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Molecular Characterization of *Streptococcus agalactiae* Isolates Harboring Small *erm*(T)-Carrying Plasmids

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Among 1,827 group B *Streptococcus* (GBS) strains collected between 2006 and 2013 by the French National Reference Center for Streptococci, 490 (26.8%) strains were erythromycin resistant. The *erm*(T) resistance gene was found in six strains belonging to capsular polysaccharides Ia, III, and V and was carried by the same mobilizable plasmid, which could be efficiently transferred by mobilization to GBS and *Enterococcus faecalis* recipients, thus promoting a broad dissemination of *erm*(T).

Streptococcus agalactiae (group B *Streptococcus* [GBS]) is responsible for serious infections in children and adults (1, 2). Although penicillins remain the drugs of choice for GBS infections, macrolides represent the main alternative, especially in patients presenting penicillin allergy. Increasing macrolide resistance among GBS is a major concern worldwide (1, 3) and may have clinical consequences when this antibiotic is given empirically. Macrolide resistance in streptococci is mainly due to target site methylation and/or efflux pumps encoded by *erm* and/or *mef* genes, respectively (4). Most methylases encountered in streptococci are encoded by *erm*(A) and *erm*(B) and confer an MLS_B phenotype leading to macrolides, lincosamides, and streptogramins B cross-resistance expressed either constitutively ($cMLS_B$) or inducibly ($iMLS_B$). Efflux pumps are encoded by *mef*(A) and *mef*(E) and confer low-level resistance to 14- and 15-membered macrolides (M phenotype). Among the 36 *erm* alleles currently described (http://faculty.washington.edu/marilynr/ermweb1.pdf), only *erm*(A), *erm*(B), *erm*(F), *erm*(Q), and *erm*(T) genes have been found in GBS (4–7). The *erm*(T) gene was first described to occur in *Lactobacillus reuteri* in 1994 (8) and was thereafter described to occur in a large range of species, including *Streptococcus pyogenes* (group A *Streptococcus* [GAS]), GBS, *Streptococcus dysgalactiae* subsp. *equisimilis*, *Streptococcus bovis*, *Streptococcus pasteurianus*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Haemophilus parasuis* (5, 9–16), where it usually confers an $iMLS_B$ phenotype. Contrary to *erm*(A) and *erm*(B), which are mostly carried by chromosome-borne transposons or integrative and conjugative elements (ICE) (9, 17), *erm*(T) is mainly plasmid borne, although it is carried by a chromosome-borne IS*1216*V-based transposon in six *S. pasteurianus* isolates (15). *erm*(T) was recently described to occur on similar small, mobilizable, broad-host-range plasmids in GBS and GAS isolates from the United States (10, 18) and in an *S. dysgalactiae* subsp. *equisimilis* isolate from Italy (9).

In the present study, we evaluated the prevalence of *erm*(T) in GBS strains collected over an 8-year period and we characterized its molecular support.

A total of 1,827 nonredundant GBS isolates were received by the French National Reference Center for Streptococci between 2006 and 2013. Antibiotic resistance was determined by disc diffusion and Etest methods, according to EUCAST guidelines (http://www.eucast.org/antimicrobial_susceptibility_testing/). A double-disc agar diffusion test (D-test) was performed by placing erythromycin (ERY) and clindamycin (CLI) 12 mm apart edge to edge to detect the $iMLS_B$ phenotype. Nonsusceptible GBS strains were screened by PCR for the presence of macrolide resistance genes *erm*(A), *erm*(B), *erm*(T), and *mef*(A/E) and the lincosamide resistance genes *lnu*(B) and *lsa*(C); the genes conferring tetracycline resistance were also characterized in all strains (19, 20). The primers used in this study are reported in Table S1 in the supplemental material. All PCR amplification products obtained from *erm*(T)-resistant isolates were subjected to bidirectional DNA sequencing. GBS plasmid DNA was purified using a Qiagen miniprep kit with previously described modifications (10) and was sequenced using primer walking. DNA database searches were carried out using GenBank BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Molecular capsular polysaccharide (CPS) typing was performed on all strains (21), and *erm*(T)-positive strains were typed by multilocus sequence typing (MLST) (22). Mating experiments were performed as previously described, using rifampin- and fucidin acid-resistant GBS BM132 and *Enterococcus faecalis* JH2-2 as recipient cells (23). Transconjugants were selected on Todd-Hewitt medium containing erythromycin (10 μg/ml), rifampin (20 μg/ml), and fucidic acid (10 μg/ml).

