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Multicenter Study of the Mechanisms of Resistance and Clonal Relationships of *Streptococcus agalactiae* Isolates Resistant to Macrolides, Lincosamides, and Ketolides in Spain

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Macrolide, lincosamide, and ketolide mechanisms of resistance and clonal relationships were characterized in a collection of 79 resistant group B streptococcus isolates obtained from neonates or pregnant women. The *erm*(B), *erm*(TR), and *mef*(A) genes were present in 62%, 30.4%, and 3.8% of the isolates, respectively. There was considerable clonal diversity among them.

Streptococcus agalactiae (group B streptococcus [GBS]) is the main cause of neonatal sepsis. In Spain, 10% to 18.5% of pregnant women are colonized by GBS in the vagina or lower rectum (3, 8; A. Andreu et al., Abstr. 12th Eur. Congr. Clin. Microbiol. Infect. Dis., Milan, Italy). For prophylactic purposes, colonized women receive penicillin G intrapartum, except for those allergic to penicillin, who receive erythromycin or clindamycin, as recommended by Spanish and U.S. guidelines (16, 17). A recent multicenter study conducted in Spain investigating GBS susceptibility has shown that penicillin, ampicillin, vancomycin, and levofloxacin are always active; however, resistance to erythromycin and azithromycin has risen to 12.45%, resistance to clindamycin has risen to 11.80%, and resistance to telithromycin has risen to 1.80% (9).

Macrolide resistance in streptococci is mainly due to a macrolide-specific efflux mechanism encoded by the *mef*(A) gene, ribosomal modification by a methylase associated with *erm* (erythromycin ribosome methylase) genes, and mutations in 23S rRNA and ribosomal proteins L4 and L22 (5, 15, 19, 20). Resistance conferred by Erm methylases can be expressed either constitutively ($cMLS_B$) or upon induction ($iMLS_B$). Drug modification by lincosamide nucleotidyltransferases, encoded by the *lin*(B) genes, which can confer resistance to lincosamides but does not affect macrolides, was first described in *Enterococcus faecium* (4) and found in *Streptococcus agalactiae* by Azavedo et al. in 2001 (7).

The aim of this study was to characterize the mechanisms of macrolide, lincosamide, and telithromycin resistance among GBS isolates collected in a multicenter study in Spain and analyze clonal relationships among the resistant isolates.

A total of 79 isolates resistant to macrolides, lincosamides, or telithromycin were studied. The isolates were collected in a multicenter study investigating the antimicrobial susceptibility of 610 GBS isolates from 25 hospitals across Spain (9). Among them, 131 were isolated between 1997 and 2002 from the blood of newborns with early onset GBS disease, and 479 were collected during 2002 from the vagina or rectum of pregnant women. No significant differences were seen between resistant isolates from newborns or pregnant women. The presence of *mef*(A), *erm*(B), *erm*(TR), and *lin*(B) genes, conferring resistance to macrolides and/or lincosamides, was detected by PCR as previously described (4, 12, 18). The following strains were used as PCR-positive controls: *Streptococcus pyogenes* containing the *mef*(A) gene, *S. pneumoniae* containing *erm*(TR), *S. pneumoniae* containing *erm*(B) (all kindly provided by R. Leclercq), and *S. agalactiae* containing *lin*(B) (kindly provided by J. de Azavedo). Pulsed-field gel electrophoresis (PFGE) was performed to analyze chromosomal DNA macrorestriction patterns. DNA was extracted as described by Gordillo et al. (10) and digested with 50 U of SmaI. The percentage of similarity between each banding pattern was determined with Dice's coincidence index, and the distance between clusters was calculated by the unweighted pair-group method with arithmetic averages (UPGMA), using TDI Lane Manager software (TDI, Spain). Statistical assessments were performed with SPSS for Windows (version 10.0). Relationships among

TABLE 1. Correlation between genes for macrolide-lincosamide resistance and phenotype in 79 GBS isolates

Gene	No. of isolates of phenotype[a]:					Total (%)
	$cMLS_B$		$iMLS_B$	M	L^r	
	Telithromycin susceptible	Telithromycin resistant				
erm(B)	39	10	0	0	0	49 (62)
erm(TR)	19	1	4	0	0	24 (30.4)
mef(A)	0	0	0	3	0	3 (3.8)
Unknown	0	0	0	0	3	3 (3.8)
Total	58	11	4	3	3	79 (100)

[a] $cMLS_B$, constitutive resistance to macrolides, lincosamides, and streptogramin B; $iMLS_B$, inducible resistance to macrolides, lincosamides, and streptogramin B; M, resistance to 14- and 15-member ring macrolides and susceptibility to lincosamides; L^r, lincosamide resistance and macrolide susceptibility.

