in the isolates under study (Table 1). *S. pneumoniae* PN150, a *mef(E)*-positive strain, was used as a control strain for PCR mapping of mef (11). *S. pyogenes* SF570K (a kanamycin-resistant derivative of *S. pyogenes* SF370), *S. pneumoniae* FP10 (a streptomycin- and chloramphenicol-resistant derivative of *S. pneumoniae* Rx1), and *S. pneumoniae* FP11 (a novobiocin- and chloramphenicol-resistant derivative of Rx1) were used as recipient strains in conjugation experiments (10, 16). Both FP10 and FP11 were defective in the competence system and were not spontaneously transformable (17).

Conjugation experiments and hybridization assay. Transferability by conjugation was assayed using *S. pyogenes* MB56Spy009 as the donor strain and *S. pneumoniae* SF370K or *S. pneumoniae* FP10 as the recipient strain. A second conjugation experiment was performed using a representative *S. pneumoniae* transconjugant from the previous experiment (L6) as the donor and *S. pneumoniae* FP11 as the recipient. Donor and recipient bacteria were grown separately at 37°C in tryptic soy broth (TSB), harvested at the end of the exponential growth phase, mixed at a 1:10 ratio, and plated onto tryptic soy agar (TSA) plates as previously described (17, 18). Transconjugants were selected on multilayer plates containing erythromycin (1 µg/ml), with the addition of other selective agents, i.e., kanamycin (1,000 µg/ml), streptomycin (1,000 µg/ml), or novobiocin (10 µg/ml), as appropriate (10, 16).

Small-restricted genomic DNAs of the donor strains, the recipient strains, and the transconjugants were examined by pulsed-field gel electrophoresis (PFGE) (17). Southern blotting and hybridization with the *mef(E)* probe were performed as previously described (16).

**TABLE 1 S. pyogenes** isolates carrying *mef(E)*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Country of origin</th>
<th>emm type</th>
<th>Sequence type</th>
<th>ICE type</th>
<th>Integration site</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB56Spy0003</td>
<td>Germany</td>
<td>12.0</td>
<td>36</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>MB56Spy0008</td>
<td>Germany</td>
<td>2.0</td>
<td>55</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MB56Spy0009</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009</td>
<td>hylA</td>
</tr>
<tr>
<td>MB56Spy0018</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>hylA</td>
</tr>
<tr>
<td>MB56Spy0019</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>hylA</td>
</tr>
<tr>
<td>MB56Spy0020</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>hylA</td>
</tr>
<tr>
<td>MB56Spy0021</td>
<td>United States</td>
<td>6.4</td>
<td>382</td>
<td>ICESpy009-like element</td>
<td>rplL</td>
</tr>
<tr>
<td>MB56Spy0022</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>hylA</td>
</tr>
<tr>
<td>MB56Spy0023</td>
<td>United States</td>
<td>6.4</td>
<td>382</td>
<td>ICESpy009-like element</td>
<td>rplL</td>
</tr>
<tr>
<td>MB56Spy0024</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>hylA</td>
</tr>
<tr>
<td>MB56Spy0025</td>
<td>United States</td>
<td>3.1</td>
<td>15</td>
<td>ICESpy009-like element</td>
<td>ND</td>
</tr>
<tr>
<td>MB56Spy0026</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>ND</td>
</tr>
<tr>
<td>MB56Spy0028</td>
<td>United States</td>
<td>12.0</td>
<td>36</td>
<td>ICESpy009-like element</td>
<td>ND</td>
</tr>
</tbody>
</table>

At least three mef(E)-Carrying ICESpy009 in *S. pyogenes*

PCR assays. To investigate the presence of an intermediate circular form of ICESpy009, two outward-facing primers (MG49 and MG47), targeting the left (L) and right (R) ends of ICESpy009, respectively, were designed and used in PCR experiments (see Table S1 in the supplemental material).