Erythromycin resistance was observed in 26.8% ($n = 490$) of the GBS isolates (Table 1), and the distributions of the $cMLS_B$, $iMLS_B$, and M phenotypes were 64% ($n = 316$), 17% ($n = 84$), and 18% ($n = 89$), respectively. Isolated resistance to clindamycin was observed in only three strains (0.7%), two of which harbored the

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TABLE 1 Macrolide-lincosamide-streptogramin resistance genes recovered from *Streptococcus agalactiae* strains according to their phenotypic patterns

Phenotypic pattern	No. of strains (%)	No. of strains with indicated resistance gene(s) (%)								
		erm(A)	*erm*(B)	*erm*(T)	*mef*(A)	*lnu*(B)	*lsa*(C)	*erm*(A) + *erm*(B)	*erm*(B) + *mef*(A)	*lnu*(B) + *mef*(A)
$iMLS_B$	84 (17)	71 (84.5)	7 (8.3)	6 (7.2)						
$cMLS_B$	316 (64)	83 (26.3)	230 (72.8)					1 (0.3)	2 (0.6)	
M	89 (18)				89 (100)					
M + Cli^r	1 (0.3)									1 (100)
Cli^r	3 (0.7)					1 (33.3)	2 (66.7)			
Total	493 (100)	154 (31.2)	237 (48.1)	6 (1.2)	89 (18.1)	1 (0.2)	2 (0.4)	1 (0.2)	2 (0.4)	1 (0.2)

lnu(B) gene, encoding a lincosamide nucleotidyltransferase recently described to occur in GBS isolates from European countries (24). However, to our knowledge, this is the first description of clindamycin-resistant GBS mediated by the *lnu*(B) gene in France. The distributions of the macrolide resistance genes and of the phenotypic patterns are detailed in Table 1. MLS resistance was more frequently encountered (*P* value, $<10^{-5}$; Fisher exact test) in CPS type V strains (59.6%), while the *mef*(A) gene was correlated (*P* value, $<10^{-5}$; Fisher exact test) with CPS type Ia (83.1%) (see Table S2 in the supplemental material). Tetracycline resistance was detected in 1,543 (84.5%) strains. The resistance determinants were *tet*(M) (1,443/1,543, 93.5%), *tet*(O) (70/1,543, 4.5%), *tet*(M) plus *tet*(O) (13/1,543, 0.8%), *tet*(M) plus *tet*(L) (10/1,543, 0.6%), *tet*(L) plus *tet*(O) (4/1,543, 0.3%), and *tet*(K) (1/1,543, 0.1%). Two tetracycline-resistant strains were negative for the *tet*(K), *tet*(L), *tet*(M), and *tet*(O) genes. Forty-four strains were phenotypically susceptible to tetracycline, although they were positive for *tet*(M) (40 strains) and *tet*(O) (4 strains) sequences. A similar observation was made for *Streptococcus pneumoniae*, where *tet*(M) pseudogenes resulting from frameshift mutations and encoding inactive truncated Tet(M) proteins have been described to occur (25). In addition, all 1,827 strains were susceptible to amoxicillin and glycopeptides. Kanamycin and gentamicin resistance represented 3.7% and 1% of the strains, respectively.

