Group A streptococci (GAS; strains of *Streptococcus pyogenes*) can cause both mild throat and skin infections and life-threatening invasive diseases such as puerperal fever, necrotizing fasciitis, and streptococcal toxic shock syndrome (STSS) (5). The incidence of invasive GAS infections has been increasing worldwide since the mid-1980s (6). Musser et al. have shown that most cases are due to an emerging clone of GAS strains causing invasive disease in France (4, 9). Serotype *emm* 1 type (11), which accounts for one-third of GAS strains belonging to this clone have resisted extensive molecular subtyping by means of standard techniques like random amplified polymorphic DNA analysis, ribotyping, multilocus enzyme electrophoresis, and pulsed-field gel electrophoresis (8). Perea Mejia et al., studying *M/emm* 1 GAS strains recovered from Mexican children with pharyngitis, showed that the *sic* gene, which encodes a complement-inhibiting protein described by Akesson et al., is unexpectedly variable in this highly clonal group (1, 12). Hoe et al. then successfully used *sic* sequencing to discriminate among *M/emm* 1 GAS strains causing invasive infections in Texas (8). As *M/emm* 1 GAS strains carrying *sic* genes are highly prevalent among invasive GAS isolates in France, notably representing about 40% of pediatric isolates, we sequenced the *sic* genes of isolates recovered in four case clusters by comparison with unrelated strains randomly selected from our collection of invasive *M/emm* 1 GAS strains in order to assess the usefulness of this approach for subtyping of *M/emm* 1 isolates in France.

All 47 clinical isolates collected throughout France from November 2006 through February 2009 were sent to the reference center for streptococci (Table 1). *M/emm* 1 type determination had been performed previously by the method of Beall et al. (2). Nine isolates belonged to four clinical clusters (A, B, C, and D), consisting of three familial clusters corresponding to two cases each (in children and adults with invasive diseases) and one cluster corresponding to three cases of endometritis in a maternity unit. The other isolates, used for comparison, were collected throughout France from 38 unrelated patients with invasive diseases. Multiplex PCR analysis of five toxin-encoding genes (*speA*, *speB*, *speC*, *smez-1*, and *ssa*) and the *sic* gene was performed as described previously (4, 10). The *sic* gene was sequenced with the primer pair sic-SEQ-RDB-F (5′-AGG TTA AGG AGA GGT CAC AAA CTA-3′) and sic-SEQ-RDB-R (5′-GTT GCT GAT GGT GTA TAT GGT GT-3′), yielding a product of 1,038 bp from *M*1 reference strain SF370 (accession number AE004092). Both strands of PCR products were sequenced by using the PCR primers and an ABI 3730xl DNA analyzer. The sequences were compared with those in the NCBI GenBank database by using the BLAST program. An allele number was attributed if the sequence was 100% identical throughout its entire length to one of the allele sequences submitted by Hoe et al. (8). Otherwise, the sequence was considered to represent a new allele and given the designation *sicND* followed by the closest allele number and a lowercase letter if several sequences were close to the same allele. The ClustalW algorithm was used to generate a dendrogram (Fig. 1). Recently, multilocus sequence typing (MLST) has been applied to strain typing of numerous bacterial species, including *S. pyogenes* (3, 7). We have checked whether this method could be used to resolve the four clusters of cases. The nine *M/emm* 1 strains belonging to the four clusters were analyzed using the MLST method of Enright et al. (http://spyogenes.mlst.net/misc/info.asp) (7) with modification of the *yqiL*-up primer sequence to 5′-TGCAACAGTAT GGACTGACCAGA-3′ according to BLAST homologies in the GenBank database.

The 47 isolates shared the *speB* and *sic* genes and the *smez-1* allele but differed by the presence of the *speA* and *speC* genes (in 94 and 32% of isolates, respectively) (Table 1). Twenty *sic* gene sequences were identified, of which six corresponded to GenBank alleles. Allele *sic1.02* was the most frequent, accounting for 32% of isolates. Twenty-four genotypes were obtained by combining the toxin gene profiles and the *sic* sequences. The most frequent genotype—the presence of allele *sic1.02* and the *speA* gene without *speC*—represented 19% of the isolates. We found no correlation between the *sic* allele or the genotype and the regional or clinical origin of the isolate (Table 1).

