ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2005, p. 5069–5074 Vol. 49, No. 12
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.12.5069–5074.2005

Erythromycin Resistance and Genetic Elements Carrying Macrolide Efflux Genes in *Streptococcus agalactiae*

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Received 26 July 2005/Returned for modification 8 September 2005/Accepted 4 October 2005

The macrolide resistance determinants and genetic elements carrying the *mef*(A) and *mef*(E) subclasses of the *mef* gene were studied with *Streptococcus agalactiae* isolated in 2003 and 2004 from 7,084 vaginorectal cultures performed to detect carrier pregnant women. The prevalence of carriage was 18% (1,276 isolates), and that of erythromycin resistance 11.0% (129 of the 1,171 isolates studied). *erm*(B), *erm*(A) subclass *erm*(TR), and the *mef* gene, either subclass *mef*(A) or *mef*(E), were found in 72 (55.8%), 41 (31.8%), and 12 (9.3%) erythromycin-resistant isolates, while 4 isolates had more than 1 erythromycin resistance gene. Of the 13 M-phenotype *mef*-containing erythromycin-resistant *S. agalactiae* isolates, 11 had the *mef*(E) subclass gene alone, one had both the *mef*(E) and the *erm*(TR) subclass genes, and one had the *mef*(A) subclass gene. *mef*(E) subclass genes were associated with the carrying element mega in 10 of the 12 *mef*(E)-containing strains, while the single *mef*(A) subclass gene found was associated with the genetic element Tn*1207.3*. The nonconjugative nature of the mega element and the clonal diversity of *mef*(E)-containing strains determined by pulsed-field gel electrophoresis suggest that transformation is the main mechanism through which this resistance gene is acquired.

Streptococcus agalactiae (Lancefield group B *Streptococcus*) is a commensal bacterium of the human digestive and genital tracts and remains an important cause of perinatal morbidity and mortality. Prevention strategies based on the detection of vaginal and rectal colonization with *S. agalactiae* followed by intrapartum administration of antibiotics to pregnant women have been demonstrated to reduce early-onset neonatal infection in the newborn (29, 32). *S. agalactiae* also causes bacteremia and skin and soft tissue infections in adults, as well as other less-common but severe infections, such as endocarditis and meningitis (15, 30).

Due to the uniform susceptibility of *S. agalactiae*, penicillin and ampicillin are currently the drugs of choice for the treatment and intrapartum prevention of neonatal infections, while clindamycin and erythromycin are recommended alternatives in cases of penicillin allergy (29, 32). There is concern about the increase of macrolide and clindamycin resistance in *S. agalactiae* associated in some countries with increases in macrolide usage (10). In most recent studies performed in Spain and other countries, erythromycin resistance rates ranged between 10% and 20% (6, 10, 15).

Among the various mechanisms of macrolide resistance found in streptococci (20), the most frequently found in human isolates are methylation of streptococcal 23S rRNA at the erythromycin-binding nucleotide target due to *erm* genes (erythromycin ribosome methylase) and a proton-dependent efflux of the drug mediated by the *mef* genes (macrolide efflux) (13, 17). The presence of the *erm* genes results in the macrolide-lincosamide-streptogramin B resistance phenotype, which may be constitutively or inducibly expressed. In *S. agalactiae*, *erm*(B) and *erm*(A) subclass *erm*(TR) genes are nearly always responsible for the macrolide-lincosamide-streptogramin B phenotype of macrolide resistance (6, 10, 15). Erythromycin resistance in streptococci mediated by the *mef* genes confers resistance only to 14- and 15-membered-ring macrolides, resulting in the M phenotype of resistance (20, 33). The *mef*(A) subclass gene was initially identified in *Streptococcus pyogenes* and the *mef*(E) subclass gene in *Streptococcus pneumoniae*, and these two subclass genes are 90% identical. Both the *mef*(A) and *mef*(E) gene subclasses have been described for erythromycin-resistant *S. agalactiae* clinical isolates (3).

