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MLS resistance phenotypes and mechanisms in β-haemolytic group B, C and G *Streptococcus* isolates in La Rioja, Spain

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Sir,

Two main mechanisms of resistance to macrolide-lincosamide-streptogramin (MLS) antibiotics have been described in group B (GBS), group C (GCS) and group G (GGS) β-haemolytic streptococci. Target site modification by rRNA methylases, encoded by *erm* genes, confers the MLS_B resistance phenotype [erythromycin-resistant–clindamycin resistant (ERY^R-CLI^R)]. The mechanism of active efflux confers a dissociate M resistance phenotype [erythromycin-resistant–clindamycin-susceptible (ERY^R-CLI^S)]. The objective of the present study was to determine the incidence of MLS resistance *in vitro* among 136 GBS, eight GCS and 28 GGS consecutive clinical isolates recovered from the Hospital San Millán (Logroño, Spain) between 1997 and 2000, and to characterize the resistance mechanisms involved. Susceptibility testing was performed for ERY, CLY, spiramycin (SPY) and virginiamycin (VGY) by the disc diffusion method. MICs were also determined for ERY, penicillin and cefotaxime using the E-test method. The MLS resistance mechanisms were determined by PCR amplification of *erm* genes [*erm*(A), *erm*(B), *erm*(C)[1] and *erm*(TR)[2]]. The efflux pump mechanism was also analysed by PCR amplification of the *msr*(A)[3] and *mef*(A/E) genes.[4] According to the new nomenclature for macrolide and MLS_B resistance determinants,[5] we consider that positive PCR amplifications obtained using either *erm*(A) and/or *erm*(TR) primers correspond to the presence of the *erm*(A) gene. Similarly, and according to Roberts *et al.*,[5] we have designated as *mef*(A) genes all the expected-size PCR products obtained with *mef*(A/E) primers. The presence of the *mre*(A) gene was also determined in all GBS isolates by PCR.

Twenty of 136 (14.7%) GBS isolates were ERY^R, and ERY MIC_{50} and MIC_{90} for these isolates were 0.094 and >256 mg/L, respectively. Penicillin and cefotaxime MIC_{90}s were 0.064 mg/L and all isolates remained susceptible to these antibiotics. All ERY^R GBS isolates with MIC in the range 4–>256 mg/L (n = 19), showed the MLS_B resistance phenotype (ERY^R-SPI^R-CLI^R), and one additional ERY^R GBS isolate (MIC 2 mg/L) showed the M phenotype (ERY^R-CLI^S). The expression of the MLS_B resistance mechanism was either inducible (six isolates) or constitutive (13 isolates) (Table). PCR analyses were performed for all ERY^R (n = 20) and for 11 of 106 ERY^S GBS isolates, in order to determine the resistance mechanisms. The *mre*(A) gene was detected by PCR and sequencing in all GBS, either with ERY^R or ERY^S phenotypes, suggesting that it could be ubiquitous in this streptococcal species, as has been reported previously.[6] Other macrolide resistance PCR analyses were negative in all 11 ERY^S isolates studied (ERY MICs in the range 0.032–0.38 mg/L). The *mef*(A) gene was detected by PCR in the only isolate with M resistance phenotype (MIC 2 mg/L), and negative results were obtained for the other macrolide resistance genes. The *erm*(B) gene was detected, either alone (11 isolates with MIC range 8–>256 mg/L) or associated with *erm*(A) (one isolate with MIC > 256 mg/L) in 12 of 13 ERY^R isolates with the constitutive MLS_B resistance phenotype. The remaining constitutive MLS_B isolate (MIC > 256 mg/L), showed only the *erm*(A) gene by PCR analysis. The presence of the *erm*(A) gene was demonstrated, either alone (five isolates with MIC range of 4–>256 mg/L) or associated with the *erm*(B) gene (one isolate with MIC > 256 mg/L) in all six ERY^R isolates with the inducible MLS_B resistance phenotype. Neither the *msr*(A), nor the *mef*(A) gene was detected in any of these MLS_B-resistant isolates. Transfer of the *mef*(A) gene was demonstrated by filter mating conjugation from the GBS isolate S385 (M resistance phenotype) to *Enterococcus faecalis* strain JH2-2. Transconjugants showed an ERY MIC of >32 mg/L and the *mef*(A) gene was shown by PCR analysis.

One of eight GCS isolates was highly ERY^R (MIC > 256 mg/L; 12.5%), as well as three of 28 GGS strains (MIC > 256 mg/L; 10.7%). ERY MIC_{50} for GCS and GGS isolates was 0.19 and 0.125 mg/L, respectively. Penicillin and cefotaxime MIC_{90}s for GCS/GGS isolates was 0.032/0.023 and 0.064/0.094 mg/L, respectively. All ERY^R GCS (n = 1) and GGS (n = 3) isolates (MIC > 256 mg/L) showed the MLS_B resistance phenotype, whereas no isolate showed the M phenotype. Expression of the MLS_B resistance mechanism was either inducible (one GGS and one GCS) or con-

Table. MICs of erythromycin and PCR amplifications for group B, C and G streptococcal clinical isolates

Erythromycin resistance group	Erythromycin MIC[a] (mg/L)	No. of isolates	Genes detected by PCR amplifications					
			ermA[b]	*ermB*	*ermC*	*msrA*	*mefA*[c]	*mreA*
Group B *Streptococcus*								
susceptible	0.032–0.38	11	–	–	–	–	–	+
M	2	1	–	–	–	–	+	+
constitutive MLS_B	>256	10	–	+	–	–	–	+
	>256	1	+	+	–	–	–	+
	>256	1	+	–	–	–	–	+
	8	1	–	+	–	–	–	+
inducible MLS_B	>256	2	+	–	–	–	–	+
	>256	1	+	+	–	–	–	+
	16	1	+	–	–	–	–	+
	8	1	+	–	–	–	–	+
	4	1	+	–	–	–	–	+
Group C *Streptococcus*								
susceptible	≤0.5	7	–	–	–	–	–	ND
inducible MLS_B	>256	1	+	–	–	–	–	ND
Group G *Streptococcus*								
susceptible	≤0.38	25	–	–	–	–	–	ND
constitutive MLS_B	>256	2	+	–	–	–	–	ND
inducible MLS_B	>256	1	+	–	–	–	–	ND

[a]MIC determined by Etest.
[b]*erm*(A) gene: a positive PCR amplification with *erm*(A)- and/or *erm*(TR)-specific primers was obtained.
[c]*mef*(A) gene: a positive PCR amplification with *mef*(A/E)-specific primers was obtained.

stitutive (two GGS). All four ERY^R GCS and GGS isolates gave positive PCR amplifications with specific *erm*(TR) primers, and weak PCR amplifications with specific *erm*(A) primers. Sequencing of the PCR products obtained with both sets of primers from one of these ERY^R isolates (GGS, S211) gave in both cases the sequence of the *erm*(TR) gene. According to the new nomenclature, we can conclude that the four ERY^R isolates belong to the *erm*(A) group, which includes both *erm*(A) and *erm*(TR) genes. Negative PCR amplifications were obtained for *erm*(B), *erm*(C), *msr*(A) and *mef*(A) genes. In ERY^S GCS and GGS isolates (MIC 0.032–0.38 mg/L), all PCR results were negative (Table).

Previous studies carried out in our group showed that the M phenotype is prevalent among ERY^R *Streptococcus pyogenes*, and curiously, this phenotype is rarely found among other streptococcal species, such as GBS, GCS and GGS, in our region. This fact should be considered when deciding empirical therapy.

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