We were also able to resolve the four clusters. Cluster A (throat isolates SP159 and SP160) involved two brothers aged...

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**Subtyping of *emm* 1 Group A Streptococci Causing Invasive Infections in France**

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By combining PCR amplification of toxin-encoding genes and *sic* gene sequencing, we distinguished 24 genotypes among 47 *M/emm* 1 group A streptococci isolated from children and adults in France in 9 cases of infection comprising four clusters and 38 unrelated invasive infection cases used as controls.
4 and 8 years, the youngest of whom died of STSS. Both isolates possessed allele sic1.78, which was not found in any of the unrelated isolates. Cluster B (isolates SP294 and SP295) corresponded to a case of bacteremic pleural empyema in a 34-year-old mother and a case of uncomplicated pharyngitis in her 1-year-old son. The two isolates shared the same genotype, characterized by the presence of speA, the absence of speC, and the same sic sequence, designated sicND-1.02a, which had no corresponding GenBank allele but was close to allele sic1.02 (Table 1). Only two of the 38 unrelated strains had this genotype. Cluster C isolates (SP309 and SP316), which corresponded to two cases of STSS with bacteremia, in a 21-year-old mother and her 1-day-old baby, carried the most frequent genotype (the presence of allele sic1.02 and the speA gene without speC), accounting for 19% of the isolates. Finally, cluster D corresponded to three cases of endometritis that occurred in a maternity unit over a 5-month period. The first was caused by an M/emm28 isolate, and the other two were caused by M/emm1 isolates (SP410 and SP440). An M/emm1 strain (SP412) was also isolated from the throat of a maternity nurse who had provided postpartum care to one of the affected women (the patient infected with isolate SP410). The two M/emm1 endometritis isolates differed by their sic alleles (sic1.117 and sic1.02, respectively), ruling out cross-infection. Thus, the three cases of endometritis that occurred during this 5-month period were unrelated. Interestingly, endometritis isolate SP410 had the same genotype (the presence of allele sic1.117 and the speA gene without speC) as the isolate from the nurse in charge of the infected patient (SP412), and this genotype was not encountered among the 38 unrelated control strains. This GAS endometritis strain was therefore likely to have been acquired in the hospital. Using the MLST method, we found that all the M/emm1 isolates in the four clusters shared the same alleles for the seven MLST genes tested and
belonged to sequence type ST28. Thus, we could not discriminate among these strains by MLST.

In conclusion, by using a combination of toxin gene profiling and sic gene sequencing, we found that French serotype M1 GAS isolates display a level of polymorphism similar to that observed in other parts of the world (8, 12, 13). In total, we identified 24 genotypes among 47 isolates. We were also able to resolve four case clusters. In our study, the MLST method...
failed to discriminate among the four clusters of cases since all the isolates tested belonged to the same sequence type, ST28. Use of this method for subtyping M/emm1 isolates of cluster D would have led to the erroneous conclusion that two cases of endometritis (caused by strains SP410 and SP440) were related and that patient-to-patient cross contamination had occurred. Indeed, according to the MLST database, ST28 represents about 94% of emm1 isolates, highlighting the clonal origin of most invasive M/emm1 GAS as shown previously by other typing methods (8, 11). Moreover, this method remains expensive and time-consuming. Thus, we think that sic sequencing coupled with toxin gene detection offers a more convenient solution for M/emm1 strain subtyping. However, the frequency of one genotype, representing 19% of M/emm1 GAS isolates in France, still hampers discrimination among invasive disease clusters.

Comparative genomic studies of several M1 GAS strains should help in the discovery of other polymorphisms useful for subtyping of strains belonging to this globally distributed clone.

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