To verify the presence of genetic elements similar to that found in *S. pyogenes* MB56Spy009 in other isolates, a series of primer pairs targeting the ICESpy009 sequence was designed for PCR mapping in order to amplify 13 overlapping fragments (see Table S1 in the supplemental material). A finer PCR mapping of mef was obtained using previously described primers (11). The chromosomal integration sites of the ICESpy009-like elements present in the *S. pyogenes* isolates were explored by using (i) primers MG49 and MG14, targeting the L and R ends of ICESpy009, respectively, combined with primers HYALSpy2 and HYALSpy1, targeting the *hylA* gene; and (ii) MG49, targeting the L end of ICESpy009, combined with RPL1L, targeting the *rplL* gene (21) (see Table S1).

Nucleotide sequence accession number. The 55,259-bp nucleotide sequence of ICESpy009 has been deposited in the GenBank database and assigned accession no. KU056701.

RESULTS

Characteristics of the isolates. All 12 *S. pyogenes* clinical isolates under study harbored *mef(E)* carried by the mega element. In 2 of the isolates, mega was larger than expected due to the presence of an insertion sequence (IS) of the IS3 family, similar to ISSmu1 (https://www-is.biotoul.fr/), that was integrated 622 nucleotide (nt) upstream of *mef(E)*, in a position not impairing the integrity of the promoter and regulatory region (22). To examine the genetic context of mef, one isolate (MB56Spy009), belonging to *emm* type 12.0 and ST36, the most common genetic background among the isolates under study (Table 1), was selected for further analysis.

Transferability of *mef(E)* from *S. pyogenes*. Conjugation experiments revealed that erythromycin resistance could be transferred from MB56Spy009 to both the *S. pyogenes* SF370K and *S. pneumoniae* FP10 recipient strains. An *S. pneumoniae* transconjugant was also able to retransfer erythromycin resistance to the *S. pneumoniae* FP11 recipient strain (Table 2). *S. pyogenes* TC1 and *S. pneumoniae* L6 transconjugants were selected for analysis by PFGE along with the donor strains.

Following PFGE separation, Southern blotting, and hybridization with a *mef(E)* probe, the size of the transferable DNA fragment was deduced by comparing the profiles of the donor strain, the recipient strains, and the transconjugants. A Smal fragment of ca. 250 kb that was present for the recipient *S. pyogenes* strain.
could not be detected for the TC1 transconjugant, for which a new SmaI fragment, of ca. 300 kb and hybridizing with the \( \text{mef}(E) \) probe, was present instead (Fig. 1). For the recipient \( S. \text{pneumoniae} \) strain, a 300-kb band was present that was missing for the L6 transconjugant, for which a new, 350-kb band positive for \( \text{mef}(E) \) was observed (Fig. 1). These results suggested that \( \text{mef}(E) \) was transferred to both transconjugants enclosed in a putative genetic element with an estimated size of approximately 50 kb.

**Identification of ICESpy009 and chromosomal integration.**

An 82.7-kb contig including \( \text{mef}(E) \) was identified and extracted from the 57 contigs obtained after MB56Spyo009 genome sequencing. Inside this contig, a 55,259-bp sequence with 39.5% GC content was considered a putative ICE and designated ICESpy009.

ICESpy009 was integrated into the chromosome at a position corresponding to nt 839,430 of MGAS9429 (accession number CP000259) (Fig. 2), inside the hyaluronate lyase precursor gene \( \text{hylA} \) of \( S. \text{pyogenes} \). A 4-bp conserved sequence (TTAA) was detected at both the left (L) and right (R) ends of the integrated ICESpy009 element, corresponding to direct repeats of putative \( \text{attL} \) and \( \text{attR} \) sequences (23) (see Fig. S1 in the supplemental material). This sequence can be considered the specific target of ICESpy009. The presence of a covalently closed circular form of ICESpy009 was revealed by PCR, and it contained a copy of the 4-bp conserved sequence corresponding to a putative \( \text{attI} \) site (see Fig. S1).

Since the most common integration site for ICEs of the ICESa2603 family is the \( \text{rplL} \) gene, a well-conserved housekeeping gene, we searched for \( \text{rplL} \) among all the contigs of the MB6Spyo009 genome. BLAST analysis showed that \( \text{rplL} \) was located inside contig 13 and that a genetic element of the 12070-RD.1 family (http://db-mml.sjtu.edu.cn/ICEberg/) was integrated at its 3’ end.

