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Mechanisms of Macrolide Resistance in Clinical Group B Streptococci Isolated in France

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Macrolide susceptibility was investigated in clinical group B streptococci obtained from neonates or pregnant women in 2000 in France. Of 490 consecutive isolates, 18% were resistant to erythromycin. The *erm*(B), *erm*(A) subclass *erm*(TR), and *mef*(A) genes were harbored by 47, 45, and 6% of these strains, respectively. Two isolates did not harbor *erm* or *mef* genes.

Group B streptococci (GBS) are a leading cause of neonatal infections. Intrapartum antibiotic prophylaxis is now recommended for colonized women to prevent neonatal GBS disease, with penicillin G being the drug of choice (1). Women allergic to β-lactam antibiotics can receive intravenous clindamycin or erythromycin (1). Although penicillin resistance in GBS has not yet been reported, isolates resistant to erythromycin and related antibiotics have been previously described (2, 10, 18, 19, 23, 24, 32).

The known mechanisms of macrolide resistance in streptococci are targets of modification by a ribosomal methylase associated with *erm* genes (17, 26, 33), a macrolide-specific efflux mechanism encoded by the *mef*(A) gene (7), and mutations in the 23S rRNA and ribosomal L4 and L22 proteins (9, 30, 31; A. Canu, B. Malbruny, M. Coquemont, T. A. Davies, P. C. Appelbaum, and R. Leclercq, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1927, p. 118, 2000). The prevalences and mechanisms of macrolide resistance have been widely reported worldwide for group A streptococci (GAS) and *Streptococcus pneumoniae* (4, 5, 8, 11, 12, 14, 16, 22, 28); relevant data on GBS are rare (3). The aims of this study were to assess the macrolide sensitivity of clinical GBS strains recently isolated in France and determine the genetic mechanisms of resistance.

In 2000, 88 erythromycin-resistant GBS isolates were identified among 490 consecutive isolates in the Paris (France) area. The isolates were recovered from genital specimens of pregnant women ($n = 67$) or from gastric fluid or ear specimens of colonized or infected newborns ($n = 21$). β-hemolytic colonies and suspected nonhemolytic colonies were identified as GBS by using a commercial agglutination technique (Murex Diagnostics, Dartford, United Kingdom). The GBS serotypes were as follows: serotype Ia, $n = 2$; serotype Ib, $n = 9$; serotype II, $n = 6$; serotype III, $n = 28$; serotype IV, $n = 10$; serotype V, $n = 26$; and nontypeable, $n = 7$.

The detection of erythromycin-resistant GBS isolates and determination of resistance phenotypes were performed as previously described (11, 27). The MICs of erythromycin azithromycin, josamycin, spiramycin, clindamycin, and streptogramin B were determined for all isolates with erythromycin inhibition zone diameters of less than 21 mm (20, 21). MICs were determined by the agar dilution method in Mueller-Hinton medium supplemented with 5% defibrinated sheep blood. The plates were incubated overnight at 35°C in air.

All erythromycin-resistant isolates were screened for erythromycin resistance genes. The *mef* and *erm* genes were detected by multiplex PCR amplification with previously described primers (5, 15, 26, 29). The internal PCR control was the *mreA* gene. The primers used to detect the *mreA* gene were 5′-AGA CAC CTC GTC TAA CCT TC-3′ and 5′-TCT GCA GGT AAG TAA GTG CG-3′ (6). *Streptococcus agalactiae* BM 132, *S. agalactiae* SBI, and *Streptococcus pyogenes* 02 C1110 were used as positive PCR controls for the *erm*(B), *mef*(A), and *erm*(A) subclass *erm*(TR) genes, respectively (3, 5, 7). Five erythromycin-susceptible GBS isolates were used as negative controls. Amplification of DNA from the positive controls with the corresponding primers yielded PCR products of the expected sizes [616, 490, 348, and 206 bp for *erm*(B), *mreA*, *erm*(A) subclass *erm*(TR), and *mef*(A), respectively] (Fig. 1). These PCR products were used for direct sequencing in an Applied Biosystems model 373 DNA sequencer by a modification of Sanger's method (25). The amplimers were found to be identical to the *erm*(B), *erm*(A) subclass *erm*(TR), and *mef*(A) genes (7, 26, 33).