In other streptococcal species, the *mef*(A) subclass gene is carried in the defective transposon Tn*1207.1* and *mef*(E) in mega (macrolide efflux genetic assembly) (18, 26, 28). In *S. pneumoniae*, mega can be inserted at different sites in the chromosome or into a transposon Tn*916*-like genetic element, forming a new composite named Tn*2009*, which also contains the tetracycline resistance *tet*(M) gene (12, 26). Tn*1207.1* has been found in *S. pneumoniae* inserted within the competence *celB* gene (11, 28), and in *S. pyogenes*, Tn*1207.1* has been described as integrated into the conjugative transposon Tn*1207.3* and into a conjugative *tet*(O)-*mef*(A) element (4, 7, 9, 26).

The primary aim of the present study was to determine the macrolide resistance genes responsible for erythromycin resistance in *S. agalactiae* isolated from pregnant women and to study the genetic elements carrying the *mef* genes in the 13 M-phenotype erythromycin-resistant *S. agalactiae* strains found. As a secondary aim, the presence of *tet*(M) and other specific genes of the newly described transposon Tn*2009* was also investigated to determine whether the ge-

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TABLE 1. Primers used for detection of erythromycin resistance determinants and genetic carrying elements in *Streptococcus agalactiae* strains

Primer group or target gene	Forward and reverse primers (5′–3′)	Amplification size (bp)	Nucleotide positions for genetic element analyzed[b]			
			Tn*1207.3* (AY657002)	Mega (AF274302)	Tn*916* (U09422)	Tn*2009* (AF376746)
erm(B)[a]	ATTGGAACAGGTAAAGGGC	442				
	GAACATCTGTGGTATGGCG					
erm(TR)	AACTTGTGGAAATGAGTCAACGG	375				
	CAGAATCTACATTAGGCTTAGGG					
Primers for analysis of Tn*1207.3* and mega						
mef(A), *mef*(E)	ATGGAAAAATACAACAATTGG	1,218	3271–3291	1125–1145		1372–1392
	TTATTTTAAATCTAATTTTCTAA(C/T)C		4464–4488	2318–2342		2565–2589
matA, *mel*[a]	TGCCTATATTCCCCAGTT	708	4808–4825	2662–2679		2909–2926
	TAATTTCCGCACCGACTA		5497–5514	3351–3368		3598–3615
orf8, *orf5*	ATGACCGCCGATAAGTTTACTAC	291	6870–6892	5074–5094		5319–5339
	GACCCAGCAAATCTTCCAAAAAC		7138–7160	5342–5364		5586–5608
orf9	ATGACCGCCGATAAGTTTACTAC	699	6870–6892			
	GTATGGGCCATTCCAAAACTAAC		7546–7568			
orf56	ATATGCGAGGGTATCTGATAGTC	495	49036–49058			
	GGCTCAATGACATAATTCTCACC		49508–49530			
Primers for analysis of mega and Tn*2009*						
orf7	TCAATTTATCAATAAATGAAATGAG	205		516–540		781–805
	ATACTTCCAATTCGATGCCAG			700–720		964–984
intTn	GGTCTTCGTATTTCAGAGTTTGG	473			17307–17329	
	GTTGCATGTGCGTAATAGTTCAG				17766–17788	
orf24	TCAAAAGGTCGTTCCCCACTC	344			260–280	
	ACGAGTGCCGATTTTGTAGCC				582–602	
orf5-orf6-tet(M)	ORF5-F: TAATACAAGTACATGACTCACC	585		5316–5337		5561–5582
	TetM-R: TCCTAAATATTGTGCGAACATC					6123–6144

[a] From reference 1.
[b] GenBank accession numbers are given in parentheses.

netic elements carrying the *mef*(E) gene were integrated into this transposon.

MATERIALS AND METHODS

Bacterial isolates. Throughout 2003 and 2004, all pregnant women in San Sebastián, Basque Country (Spain), were screened at weeks 35 to 37 for vaginorectal carriage of *S. agalactiae* using the selective and differential "Granada medium" (27). Duplicate specimens were not included in the study. The characteristic red-orange colonies produced by *S. agalactiae* in Granada medium were tested for erythromycin susceptibility by the disk diffusion method according to CLSI (formerly NCCLS) methods and criteria (24). MICs of erythromycin and other antimicrobial agents were determined by the broth microdilution method using Sensititre microtiter trays (Sensititre; Trek Diagnostics Systems, West Sussex, England) and cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood and were interpreted according to the CLSI criteria (23).