When the insertions of ICESpy009 in the transconjugants \( S. \text{pyogenes} \) TC1 and \( S. \text{pneumoniae} \) L6 were explored, PCR assays indicated that the ICE was not integrated into \( \text{hylA} \), as in the donor strain, but into the common integration site of the ICESa2603 family, i.e., \( \text{rplL} \).

**Characterization of ICESpy009 and comparative analysis.**

The annotated sequence of ICESpy009 was obtained by use of the

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**TABLE 2** Frequencies of conjugation of ICESpy009 to \( S. \text{pyogenes} \) and \( S. \text{pneumoniae} \) recipient strains

<table>
<thead>
<tr>
<th>Donor strain (species)</th>
<th>Recipient strain (species)</th>
<th>Transfer frequency&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Representative transconjugant</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB56Spyo009 (( S. \text{pyogenes} ))</td>
<td>SF370K (( S. \text{pyogenes} ))</td>
<td>( 5.3 \times 10^{-8} )</td>
<td>TC1</td>
</tr>
<tr>
<td>FP10 (( S. \text{pneumoniae} ))</td>
<td>( 4.0 \times 10^{-7} )</td>
<td>L6</td>
<td></td>
</tr>
<tr>
<td>L6 (( S. \text{pneumoniae} ) transconjugant)</td>
<td>FP11 (( S. \text{pneumoniae} ))</td>
<td>( 4.2 \times 10^{-5} )</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of transconjugants per donor cell.

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**FIG 1** Analysis of MB56Spyo009 and the transconjugants obtained in conjugation experiments. (A and C) PFGE patterns of SmaI-digested genomic DNAs. (B and D) Hybridization assays with the \( \text{mef}(E) \) probe. Lanes: 1, MB56Spyo009 donor strain; 2, \( S. \text{pyogenes} \) SF370K recipient strain; 3, \( S. \text{pyogenes} \) TC1 transconjugant; 4, \( S. \text{pneumoniae} \) FP10 recipient strain; 5, \( S. \text{pneumoniae} \) L6 transconjugant; M, lambda ladder. The arrows indicate the bands containing the transferred DNA fragment.
Web-based RAST service (19). ICESpy009 was found to contain 61 open reading frames (ORFs), 47 of them with the same direction of transcription as that of \textit{mef}(E) and \textit{mel}, the two components of the macrolide efflux system. The genetic organization of ICESpy009 is reported in Fig. 2.

A putative function was defined for each of the ORFs identified, with the exception of 21 ORFs, as detailed in Table S2 in the supplemental material. On the basis of their putative functions, the ORFs of ICESpy009 were found to be organized in a typical modular structure (12), including a conjugation module, a recombination module, and a module with ORFs encoding antibiotic resistance as an accessory function (23).

Among the relevant ORFs in the left region of ICESpy009, ORF2 and ORF3 encoded a replication initiation protein and a DNA-cytosine methyltransferase, respectively, the latter of which was similar to that described for Tn5252 (24), probably involved in methylation of DNA for protection of the recipient from restriction systems before DNA transfer. Close to these ORFs, the conjugation module included ORF9, ORF11, ORF13, and ORF14, showing 75 to 87% identity and 87 to 96% similarity to 4 conserved proteins, VirD4, VirB6, VirB4, and VirB1-like protein, of \textit{Streptococcus suis}05ZYH33 (accession no. NC_009442). These genes are likely involved in the type IVC secretion system, recently described for streptococci (25), participating in conjugal transfer of the ICE with other members of the conjugation machinery, such as the relaxase (26). A feature common to the Tn5253 family is the presence of other ORFs downstream of the type IVC secretion system cluster that encode putative proteins similar to a

