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Short communication

Macrolide resistance phenotypes and mechanisms of resistance in *Streptococcus pyogenes* in La Rioja, Spain

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Abstract

One hundred and thirty seven consecutive clinical *Streptococcus pyogenes* isolates were evaluated for macrolide–lincosamide–streptogramin resistance (MLS). Forty of these isolates were resistant to erythromycin (29.2%), 36 of them showed the new M resistance phenotype (erythromycin resistant and clindamycin susceptible) and four isolates had the MLS_B resistance phenotype (erythromycin and clindamycin resistant). In all 36 isolates with the M resistance phenotype, the *mef* gene was identified by polymerase chain reaction (PCR). In two of the four *S. pyogenes* isolates with the MLS_B phenotype, both *ermB* and *ermTR* genes were found; negative results were obtained with the other two isolates which might possess a new mechanism of high level resistance against erythromycin not previously described. In summary, a high rate of erythromycin resistance was found in *S. pyogenes* isolates and the active efflux pump mediated by the *mef* gene was the mechanism most frequently involved.

Keywords: Streptococcus pyogenes; Macrolides; Mechanisms of resistance

1. Introduction

Streptococcus pyogenes is a pathogen that frequently colonizes and causes infections of the upper respiratory tract such as tonsillitis and can also cause skin or systemic infections [1]. Macrolides are the drug of choice for patients with streptococcal infections who cannot take penicillin because of allergy.

Three different mechanisms have been found to be implicated in acquired erythromycin resistance in bacteria: target site modification, active efflux of erythromycin and enzymatic inactivation of the antibiotic [2]. The first two mechanisms have been described in *S. pyogenes* [2–4]. Target site modification is due to the presence of an rRNA methylase that modifies an adenine residue in 23S rRNA. Methylation probably results in a conformational change in the ribosome leading to reduced binding of macrolide, lincosamide and streptogramin B (MLS_B) antibiotics to their target site in the 50S ribosomal subunit. MLS_B resistance can be expressed constitutively or inducibly. Different types of erythromycin–resistance–methylases, encoded by *erm* genes, are produced by bacteria. In *S. pyogenes*, genes belonging to the *erm*AM class have been detected [2] and recently, a new *erm*TR gene has also been demonstrated in this bacterial species [3]. Active efflux has also been shown as a common mechanism of erythromycin resistance in *S. pyogenes*, resulting in a dissociate M resistance phenotype (erythromycin resistance and clindamycin susceptibility). This kind of resistance is mediated by the *mef*A gene that has been recently sequenced [4]. In Spain, few clones could be involved in erythromycin resistant *S. pyogenes* strains with the M phenotype [5]. So far, no erythromycin inactivating enzyme has been described for *S. pyogenes*.

The goal of this study was to determine the MLS resistance phenotypes in a series of 137 *S. pyogenes* clinical isolates and to characterize the mechanisms of resistance involved.

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2. Material and methods

2.1. Bacterial isolates

One hundred and thirty seven clinical *S. pyogenes* isolates were studied. They were collected in the Hospital San Millán, Logroño, during the period 1996–1997. The origins of the isolates were: the upper respiratory tract ($n = 84$), skin/wound ($n = 12$), blood ($n = 2$), urinary tract ($n = 5$), vagina ($n = 23$) and others ($n = 11$). Fifty eight percent of the isolates studied were collected from children of ≤ 10 years of age.

2.2. Susceptibility testing

Susceptibility testing was performed for erythromycin, clindamycin, and spiramycin (Sigma, St. Louis, MO) using a disk diffusion method [6] on Müeller–Hinton–Agar (Difco, Detroit, MI) supplemented with 5% sheep blood (MHA-B); the plates were incubated for 24 h at 37°C in the presence of 5% CO_2. The breakpoint used for spiramycin was as previously recommended [7]. MICs were also determined for erythromycin, penicillin and cefotaxime using the E-test method (AB Biodisk, Solna, Sweeden) on MHA-B in the presence of 5% CO_2.

The resistance phenotypes of erythromycin-resistant *S. pyogenes* isolates were determined by the double-disk test with erythromycin and clindamycin disks (separated by 12 mm) as described previously [8]. Blunting of the clindamycin inhibition zone near to the erythromycin disk indicated an inducible type of MLS_B resistance and resistance to both erythromycin and clindamycin indicated a constitutive type of MLS_B resistance. Susceptibility to clindamycin with no blunting indicated the M resistance phenotype.

2.3. Erythromycin resistance mechanisms

The MLS resistance mechanism was determined by polymerase chain reaction (PCR) by amplification of *erm* genes, using degenerate *erm* primers (E_1 5′-GARATIGGIIIIGGIAAGAGGICA-3′; E_2 5′-AAYTGRTTYTTIGTRAA-3′) [9], as well as specific primers for *erm*A (A_1 5′-TCTAAAAAGCATGTAAAAGAA-3′; A_2 5′-CTTCGATAGTTTATTAATATTAGT-3′), *erm*B (B_1 5′-GAAAAGRTACTCAACCAAATA-3′; B_2 5′-AGTAACGGTACTTAAATTGTTTAC-3′), *erm*C (C_1 5′-TCAAAACATAATATAGATAAA-3′; C_2 5′-GCTAATATTGTTTAAATCGTCAAT-3′) [10] and *erm*TR genes (TR_1 5′-ATAGAAATTGGGTCAGGAAAAGG-3′; TR_2 5′-TTGATTTTTAGTAAAAAG-3′) [11]. The conditions used in each case have been described previously [9,11,12]. The efflux-pump mechanism was determined by PCR using primers and specific conditions for amplification of *mef*A/E genes (A/E_1 5′-AGTATCATTAATCACTAGTGC-3′; A/E_2 5′-TTCTTCTGGTACTAAAAGTGG-3′) [10,12]. Positive and negative controls were used in all experiments. Genomic DNA for the PCR reactions was obtained by the Instagene matrix system (BioRad) according to the manufacturer's instructions. Total DNA of *S. pyogenes* was obtained by the method of Caparon and Scott [13] and used in dot blot hybridization at 50°C with the *erm*B and *erm*TR PCR products of *Enterococcus faecium* E134 and group G *Streptococcus* S211 as probes, respectively (labelled with digoxigenine, Boehringer Mannheim).

