ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2001, p. 2400–2402 Vol. 45, No. 8
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.8.2400–2402.2001

Antibiotic Susceptibility and Mechanisms of Erythromycin Resistance in Clinical Isolates of *Streptococcus agalactiae*: French Multicenter Study

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Received 7 February 2001/Returned for modification 7 April 2001/Accepted 9 May 2001

Among 126 *Streptococcus agalactiae* isolates collected in 10 French laboratories in 1999, 27 (21.4%) had macrolide resistance related to the presence of *erm*(B) (11 strains), *erm*(A) subclass *erm*(TR) (10 strains), and *mef*(A) genes (2 strains) and the presence of combinations of *erm*(B) and *erm*(A) genes or *mef*(A) genes (3 strains).

Streptococcus agalactiae or group B streptococcus (GBS) is one of the pathogens most frequently responsible for peripartum maternal and neonatal infections. Aminopenicillin is recommended as a first-line intrapartum chemoprophylaxis for prevention of GBS infection in neonates. Erythromycin and clindamycin are alternatives to the penicillins in cases of intolerance (6). While aminopenicillins are still active against the vast majority of GBS strains, resistance to erythromycin has been reported as far back as 1962 in the United States and has emerged during the last decade in several countries (11, 15). In GBS, resistance to macrolides is conferred either by methylases encoded by *erm* genes, that modify the ribosomal target of macrolides or by pumps that efflux these antibiotics. Ribosomal modification by methylase was the first erythromycin resistance mechanism reported in GBS and results in cross-resistance to macrolide-lincosamide-streptogramin B (MLS_B). In streptococci, MLS_B resistance can be mediated by two classes of methylase genes, i.e., the conventional *erm*(B) (*ermAM*) determinant and the recently described *erm*(TR) gene, which is considered to be a subset of the *erm*(A) class (18). Macrolide efflux is mediated by a membrane-bound protein encoded by the *mef*(A) gene and gives rise to the so-called M phenotype characterized by resistance to 14- and 15-membered ring macrolides, while lincosamides, streptogramins, and 16-membered ring macrolides remain active even after induction with erythromycin (8). There have not been many studies investigating the macrolide resistance mechanisms in *S. agalactiae* (5, 17). The aim of this multicenter study was to determine the susceptibility to antibiotics of GBS isolated recently in the community and to characterize the mechanisms of macrolide resistance in erythromycin-resistant strains.

In February 1999, all consecutive clinical strains from outpatients identified as GBS and *S. agalactiae* by latex agglutination assay and API 20 STREPT gallery, respectively, were collected in 10 French private laboratories located in nine different cities in the Paris area and southeastern and southwestern France (one, two, and seven laboratories, respectively) and sent to the hospital Begin laboratory for investigation. The MICs of penicillin G, amoxicillin, cefotaxime, erythromycin, clindamycin, pristinamycin (a streptogramin antibiotic), tetracycline, and rifampin were determined by the agar dilution method with Mueller-Hinton medium supplemented with 5% defibrinated sheep blood (Bio-Rad, Marnes la Coquette, France) as recommended by the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) (9). Results were interpreted according to the recommendations of the CA-SFM (breakpoints are shown in Table 1) (10).

The resistance phenotypes of erythromycin-resistant GBS were determined by the disk diffusion method on Mueller-Hinton agar supplemented with 5% defibrinated horse blood (Bio-Rad) on erythromycin and clindamycin disks. Blunting of the clindamycin inhibition zone proximal to the erythromycin disk indicated an inducible type of MLS_B resistance (12, 13, 19). Resistance to both erythromycin and clindamycin indicated an MLS_B cross-resistance. Susceptibility to clindamycin with no blunting defined the M phenotype (efflux mechanism) (8, 12).

The *mef*(A), *erm*(TR), *erm*(B), and *msr*(A) genes were detected after PCR amplification and hybridization as previously described (1, 20). *Streptococcus pneumoniae* HM28 containing the *erm*(B) gene, *Staphylococcus aureus* HM1054/R [*erm*(C)], *S. aureus* HM1051 [*erm*(A)], *Streptococcus pyogenes* UCN1 [*erm*(TR)], and *Staphylococcus saprophyticus* HM1053 [*msr*(A)] from our collection and *S. pneumoniae* O2J1175 [*mef*(A)] were used as controls in PCR experiments.