Among the 88 GBS erythromycin-resistant isolates, 71, 23 and 6% expressed the inducible macrolide-lincosamide-streptogramin B (MLS_B), constitutive MLS_B, and M resistance phenotypes, respectively. Table 1 shows MICs for the isolates according to erythromycin resistance genotype. PCR amplification showed that all the resistant isolates with the constitutive MLS_B, inducible MLS_B, and M phenotypes harbored the *erm*(B) or *erm*(A) subclass *erm*(TR) and *mef*(A) genes, respectively. All strains carried the *mreA* gene, but two erythromycin-resistant strains did not yield amplified products with the *erm* and *mef* primers tested; the mechanisms of resistance are under investigation. The MICs of various drugs for these two isolates were as follows: ≥128 μg/ml for all macrolides and

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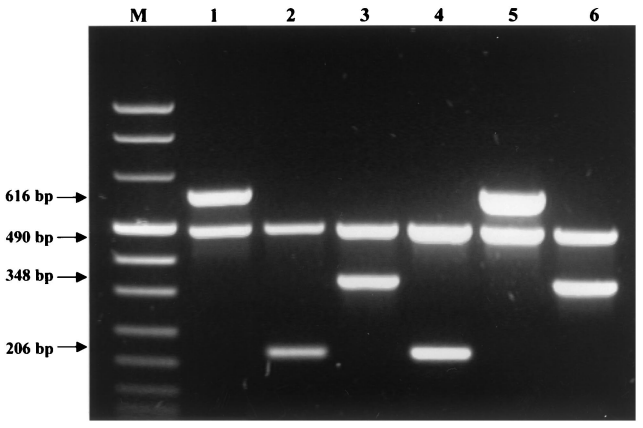

FIG. 1. PCR analysis of erythromycin-resistant control strains. Primers specific for the detection of *erm*(B) (lane 1), *mef*(A) (lane 2), and *erm*(A) subclass *erm*(TR) (lane 3) were used, followed by three representative clinical isolates (lanes 4, 5, and 6). Lanes 1 to 6, internal PCR control, the *mreA* gene, Lane M, DNA molecular size marker VIII (Boehringer Mannheim).

clindamycin and 16 μg/ml for streptogramin B for the first isolate and 32 μg/ml for macrolides, ≥128 μg/ml for clindamycin, and 8 μg/ml for streptogramin B for the second isolate. The distributions of the erythromycin resistance genes are shown in Table 2 according to serotype.

Erythromycin resistance in GBS has mainly been investigated in North America. In the most recent studies, the rates of resistance ranged from 4 to 25% (2, 10, 18, 19, 23, 24, 32). In our study of GBS isolates of similar origins collected in the Paris area in 2000, the prevalence of erythromycin resistance was 18%. A previous North American study has shown an increase in GBS erythromycin resistance from 1995 to 1998, which could be related to the implementation of American guidelines recommending intrapartum antibiotic prophylaxis for GBS infection (1). In our institutions, the level of GBS erythromycin resistance varied only from 16% in 1997 to 18% in 2000, with no significant change in the consumption of macrolides during the last 5 years (E. Bingen, unpublished data).