Serotyping was performed by coagglutination according to the manufacturer's instructions (ESSUM group B *Streptococcus* serotyping test; Bacterum AB, Umeå, Sweden).

PCR. The presence of the erythromycin resistance genes *erm*(B), *erm*(A) subclass *erm*(TR), and *mef* was studied with erythromycin-resistant isolates by PCR (Table 1). *S. pneumoniae* ATCC 700676 and ATCC 700677 were used as PCR-positive controls for the *mef* and *erm*(B) genes, respectively. *S. agalactiae* clinical isolate B222703 was used as a positive control in the PCR used to detect the *erm*(A) subclass *erm*(TR) gene. The presence of the *erm*(TR) subclass gene in the control isolate B222703 was confirmed after sequencing both strands of the amplification product with the same primers used for the amplification.

For *mef*-containing strains, another PCR was performed to study the presence of the *msr*(A) homologue, a gene encoding a protein that mediates resistance to macrolides and streptogramin B in staphylococci (18). These homologues are named *matA* and *mel* according to the names provided in GenBank and in other streptococcal species are located downstream of *mef*(A) and *mef*(E), respectively (Fig. 1). After DNA extraction using QIAamp spin columns (QIAGEN, Chatsworth, CA), amplification was performed using the specific primers described in Table 1. PCRs were performed at a final volume of 50 μl using approximately 30 to 50 ng of genomic DNA as a template, 1 U of Ampli*Taq* Gold DNA polymerase (Roche, Branchburg, NJ), 200 μM deoxynucleoside triphosphates, 1× PCR buffer, 3 mM $MgCl_2$, and 100 ng of each primer in a GeneAmp PCR system 2700 thermocycler (PE Applied Biosystems, Foster City, California). PCR conditions for amplification of the these four genes comprised an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 1 min. A final elongation step at 72°C for 10 min was carried out after amplification cycles.

On *mef*-containing isolates, distinct regions of the genetic elements carrying the *mef*(A) and *mef*(E) genes were studied by means of PCR. The presence of transposon Tn*1207.3* was studied using primers that hybridized with its *orf8*, *orf9*, and *orf56* genes. *orf8* is also present in Tn*1207.1*, but *orf9* and *orf56* are exclusive to transposon Tn*1207.3* (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its *orf5* and *orf7* genes on the basis of the *S. pneumoniae* sequences of mega and Tn*2009*. To study whether mega was inserted into a transposon Tn*916*-like element, a PCR with two specific sets of primers that hybridized with the *intTn* (the characteristic integrase gene of transposons of the Tn*916*-Tn*1545* family) and *orf24* genes from the Tn*916* family was performed. With strains showing these genes, another PCR was performed with primers that hybridized the 3′-end sequence of mega (*orf5* gene) and the 5′-end sequence of *tet*(M) on the basis of the transposon Tn*2009* sequence (Fig. 1). This PCR amplifies a 585-bp fragment of DNA including fragments of the *orf5*, *orf6*, and *tet*(M) genes, a fragment that in this study was arbitrarily named "*orf5-orf6-tet*(M)." The conditions for all of these last PCRs included 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C or 65°C for 30 s, and elongation at 72°C for 1 min.

The presence of the *tet*(M) and *tet*(O) genes of tetracycline resistance was determined using previously described primers and conditions (14, 25).

A previously described *S. pneumoniae* E-1824-J erythromycin-resistant clinical isolate (22) was used as a positive control for the PCRs detecting the *orf5* and