FIG 2 Schematic representation of ICESpy009. (A) Genetic structure and relevant ORFs of ICESpy009. The integration site (\textit{hyLA}) and the nucleotide positions in the MGAS9429 chromosome (accession number CP000259) are indicated. The ORF numbers of the relevant ORFs (named in Table S2 in the supplemental material) are reported below the corresponding arrows in the schematic representation. (B) Multiple-sequence alignment of ICESpy009 (1) with the following ICEs belonging to the ICE\textit{Sa}2306 family and observed in different streptococcal species: ICE\textit{Sth}JIM8232-1 (\textit{Streptococcus thermophilus}) (2), ICE\textit{Sa}2603 (\textit{Streptococcus agalactiae}) (3), ICE\textit{Sdy}12394-1 (\textit{Streptococcus dysgalactiae} subsp. \textit{equisimilis}) (4), ICE\textit{Sde}3396 (\textit{S. dysgalactiae} subsp. \textit{equisimilis}) (5), ICE\textit{Ssu}(BM407)2 (\textit{Streptococcus suis}) (6), and ICE\textit{Ssu}(SC84) (\textit{S. suis}) (7). The figure was generated by Mauve aligner (http://darlinglab.org/mauve). Each contiguously colored region represents a local collinear block (LCB) corresponding to a homologous region shared by the aligned sequences. Inside the LCB, the colored area is higher in areas where the similarity is high; areas of low similarity are identified by white portions.
glucan-binding protein (ORF16), a calcium-binding protein (ORF17), and an SNF2 family protein/helicase (ORF19) (27). Downstream, ORF35, ORF36, and ORF37 were found to be similar to ORF10, ORF9, and ORF4 (relaxase) of Tn5252, respectively (28). The 5,511-bp mega element, including the macrolide resistance genes mef(E) (ORF40) and mel (ORF41), spanned nt 39,118 to nt 44,628 of the ICESpy009 sequence. Immediately downstream of mega, a ca. 8-kb DNA region that did not show identity to any DNA sequences in GenBank was identified. The deduced ORFs showed homology with putative aminoglycoside phosphotransferases (ORFs 48 and 49) and with ABC-type multidrug transport proteins (ORFs 50 to 52). This region may represent an accessory module containing genes possibly involved in antibiotic resistance.

At the right end of ICESpy009, the recombination module contained an integrase gene (ORF61) that, by comparison at the ICEberg website (http://db-mml.sjtu.edu.cn/ICEberg/), showed 88% amino acid identity and 94% similarity to the integrase of ICEStn(SC84) of S. suis SC84 (accession no. CAZS1585). This integrase corresponds to a tyrosine family site-specific recombinase related to integrases characterizing the ICESa2603 family. Nucleotide alignment of ICESpy009 with ICEs of the ICESa2603 family showed similarity with large regions, mainly in the conjugation module (Fig. 2B). By BLASTN analysis using the whole-genome shotgun database at the NCBI website, sequences corresponding to ICESpy009 were also found in draft genomes. Nucleotide identity was obtained between ICESpy009 and regions inside (i) supercontig 1 of S. pyogenes ABC020015285 (accession no. JIFU01000001) (100% identity), (ii) contig 2 of Streptococcus sanguinis SK1056 (accession no. AFFLO1000002) (99% identity), and (iii) contig 8 of S. pneumoniae GA02254 (accession no. AIKJ01000009) (99% identity). These findings show that ICESpy009 is shared by at least three different streptococcal species.

Detection of ICESpy009 and integration sites in S. pyogenes clinical isolates. PCR mapping targeting ICESpy009 revealed that an ICESpy009-like genetic element was present in 5 of the 12 S. pyogenes clinical isolates under study, besides MB56Spyo009. In two isolates (MB56Spyo019 and MB56Spyo023), the ICESpy009-like element was found to be integrated into the same chromosomal site (hylA) as the ICE identified in MB56Spyo009, while in two other isolates (MB56Spyo021 and MB56Spyo024), the ICESpy009-like element was found to be integrated at the 3′ end of the rplL gene. For one strain (MB56Spyo025), the integration site remained undefined (Table 1).