TABLE 1. MICs of macrolides and related agents for 86 erythromycin-resistant GBS isolates according to known mechanisms of resistance

Antimicrobial agent	MIC (μg/ml)[a]		
	50%	90%	Range
erm(B) (*n* = 41)			
Erythromycin	≥128	≥128	2–≥128
Azithromycin	≥128	≥128	1–≥128
Josamycin	≥128	≥128	2–≥128
Spiramycin	≥128	≥128	2–≥128
Clindamycin	≥128	≥128	0.06–≥128
Streptogramin B	32	128	1–≥128
erm(A) subclass *erm*(TR) (n = 40)			
Erythromycin	4	32	1–≥128
Azithromycin	8	64	1–≥128
Josamycin	2	32	0.5–≥128
Spiramycin	2	32	0.5–64
Clindamycin	0.064	≥128	0.064–≥128
Streptogramin B	4	8	2–16
mef(A) (*n* = 5)			
Erythromycin	2	2	2
Azithromycin	2	4	1–4
Josamycin	0.5	1	0.5–1
Spiramycin	0.5	1	0.5–1
Clindamycin	≤0.032	0.064	≤0.032–≤0.064
Streptogramin B	2	4	2–4

[a] 50 and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

TABLE 2. Serotype distribution according to genetic mechanism of macrolide resistance in 88 erythromycin-resistant GBS isolates

Genetic mechanism of resistance	No. of isolates belonging to serotype:						
	Ia	Ib	II	III	IV	V	NT[a]
erm(B) (*n* = 41)		4	3	15	3	13	3
erm(A) subclass *erm*(TR) (*n* = 40)		4	3	10	6	13	4
mef(A) (*n* = 5)	2			3			
Unknown (*n* = 2)		1			1		
Total (%)	2 (2)	9 (10)	6 (7)	28 (32)	10 (11)	26 (30)	7 (8)

[a] NT, nontypeable.

While the prevalence and mechanisms of erythromycin resistance in *S. pneumoniae* and GAS have been widely investigated (4, 5, 8, 12, 14, 22, 28), to our knowledge such data are not available for GBS. In our study, erythromycin resistance in GBS was mainly associated with the *erm*(B) and *erm*(A) subclass *erm*(TR) genes (47 and 45% of isolates, respectively), with only 6% of isolates harboring the *mef*(A) gene. None of the strains carried both *erm*(A) and *erm*(B) or both *mef* and *erm*, as previously observed with GAS isolates (13). The *mreA* gene, initially considered a novel macrolide efflux gene, was detected for all our strains (6). Indeed, the *mreA* gene is now considered a housekeeping gene for the GBS species (G. Clarebout, and R. Leclercq, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 840, p. 115, 1999). Erythromycin resistance in two of our strains was not associated with either the *mef* or the *erm* genes. Similar results have recently been reported with GAS isolates (22). Such resistance in beta-hemolytic streptococci may be related to mutations in ribosomal proteins, as previously reported for *S. pneumoniae* (9, 30, 31).

Interestingly, the mechanisms of macrolide resistance in our GBS isolates differed from those previously described for pneumococcal and GAS isolates in France (5, 11). While erythromycin resistance in pneumococci is mainly associated with *erm*(B), erythromycin-resistant GAS strains bore the *erm*(B) or *mef*(A) gene and, sporadically, the *erm*(A) subclass *erm*(TR) gene. Insufficient data are available to compare the genetic mechanisms underlying erythromycin resistance in GBS in France and elsewhere. However, several recent North American studies showed a rate of erythromycin- and clindamycin-resistant GBS of 4 to 16% (2, 10, 18, 19), pointing to the involvement of the *erm*(B) and/or *erm*(A) subclass *erm*(TR) genes.

Our study shows that erythromycin resistance is not equally distributed among the different GBS serotypes, with higher rates being associated with serotypes III and V. This is a matter of concern, as these serotypes are usually associated with in-

vasive strains. Thus, antibiotic intrapartum prophylaxis for patients allergic to penicillin must be guided by macrolide susceptibility testing of each GBS isolate.

Surveillance of macrolides and patterns of resistance in GBS, associated with a survey of macrolide consumption, should continue.