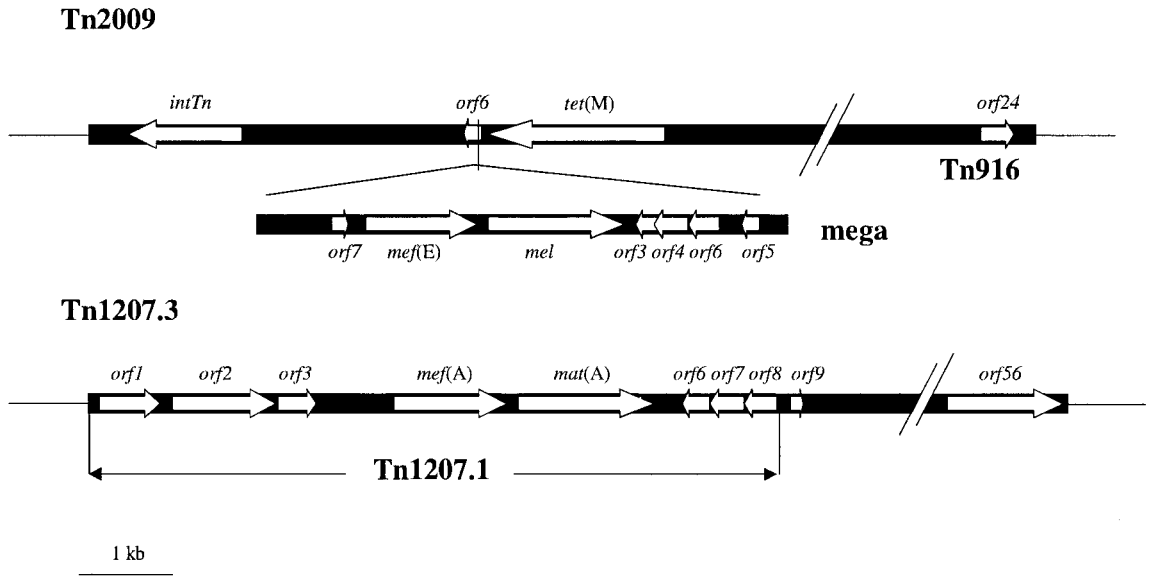

FIG. 1. Schematic representation of mega inserted into Tn*2009* and of Tn*1207.1* integrated into Tn*1207.3*, adapted from references 12 and 26, respectively. Relevant ORFs, to which PCRs were performed in each genetic element (see Table 1), are represented by white arrows.

orf7 genes from mega, the *intTn* and *orf24* genes of the Tn*916* family, and the *orf5-orf6-tet*(M) fragment of Tn*2009*.

When the PCR products yielded the expected size, the specificities of the amplicons obtained were assessed by sequencing, using the same primers as those used for amplification. The sequences were then compared with the *S. pyogenes* sequence of Tn*1207.3* (GenBank accession number AY657002), with the *S. pneumoniae* sequences of Tn*1207.1* (GenBank accession number AF227520), mega (GenBank accession number AF274302), and Tn*2009* (GenBank accession number AF376746), and with the *Enterococcus faecalis* sequence of Tn*916* (GenBank accession number U09422) using the BLAST software available at the web site of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed on *mef*(E)-containing isolates as described previously in protocols (5) with minor modifications. Briefly, genomic DNA was extracted in agarose plugs after incubation with lysozyme, mutanolysin, and proteinase K. The DNA-containing agar plugs were treated with the restriction endonuclease SmaI. The resultant DNA fragments were separated by PFGE in 1% agarose gels in a CHEF-DRIII system (Bio-Rad, Hercules, Ca) with pulse times increasing from 2 to 30 s over 23 h at a voltage gradient of 6 V/cm. The gels were stained with ethidium bromide, and analysis of SmaI restriction profiles was performed with Diversity Database fingerprinting software version 2 (Bio-Rad). A dendrogram was constructed by the unweighted pair group method with arithmetic averages, the Dice coefficient, and a position tolerance of 1%.

RESULTS

Erythromycin resistance and mechanism of resistance. From January 2003 to December 2004, vaginorectal screening for *S. agalactiae* was performed on 7,084 pregnant women (3,629 in 2003 and 3,455 in 2004), and *S. agalactiae* was detected in 1,276 women, 657 in 2003 and 619 in 2004 (overall prevalence of carriage, 18.0%). As determined by disk diffusion, 129 of the 1,171 *S. agalactiae* isolates available for study (11.0%) were resistant to erythromycin.

Of the 129 erythromycin-resistant *S. agalactiae* isolates, 72 (55.8%) had the *erm*(B) gene, 41 (31.8%) had the *erm*(A) subclass *erm*(TR) gene, 12 (9.3%) had the *mef* gene, 3 (2.3%) had both the *erm*(B) and *erm*(A) subclass *erm*(TR) genes, and 1 (0.8%) isolate had both the *erm*(A) subclass *erm*(TR) and *mef*(E) subclass genes. Of the 12 isolates with only the *mef* gene, 11 had the *mef*(E) subclass gene and one had the *mef*(A) subclass gene. The broth microdilution method showed that all of these 13 *mef*-containing *S. agalactiae* isolates were susceptible to penicillin, trimethoprim-sulfamethoxazole, and rifampin and were resistant to tetracycline (Table 2).