The *erm*(T) gene was detected in six strains responsible for invasive infections (for adults, $n = 2$; for infants, $n = 4$) (Table 2), all exhibiting an $iMLS_B$ phenotype. All strains from infants were CPS type III and belonged to hypervirulent GBS clonal complex 17 (CC17), a clone associated with neonatal invasive infections (26). The two remaining strains from adults were CPS types Ia and V. These results indicate that the population of GBS isolates containing *erm*(T) is associated with various genetic backgrounds. Previous studies also reported heterogeneous populations of *erm*(T)-carrying GBS isolates (5, 10); conversely, clonal spread of *erm*(T)-carrying GAS isolates has been described (10). All six *erm*(T)-positive strains likely contained a plasmid harboring the *erm*(T) gene, as suggested by the sequence analysis of a 4,770-bp PCR fragment obtained using *erm*(T) divergent primers (see Fig. S1 in the supplemental material). To complete this analysis, we purified and sequenced the *erm*(T)-carrying plasmid from strain CCH20130208. This 4,972-bp plasmid, designated pCCH208 (GenBank accession no. KJ778678), has an average G+C content of 37.3% and carries the replication initiation protein gene *rep*, the relaxase gene *mob*, and *erm*(T), preceded by the gene encoding its leader peptide (Fig. S2). BLAST analysis revealed that the highest similarity score (100% coverage and 99% identity) was obtained with (i) plasmids pRW35 (GenBank accession no. EU192194) and pGA2000 (GenBank accession no. JF308631) from GAS strains from the United States (10, 18), (ii) plasmids pGB2001 (GenBank accession no. JF308630) and pGB2002 (GenBank accession no. JF308629) from GBS strains from the United States (10), and (iii) plasmid p5580 (GenBank accession no. HE862394) from an *S. dysgalactiae* subsp. *equisimilis* isolate from Italy (9). The *rep*, *mob*, and *erm*(T) genes from pCCH208 and these five plasmids exhibited 100% sequence identity.

Importantly, we demonstrated that all six *erm*(T) GBS strains could transfer *erm*(T) to *E. faecalis* JH2-2 in mating experiments but that only five were donor proficient when GBS BM132 was used as a recipient strain (Table 2). Plasmid transfer from the GBS CCH209800700 donor to the GBS BM132 recipient was repeatedly never observed with the use of different mating conditions. All *erm*(T) transconjugants exhibited the $iMLS_B$ phenotype of the parental strains, with an erythromycin MIC of >256 μg/ml and a clindamycin MIC ranging from 0.047 to 0.064 μg/ml in the GBS BM132 genetic background. The average transfer frequency obtained was 3.5×10^{-6} transconjugants per recipient, a value slightly inferior to that (5×10^{-5} transconjugants per recipient) observed by Palmieri et al. (9) with an *erm*(T)-positive *S. dysgalactiae* subsp. *equisimilis* donor. In their study, the *erm*(T) plasmid was

TABLE 2 Characteristics of *erm*(T)-positive *Streptococcus agalactiae* isolates[a]

Strain	Yr of isolation	Age of patient	Site of isolation	MLST	CPS type	Transfer to *S. agalactiae* BM132
CCH209800120	2009	1 day	Blood culture	ST17	III	+
CCH209800250	2009	23 days	Blood culture	ST17	III	+
CCH209800700	2009	63 yr	Blood culture	ST1	V	−
CCH20130208	2013	76 yr	Cutaneous abscess	ST88	Ia	+
CCH20131128	2013	1 day	Blood culture	ST17	III	+
CCH20131153	2013	30 days	CSF	ST17	III	+

[a] The MICs of erythromycin and clindamycin for all six strains were >256 and 0.047 μg/ml, respectively. CCH209800250 exhibited kanamycin resistance. All six strains could transfer *erm*(T) to *E. faecalis* JH2-2. CSF, cerebrospinal fluid; ST, sequence type; CPS, capsular polysaccharide.

mobilized in *trans* by a coresident ICE belonging to the ICE*Sa2603* family. However, we failed to detect the presence of these elements in our *erm*(T) GBS donors with the use of a PCR assay targeting their specific integrase gene. It is, however, conceivable that in our GBS strains, *erm*(T) mobilization was mediated by an ICE belonging to another family but encoding proteins for related conjugative functions. Although nonconjugative, this plasmid could be efficiently transferred by mobilization to GBS and *E. faecalis* recipients, thus promoting a broad dissemination of *erm*(T). Microbiologists should be aware of the possible presence of the *erm*(T) gene in GBS when confronted with an iMLS$_B$ phenotype that could not be due to the presence of *erm*(A) or *erm*(B) determinants.

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