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REFERENCES

1. **American Academy of Pediatrics, Committee on Infectious Diseases and Committee on Fetus and Newborn.** 1997. Revised guidelines for prevention of early-onset group B streptococcal (GBS) infection. Pediatrics **99:**489–496.
2. **Andrews, J. I., D. J. Diekema, S. K. Hunter, P. R. Rhomberg, M. A. Pfaller, R. N. Jones, and G. V. Doern.** 2000. Group B streptococci causing neonatal bloodstream infection: antimicrobial susceptibility and serotyping results from SENTRY centers in the western hemisphere. Am. J. Obstet. Gynecol. **183:**859–862.
3. **Arpin, C., H. Daube, F. Tessier, and C. Quentin.** 1999. Presence of *mefA* and *mefE* genes in *Streptococcus agalactiae*. Antimicrob. Agents Chemother. **43:** 944–946.
4. **Baquero, F., J. A. García-Rodríguez, J. García de Lomas, L. Aguilar, and The Spanish Surveillance Group for Respiratory Pathogens.** 1999. Antimicrobial resistance of 1,113 *Streptococcus pneumoniae* isolates from patients with respiratory tract infections in Spain: results of a 1-year (1996–1997) multicenter surveillance study. Antimicrob. Agents Chemother. **43:**357–359.
5. **Bingen, E., F. Fitoussi, C. Doit, R. Cohen, A. Tanna, R. George, C. Loukil, N. Brahimi, I. Le Thomas, and D. Deforche.** 2000. Resistance to macrolides in *Streptococcus pyogenes* in France in pediatric patients. Antimicrob. Agents Chemother. **44:**1453–1457.
6. **Clancy, J., F. Dib-Hajj, J. W. Petitpas, and W. Yuan.** 1997. Cloning and characterization of a novel macrolide efflux gene, *mreA*, from *Streptococcus agalactiae* Antimicrob. Agents Chemother. **41:**2719–2723.
7. **Clancy, J., J. Petitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and J. A. Retsema.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mef*A, from *Streptococcus pyogenes*. Mol. Microbiol. **22:**867–879.
8. **Cornaglia, G.** 1999. Macrolide resistance and *Streptococcus pyogenes*: molecular basis, epidemiology and clinical significance. Rev. Med. Microbiol. **10:** 245–258.
9. **Depardieu, F., and P. Courvalin.** 2001. Mutation in 23S rRNA responsible for resistance to 16-membered macrolides and streptogramins in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **45:**319–323.
10. **Fernandez, M., M. E. Hickman, and C. J. Baker.** 1998. Antimicrobial susceptibilities of group B streptococci isolated between 1992 and 1996 from patients with bacteremia or meningitis. Antimicrob. Agents Chemother. **42:**1517–1519.
11. **Fitoussi, F., C. Doit, P. Geslin, N. Brahimi, and E. Bingen.** 2001. Mechanisms of macrolide resistance in clinical pneumococcal isolates in France. Antimicrob. Agents Chemother. **45:**636–638.
12. **Geslin, P.** 1998. Rapport d'activité année 1997. Centre National de Référence des Pneumocoques, Creteil, France.
13. **Giovanetti, E., M. P. Montanari, M. Mingoia, and P. E. Varaldo.** 1999. Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains in Italy and heterogeneity of inducibly resistant strains. Antimicrob. Agents Chemother. **43:**1935–1940.
14. **Johnston, N. J., J. C. de Azavedo, J. D. Kellner, and D. E. Low.** 1998. Prevalence and characterization of the mechanisms of macrolide, lincosamide, and streptogramin resistance in isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **42:**2425–2426.
15. **Klugman, K. P., T. Capper, C. A. Widdowson, H. J. Koornhof, and W. Moser.** 1998. Increased activity of 16-membered lactone ring macrolides against erythromycin-resistant *Streptococcus pyogenes* and *Streptococcus pneumoniae*: characterization of South African isolates. J. Antimicrob. Chemother. **42:**729–734.