Detection of mega genetic element in *mef*(E)-containing strains. In the 12 erythromycin-resistant *S. agalactiae* strains with the *mef*(E) gene, the *mel* and *orf5* genes from mega were also detected (Table 3). Two strains failed to amplify *orf7* from mega. The sequences of the *mef*(E), *mel*, *orf5*, and *orf7* genes of these *S. agalactiae* strains demonstrated a similarity of >99% to the corresponding sequences of mega and Tn*2009* described for *S. pneumoniae*.

All *mef*(E)-containing isolates were also tetracycline resistant. Of these, 12 had the *tet*(M) gene and one had the *tet*(O) gene. In four of the *tet*(M)-containing strains, the *intTn* and *orf24* genes of the transposon Tn*916* family were detected, but no amplification was obtained with the primers designed to amplify the "*orf5-orf6-tet*(M)" fragment of transposon Tn*2009* that was detected in the *S. pneumoniae* strain used as a control.

Detection of Tn*1207.3* in the *mef*(A)-containing strain. In the single *S. agalactiae* isolate that contained the *mef*(A) subclass gene—strain B222296—the following genes of Tn*1207.3* were detected: *mef*(A), *matA*, *orf8* (encoding an UmuC/MucB-like protein), *orf9* (encoding an unknown product), and *orf56* (encoding a site-specific recombinase). All of these genes demonstrated a similarity of >99% to the sequences of the same corresponding fragments of Tn*1207.3* described for *S. pyogenes* at GenBank. All these sequences were also found in a *mef*(A)-containing *S. pyogenes* clinical isolate used as control for these PCRs (data not shown).

Serotyping and PFGE. Serotyping showed that serotypes 1b and III were the most frequent among *mef*(E)-containing isolates (Table 2). By PFGE, the 11 strains that had the *mef*(E)

TABLE 2. Serotypes and antibiotic MICs of 13 *S. agalactiae* strains with the M phenotype of macrolide resistance isolated from vaginorectally colonized pregnant women

Isolate	Strain no.	Isolation date (mo/day/yr)	Serotype	*mef* gene	MIC (μg/ml) of antibiotic[a]										
					Pen	Amp	Ery	Azi	Cli	Tet	Rif	Chl	Sxt	Cip	Lev
1	B223605	2/07/2003	1a	*mef*(E)	<0.03	<0.25	4	4	<0.25	>4	<1	<4	<0.5/9.5	1	1
2	B220857	6/05/2003	1b	*mef*(E)	<0.03	<0.25	4	4	<0.25	>4	<1	<4	<0.5/9.5	1	1
3	B221331	7/17/2003	1b	*mef*(E)	<0.03	<0.25	8	4	<0.25	>4	<1	<4	<0.5/9.5	1	1
4	B222561	11/20/2003	1b	*mef*(E)	<0.03	<0.25	4	4	<0.25	>4	<1	<4	<0.5/9.5	1	1
5	B222696	12/04/2003	1a	*mef*(A)	<0.03	<0.25	4	4	<0.25	>4	<1	<4	<0.5/9.5	2	2
6	B220756	6/24/2004	III	*mef*(E)	<0.03	<0.25	4	>4	<0.25	>4	<1	>8	<0.5/9.5	1	1
7	B220783	6/28/2004	1b	*mef*(E)	<0.03	<0.25	4	>4	<0.25	>4	<1	<4	<0.5/9.5	2	1
8	B220815	6/30/2004	III	*mef*(E)	<0.03	<0.25	4	>4	<0.25	>4	<1	<4	<0.5/9.5	1	1
9	B220840	7/04/2004	IV	*mef*(E)	<0.03	<0.25	4	>4	<0.25	>4	<1	<4	<0.5/9.5	1	1
10	B220867	7/06/2004	III	*mef*(E)	<0.03	<0.25	4	>4	<0.25	>4	<1	<2	<0.5/9.5	1	1
11	B221495	9/03/2004	II	*mef*(E)	0.06	<0.25	8	4	<0.25	>4	<1	<2	<0.5/9.5	1	1
12	B222129	11/04/2004	V	*mef*(E)	<0.03	<0.25	4	>4	<0.25	>4	<1	<2	<0.5/9.5	1	1
13	B222261	11/18/2004	1b	*mef*(E)	<0.03	<0.25	8	>4	<0.25	>4	<1	<2	<0.5/9.5	<0.5	<0.5