DISCUSSION

The mef(E) gene is rare in S. pyogenes, as previously reported for a global collection where only 21 of 28,892 clinical isolates were mef(E) positive (9). To define the genetic element carrying mef(E) in S. pyogenes, in the current study we analyzed 12 isolates selected from the 21 previously reported isolates, excluding the isolates carrying mef(E) in association with tet(M), previously characterized (10).

We confirmed that in these isolates, mef(E) and mel, encoding the two components of the macrolide efflux system, were carried by the well-known mega element. Sequencing of one of the isolates, MB56Spyo009, revealed that mega was included in a novel genetic context, integrated inside an ICE (ICESpy009) belonging to the ICESa2603 family (http://db-mml.sjtu.edu.cn/ICEberg/).

So far, all the composite genetic elements comprising mega had belonged to the Tn916 family. These included Tn2009 (11), carrying the resistance determinants mef(E)/mel and tet(M), and Tn2010 (29) and Tn2017 (30), both carrying ermm(B) in addition to mef(E)/mel and tet(M). Among these elements, Tn2009 was also detected in S. pyogenes (10). In both S. pneumoniae and S. pyogenes, Tn2009 appeared to be incapable of conjugative transfer.

In this study, we found that ICESpy009 of MB56Spyo009 was transferred by conjugation from S. pyogenes to both S. pyogenes and S. pneumoniae recipients, representing the first evidence of conjugative mobilization of mega from S. pyogenes. This feature is probably due to the nature of ICESpy009, a composite element belonging to the ICESa2603 family, which includes ICEs identified in different streptococcal species, including Streptococcus agalactiae, Streptococcus dysgalactiae subsp. equisimilis, S. suis, Streptococcus pneumoniae, and Streptococcus thermophilus, that are able to undergo horizontal transmission among streptococci (31, 32).

It is conceivable that the backbone of this ICE family is composed of efficient modules that allow the mobility of the genetic elements carrying them. The ORFs contained in the conjugation module of the ICESa2603 family are similar to those found in another important ICE family, the Tn5253 family. Genetic elements of the Tn5253 family are widely distributed among streptococci and are generally transferable (13, 33–35). Functional and successful modules shared between these two different ICE families may be responsible for the conjugative transfer ability observed for both.

In ICESpy009, ORFs encoding putative aminoglycoside phosphotransferases and ABC transporters are located downstream of mega. Although the functions of these putative ORFs remain to be elucidated, this region may represent a novel accessory resistance module among those already described for the ICESa2603 family (36).

By PCR mapping, we identified an ICESpy009-like element in 5 other isolates under study, indicating that this genetic context of mega is not unique to MB56Spyo009. As a further confirmation, a genetic element identical to ICESpy009 is present in the unfinished genome of S. pyogenes ABC020015285.

In MB56Spyo009, S. pyogenes ABC020015285, and 2 other isolates under study, the ICESpy009 element was found to be integrated into hylA. This gene encodes hyaluronidase, the enzyme that cleaves the hyaluronic acid (HA) of bacterial capsule and mammalian tissue and permits bacteria to utilize HA as an energy source (37). This represents a novel integration site for an ICE of the ICESa2603 family, for which the most common integration site is the rplL gene (36), and it adds to the list of sites where ICEs encoding tyrosine recombinases can be integrated (23). hylA can represent an alternative integration site for ICESpy009 when rplL is occupied by another integrated element, as in the case of MB56Spyo009. Interestingly, in the S. pyogenes and S. pneumoniae transconjugants and in two S. pyogenes clinical isolates, ICEESpy009 was found to be integrated at the common integration site, i.e., rplL.

In addition to S. pyogenes, sequences identical to ICESpy009 are also present in the genomes of two strains of other streptococcal species, S. sanguinis SK1056 and S. pneumoniae GA02254, the latter of which belongs to a group of 120 recently sequenced pneumococci (35). The presence of ICESpy009 in different streptococcal species, the evidence of its transferability between S. pyogenes and S. pneumoniae, and the transferability of the ICESa2603 fami-
ily members among different species of streptococci suggest that this genetic element, although rare, has the potential for dissemination within the genus Streptococcus.

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