16. **Lagrou, K., W. E. Peetermans, J. Verhaegen, S. Van Lierde, L. Verbist, and J. Van Eldere.** 2000. Macrolide resistance in Belgian *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **45:**119–121.
17. **Leclercq, R., and P. Courvalin.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob. Agents Chemother. **35:**1267–1272.
18. **Lin, F.-Y., C. P. H., Azimi, L. E. Weisman, J. B. Philips III, J. Regan, P. Clark, G. G. Rhoads, J. Clemens, J. Troendle, E. Pratt, R. A. Brenner, and V. Gill.** 2000. Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995–1998. Clin. Infect. Dis. **31:**76–79.
19. **Morales, W. J., S. S. Dickey, P. Bornick, and D. V. Lim.** 1999. Change in antibiotic resistance of group B *Streptococcus*: impact on intrapartum management. Am. J. Obstet. Gynecol. **181:**310–314.
20. **National Committee for Clinical Laboratory Standards.** 2000. Performance standards for antimicrobial disk susceptibility tests; approved standard M2–A7, 7th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
21. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7–A5, 5th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
22. **Palavecino, E. L., I. Riedel, X. Berrios, S. Bajaksouzian, D. Johnson, E. Kaplan, and M. R. Jacobs.** 2001. Prevalence and mechanisms of macrolide resistance in *Streptococcus pyogenes* in Santiago, Chile. Antimicrob. Agents Chemother. **45:**339–341.
23. **Pearlman, M. D., C. L. Pierson, and R. G. Faix.** 1998. Frequent resistance of clinical group B streptococci isolates to clindamycin and erythromycin. Obstet. Gynecol. **92:**258–261.
24. **Rouse, D. J., W. W. Andrews, F. Y. C. Lin, C. W. Mott, J. C. Ware, and J. B. Philips III.** 1998. Antibiotic susceptibility profile of group B *Streptococcus* acquired vertically. Obstet. Gynecol. **92:**931–934.
25. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
26. **Seppälä, H., M. Skurnik, H. Soini, M. C. Roberts, and P. Huovinen.** 1998. A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes*. Antimicrob. Agents Chemother. **42:**257–262.
27. **Seppäla, H., A. Nissinen, Q. Yu, and P. Huovinen.** 1993. Three different phenotypes of erythromycin-resistant *Streptococcus pyogenes* in Finland. J. Antimicrob. Chemother. **32:**885–891.
28. **Shortridge, V. D., G. V. Doern, A. B. Brueggemann, J. M. Beyer, and R. K. Flamm.** 1999. Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from a multicenter antibiotic surveillance study conducted in the United States in 1994–1995. Clin. Infect. Dis. **29:**1186–1188.
29. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
30. **Tait-Kamradt, A., T. Davies, P. C. Appelbaum, F. Depardieu, P. Courvalin, J. Petitpas, L. Wondrack, A. Walker, M. R. Jacobs, and J. Sutcliffe.** 2000. Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. Antimicrob. Agents Chemother. **44:**3395–3401.
31. **Tait-Kamradt, A., T. Davies, M. Cronan, M. R. Jacobs, P. C. Appelbaum, and J. Sutcliffe.** 2000. Mutations in 23S rRNA and ribosomal protein L4 account for resistance in pneumococcal strains selected in vitro by macrolide passage. Antimicrob. Agents Chemother. **44:**2118–2125.
32. **Tyrrell, G. J., L. D. Senzilet, J. S. Spika, D. A. Kertesz, M. Alagaratnam, M. Lovgren, J. A. Talbot, and the Sentinel Health Unit Surveillance System Site Coordinators.** 2000. Invasive disease due to group B streptococcal infection in adults: results from a Canadian, population-based, active-laboratory surveillance study—1996. J. Infect. Dis. **182:**168–173
33. **Weisblum, B.** 1995. Erythromycin resistance by ribosome modification. Antimicrob. Agents Chemother. **39:**577–585.