[a] Pen, penicillin; Amp, ampicillin; Ery, erythromycin; Azi, azithromycin; Cli, clindamycin; Tet, tetracycline; Rif, rifampin; Chl, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole; Cip, ciprofloxacin; Lev, levofloxacin.

gene alone showed a different pattern (Fig. 2). Homology among their PFGE patterns was ≤80%.

DISCUSSION

A collection of 1,276 commensal *S. agalactiae* isolates isolated from healthy carrier pregnant women were studied for the prevalence of erythromycin resistance, the presence of the most frequent determinants of macrolide resistance, and the *mef*(A) and *mef*(E) genes carrying genetic elements. Both the prevalence of vaginorectal healthy carriers (18%) and the rate of macrolide-resistant isolates (11%) were in agreement with the results reported in other studies (10, 15, 19). The most frequent gene involved in erythromycin resistance was *erm*(B), followed by *erm*(A) subclass *erm*(TR), while, as found in other studies, the prevalence of *mef*-containing *S. agalactiae* was low (12, 16, 17). The high prevalence of *S. agalactiae* isolates containing the *erm*(B) and *erm*(A) subclass *erm*(TR) genes in carrier pregnant women is of concern, since for these patients preventive treatment with either macrolides or clindamycin could lead to therapeutic failures with a consequent risk of neonatal infection in the newborn.

Using serotyping and PFGE after restriction with SmaI, no homology was found among *mef*(E)-containing strains, suggesting that, as occurs with other gram-positive bacterial species, horizontal transfer may be the main route through which this mechanism of erythromycin resistance is acquired (8, 31). For several bacterial species found in the intestinal tract, including *Enterococcus* spp., viridans group streptococci, and other gram-positive and even gram-negative bacteria, the presence of the *mef* genes has been described (2, 21). These species could act as reservoirs and donors of the *mef* genes of resistance to *S. agalalatiae*.

Amplification by PCR of different open reading frame (ORF) genes of the *mef*(A)- and *mef*(E)-carrying genetic elements was performed to determine whether these elements, which have been found in other streptococci, were present in *S. agalactiae* strains. In the 12 strains with the *mef*(E) subclass gene, *mef*(E) was always associated with the *mel* resistance efflux gene described for mega. In 10 strains, the *orf7* gene,

TABLE 3. PCR amplification of specific DNA fragments and *tet*(M) and *tet*(O) genes in M-phenotype erythromycin-resistant *S. agalactiae* strains

Isolate	Strain no.	Presence of:													
		ORFs of Tn*1207.3*					ORFs of mega				ORFs of Tn*916* and Tn*2009*			*tet*(M)	*tet*(O)
		mef(A)	*matA*	*orf8*	*orf9*	*orf56*	*mef*(E)	*mel*	*orf5*	*orf7*	*intTn*	*orf24*	*orf5-orf6-tet*(M)		
1	B223605	−	−	−	−	−	+	+	+	+	−	−	−	+	−
2	B220857	−	−	−	−	−	+	+	+	+	−	−	−	−	+
3	B221331	−	−	−	−	−	+	+	+	+	−	−	−	+	−
4	B222561	−	−	−	−	−	+	+	+	+	+	+	−	+	−
5	B222696	+	+	+	+	+	−	−	−	−	−	−	−	+	−
6	B220756	−	−	−	−	−	+	+	+	−	+	+	−	+	−
7	B220783	−	−	−	−	−	+	+	+	+	+	−	−	+	−
8	B220815	−	−	−	−	−	+	+	+	+	−	−	−	+	−
9	B220840	−	−	−	−	−	+	+	+	+	−	−	−	+	−
10	B220867	−	−	−	−	−	+	+	+	+	−	−	−	+	−
11	B221495	−	−	−	−	−	+	+	+	+	+	+	−	+	−
12	B222129	−	−	−	−	−	+	+	+	−	−	−	−	+	−
13	B222261	−	−	−	−	−	+	+	+	+	+	+	−	+	−