One hundred and twenty-six GBS strains were obtained, of which 63.4% were isolated from cases of genital tract infection or colonization, 16.1% were isolated from the urinary tract, and 6.5% were isolated from superficial pus. A summary of

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TABLE 1. In vitro activities of eight antibiotics for 126 *S. agalactiae* isolates

Antimicrobial agent	MIC (μg/ml)[a]				CA-FSM breakpoint[b] (μg/ml)	Susceptibility (%)
	Range	Mean	50%	90%		
Penicillin G	0.03–0.06	0.03	0.03	0.03	≤0.25	100
Amoxicillin	0.03–0.06	0.05	0.06	0.06	≤4	100
Erythromycin	0.03–>64	0.32	0.12	8	≤1	78.6
Clindamycin	0.03–>64	0.31	0.06	128	≤2	82.5
Pristinamycin	0.03–2	0.06	0.06	0.125	≤1	100
Tetracycline	0.03–>64	25.1	32	64	≤4	11.9
Co-trimoxazole	1–64	2.1	2	4	≤2/38	89.4
Rifampin	0.125–2	0.14	0.125	0.125	≤4	100

[a] 50 and 90%, MIC_{50} and MIC_{90}, respectively.
[b] Susceptibility breakpoint recommended by the CA-SFM (8).

MIC data (geometric mean range, MIC at which 50% of strains tested are inhibited [MIC_{50}], and MIC_{90}) on 11 antibiotics for these strains are listed in Table 1. Twenty-seven GBS (21.4%) showed decreased susceptibility to erythromycin. The rates of resistance varied from 11 to 50% among the participant centers. Nineteen strains (70.4% of erythromycin-resistant strains) expressed the MLS_B phenotype, six strains (22.2%) expressed the inducible MLS_B phenotype, and two strains (7.4%) expressed the M phenotype. Distribution of erythromycin resistance genes according to erythromycin resistance phenotypes is reported in Table 2. The *erm*(B) and *erm*(TR) genes were prevalent, and three strains harbored combinations of resistance genes.

One isolate resistant to erythromycin and clindamycin (MICs, 4 and 8 μg/ml, respectively) did not harbor any of the target genes. No hybridization with any probe was observed for five randomly selected erythromycin-susceptible strains used as controls. Our multicenter study confirms the high level of activity of penicillin G and amoxicillin against GBS (3, 14–16). In this species, beta-lactam resistance has rarely been described (4, 23). If beta-lactam susceptibility patterns have remained unchanged, then the rate of erythromycin resistance in GBS is increasing: 1.2% for the period 1980 to 1993 versus 18% for the period 1997 to 1998 in a North American study (15). Compared to this report and other recent studies, the rates of erythromycin and clindamycin resistance were high in our experience: respectively, 21.4% versus 4.9 to 20.2% for erythromycin and 18% versus 0.7 to 15% for clindamycin (3, 14–16).

TABLE 2. Distribution of erythromycin resistance phenotypes according to macrolide resistance genotype

Erythromycin resistance gene(s)	No. of strains with erythromycin resistance phenotype:			Total no. of strains
	MLS_B	Inducible MLS_B	M	
erm(B)	10	1	0	11
erm(TR)	6	4	0	10
erm(B) + *erm*(TR)	1	0	0	1
mef(A)	0	0	2	2
erm(B) + *mef*(A)	2	0	0	2
Absence[a]	1	0	0	1
Total	19	6	2	27

[a] Absence, lack of amplification by PCR using primers specific for *erm*(B), *erm*(TR), *mef*(A), and *msr*(A) genes.

Among the erythromycin-resistant strains, *erm*(B) genes and to a lesser extent *erm*(TR) genes were widely distributed. Resistance genes were combined in three strains. Combinations of *erm*(B) and *mef*(E) genes have been reported previously in pneumococci isolated in France (1). We did not study the relatedness of the strains by molecular techniques, but the erythromycin-resistant isolates were distributed in all the participant centers. The active efflux pump mediated by the *mef*(A) gene has already been reported in a previous French study (2) but is far less frequent in French GBS isolates than in isolates belonging to other beta-hemolytic streptococcal species, including *S. pyogenes* (12, 23). The presence of the *mreA* gene, described as a novel macrolide efflux gene (7), has not been investigated because it is an intrinsic gene that encodes riboflavin kinase and is found in all *S. agalactiae* strains (G. Clarebout and R. Leclercq, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 840, 1999). In our study, one erythromycin-resistant strain with the MLS_B phenotype had a negative PCR result. This strain might possess other macrolide resistance mechanisms such as mutation in 23S rRNA or one of the ribosomal proteins already described in *S. pneumoniae* (21).

The level of erythromycin and clindamycin resistance in French GBS isolates is of concern and leads to the recommendation that alternative prophylactic therapy for pregnant women who are penicillin intolerant should be guided by susceptibility testing.

We thank Bruno Moutinho for technical assistance, and we are grateful to Joyce Sutcliffe for providing *S. pneumoniae* reference strain O2J1175.

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