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TABLE 2. Association between phenotype, genotype, and MICs among 76 GBS isolates resistant to erythromycin and 11 resistant to telithromycin[a]

Phenotype	Genotype	Erythromycin				Telithromycin			
		n	MIC_{50}	MIC_{90}	MIC range	*n*	MIC_{50}	MIC_{90}	MIC range
$cMLS_B$	*erm*(B)	49	>32	>32	4–>32	10	4	8	4–>32
	erm(TR)	20	>32	>32	4–>32	1	4	4	4
$iMLS_B$	*erm*(B)	0							
	erm(TR)	4	1	4	0.5–4				
M	*mef*(A)	3	4	4	4				

[a] All MICs are given in μg/ml.

clusters and macrolide resistance genes were analyzed using Fisher's exact test. *P* values of less than 0.05 were considered statistically significant.

The 79 GBS isolates studied include 76 macrolide- and lincosamide-resistant isolates: 69 displayed a $cMLS_B$ phenotype of resistance, 4 an $iMLS_b$ phenotype, and 3 the efflux pump phenotype. The remaining three isolates were clindamycin resistant but macrolide susceptible. Among the 69 isolates with a $cMLS_B$ phenotype, 11 were telithromycin resistant, with MICs ranging from 4 to >32 mg/liter (see Table 2). The interpretative categories used for each antibiotic followed NCCLS recommendations (13). The MIC breakpoint for telithromycin was taken from Comité de l'Antibiogramme de la Societé Française de Microbiologie recommendations (6).

The distribution of genes encoding macrolide-lincosamide resistance according to phenotype is reported in Table 1. The *erm*(B) gene was present in 62% of macrolide-resistant isolates, *erm*(TR) in 30.4%, *mef*(A) in 3.8%, and *lin*(B) in 0%. Among GBS isolates with constitutive resistance to MLS_B antibiotics, 71% showed the *erm*(B) gene and 29% the *erm*(TR) gene. All four GBS isolates with an inducible resistant phenotype presented the *erm*(TR) gene. The three isolates with a phenotype typical of efflux pump had the *mef*(A) gene. Among the 11 telithromycin-resistant isolates, 10 harbored the *erm*(B) gene and 1 harbored the *erm*(TR) gene.

Among GBS isolates presenting the $cMLS_B$ resistance phenotype, those associated with *erm*(B) had MICs of erythromycin identical to those associated with *erm*(TR) (Table 2). However, isolates with *erm*(TR) and an inducible phenotype presented lower MICs.

Erythromycin- and lincosamide-resistant GBS isolates showed high clonal diversity. Among the 79 isolates studied, 48 different PFGE patterns were found. Fourteen isolates were repeatedly nontypeable by PFGE, because of incomplete DNA digestion. Four main clusters were defined at 50% homology (CI to CIV): cluster I contained 64.7% of the isolates, CII contained 32.3%, and CIII and CIV contained one isolate each. The CI isolates mainly included those with the *erm*(B) gene ($P = 0.012$, χ^2 test), and the CII isolates mainly included those with the *erm*(TR) gene ($P = 0.002$, χ^2 test). Resistant isolates causing colonization and sepsis were distributed equally in the different clusters. The three isolates resistant to clindamycin and susceptible to erythromycin and the 11 telithromycin-resistant isolates showed no cluster association.

Our results agree with previous studies conducted in Spain reporting that Erm(B) methylase is the main cause of macrolide resistance in GBS, followed by Erm(TR) (1, 14). This mechanism also predominates in other countries, except in Canada and the United States, where Erm(TR) methylase is the main mechanism (2, 7, 11). In our study, none of the isolates harbored more than one gene for macrolide resistance. However, Betriu et al. (1) found that 26.92% of isolates showed various combinations, mainly *erm*(B) with *erm*(A); nonetheless, the origin of the isolates studied was related not only to neonatal sepsis but also to skin and soft tissues, urine, respiratory tract, and others.

The uncommon phenotype of resistance to clindamycin but susceptibility to macrolides has been found by other groups in Spanish isolates (A. B. Campo-Esquisabel, E. Ugalde, A. Portillo, M. A. Martinez-Bernal, and L. Martinez-Martinez, Abstr. 11th Spanish Congr. Clin. Microb. Infect. Dis, abstr. 4, 2004). In 2001, de Azavedo et al. reported a GBS isolate with this type of resistance encoded by the *lin*(B) gene of *Enterococcus faecium*, which codes for a lincosamide-inactivating nucleotidyltransferase (7). However, our three resistant isolates did not present the *lin*(B) gene or the other genes conferring resistance to macrolides and lincosamides.

The presence of the previously unreported 11 (1.4%) telithromycin-resistant GBS isolates implies the need to investigate the mechanism of resistance involved and its dissemination.

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