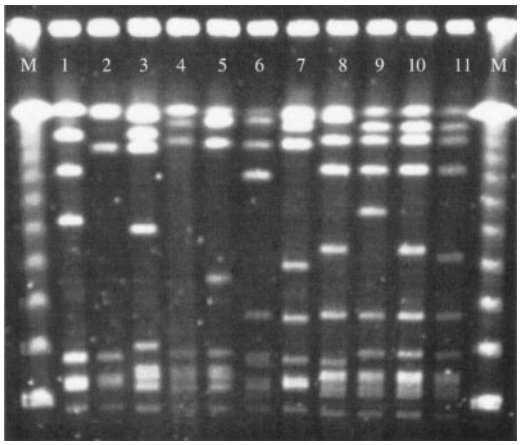

FIG. 2. PFGE after SmaI restriction of *mef*(E) containing *S. agalactiae* isolates. M: molecular weight DNA marker (48.5-Kb Lambda ladder, Amersham Biosciences, Piscataway, NJ USA). Lines 1 to 11: PFGE patterns of isolates 2, 7, 6, 10, 8, 13, 11, 4, 3, 1, 12 as described in Table 2, respectively.

located upstream of *mef*(E) at the 5′ end of the mega sequence, and the *orf5* gene, located at the 3′ end of the mega sequence, were also detected. Adding the four DNA fragments of mega sequenced in *mef*(E)-containing *S. agalactiae* strains, *orf*7, *mef*(E), *mel*, and *orf*5, nearly half of the mega element was sequenced, showing a similarity of >99% with the mega sequence of *S. pneumoniae* at GenBank. The detection of different parts of the mega element, together with the *mef*(E) and *mel* genes, strongly suggested that, as occurs in *S. pneumoniae* and other streptococcal species containing the *mef*(E) subclass gene (1, 12, 18, 26), in the majority of the *mef*(E)-containing *S. agalactiae* isolates studied the *mef*(E) subclass gene was carried in mega.

The absence of the *intTn* and *orf24* genes in 8 of the 12 *mef*(E)-containing strains ruled out the presence of a Tn*916*-like element. In the four remaining *mef*(E)-containing *S. agalactiae* strains in which the *intTn* and *orf24* genes were detected, the failure to demonstrate the location of mega upstream of *tet*(M) indicated that mega was not carried, at least not in the same orientation as that described for the Tn*2009*-like transposon.

Erythromycin-resistant *S. agalactiae* isolates with the M phenotype usually have the *mef*(E) subclass gene, while *mef*(A) subclass-containing isolates are rarely found (3). We found only one strain with *mef*(A), in which the *matA* and the *orf8* genes described for the Tn*1207.1* genetic carrying element were also detected. The detection of the *orf9* and *orf56* genes of the 5′- and 3′-end regions of transposon Tn*1207.3* in this *mef*(A)-containing strain indicated that Tn*1207.1* was integrated into the conjugative transposon Tn*1207.3*. In addition, the detection of the *tet*(M) gene and not of *tet*(O) in this erythromycin- and tetracycline-resistant strain ruled out the carriage of Tn*1207.1* into the recently described *tet*(O)-*mef*(A) element (7).

As far as we know, this is the first report of the presence of mega and Tn*1207.1* integrated into Tn*1207.3* in *S. agalactiae*. These two genetic elements have been described previously for other pathogenic and non-pathogenic streptococcal species, such as *S. pneumoniae*, *S. pyogenes*, and viridans group streptococci (4, 12, 18, 26, 28).

In conclusion, in *S. agalactiae* isolated from pregnant carrier women, the rates of prevalence, erythromycin resistance, and determinants of macrolide resistance found were similar to those described in other studies. In erythromycin-resistant *S. agalactiae* with the M phenotype, the presence of the *mef*(E) and *mef*(A) genes was associated with the mega- and Tn*1207.3*-carrying elements, respectively. The clonal diversity of *mef*(E)-containing strains and the described lack of conjugation of the mega element (18, 26) suggest that transformation might be the main mechanism through which this genetic element of resistance is acquired.

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