3. Results and discussion

Susceptibility to MLS antibiotics was determined in 137 clinical *S. pyogenes* isolates. The MIC_{50} and MIC_{90} of erythromycin against these streptococcal isolates were 0.125 and 16 mg/l, respectively. Forty of them were resistant to erythromycin (29.2%) (Table 1). The rate of erythromycin resistance in *S. pyogenes* found in this study was very high. Over the last few years an increase of macrolide resistance in *S. pyogenes* has been observed by different authors in Europe and Australia [14–28], probably due to the increase in macrolide consumption [19]. In the USA, the prevalence of erythromycin-resistant *S. pyogenes* is low (1–4%) [29–31] and a low erythromycin resistance rate has also been detected in *S. pyogenes* from Israel [32,33].

Thirty six of 40 erythromycin-resistant isolates (90%) showed the M phenotype (erythromycin resistant and clindamycin and spiramycin susceptible). These isolates showed a low level of erythromycin resistance (MIC of

Table 1
Susceptibility of 137 *Streptococcus pyogenes* clinical isolates to erythromycin, penicillin and cefotaxime*

Antibiotics	*S. pyogenes*
Erythromycin	
Range	≤0.03–>256
MIC_{50}	0.125
MIC_{90}	16
% R (MIC≥1)	30
Penicillin	
Range	≤0.0035–0.03
MIC_{50}	0.015
MIC_{90}	0.015
% I/R (MIC≥0.125)	0
Cefotaxime	
Range	≤0.0035–0.125
MIC_{50}	0.015
MIC_{90}	0.03
% I/R (MIC≥1)	0

* MIC by E-test (in mg/l). R, resistant; S, susceptible; I, intermediate.

Table 2
MLS resistance phenotypes and mechanisms involved in 137 clinical isolates of *Streptococcus pyogenes*

MLS resistance phenotypes[a]	Erythromycin MIC (mg/l)	No. strains	PCR amplification					
			degenerated *erm*	*erm*A	*erm*B	*erm*C	*erm*TR	*mef*A/E
MLS_I	>256	2	+	−	+	−	+	−
MLS_c	>256	2	−	−	−	−	−	−
M	8–64	36	−	−	−	−	−	+
Susceptible	≤0.03–0.5	97	−	−	−	−	−	−

[a] MLS, resistance to erythromycin, clindamycin and streptogramin-B; i, inducible; c, constitutive. M, resistance to erythromycin but not to clindamycin.

8–64 mg/l). The high incidence of the M resistance phenotype found in this study agrees with the results from other groups in Europe [16,18–20,34,35]. Only four of the total 40 *S. pyogenes* isolates showed the MLS_B resistance phenotype (resistance to erythromycin, spiramycin and clindamycin), two of them constitutive and the other two inducible, as demonstrated by double-disk experiments. All four isolates showed a high level of erythromycin resistance (MIC of >256 mg/l).

When PCR reactions were performed using genomic DNA from the 36 erythromycin-resistant *S. pyogenes* isolates with M phenotype, a fragment of the size expected (348 bp) was obtained in all these isolates using *mef*A/E primers, suggesting the presence of the *mef* gene previously described for *S. pyogenes* [4]. PCR amplifications with degenerate or specific *erm* primers gave negative results in these isolates (Table 2). The *mef*A gene has been detected in *S. pyogenes* strains with the M resistance phenotype [5,12,18,19,36].

In two of the four erythromycin-resistant *S. pyogenes* isolates with the MLS_B resistance phenotype, both with inducible resistance, a positive PCR reaction was obtained with degenerate *erm* and specific *erm*B and *erm*TR primers and a negative result was obtained with *erm*A, *erm*C and *mef*A/E primers. Kataja et al. [36] detected the *erm*TR gene in strains with the MLS_B inducible phenotype. The other two isolates with the MLS_B constitutive resistance phenotype showed negative amplifications in all the PCR reactions carried out, even those performed with degenerate *erm* primers. Negative results were also obtained when total DNA of these two isolates were used in dot blot hybridization experiments with *erm*B and *erm*TR PCR products as probes; negative and positive controls were included. Interestingly, these two isolates showed a very high level of erythromycin resistance (MIC of >256 mg/l), and these results indicate that a new resistance mechanism could be involved. Other authors refer to an erythromycin-resistant *S. pyogenes* strain with a MLS_B resistance phenotype with an unknown mechanism (not *erm*A/B/C/TR or *mef* related) [37]. All the erythromycin-susceptible *S. pyogenes* isolates showed negative results in the PCR reactions for amplification of *erm* and *mef*A/E genes.

Susceptibility to penicillin and cefotaxime was very high in all *S. pyogenes* isolates studied and MIC_{90} values of 0.015 and 0.03 mg/l, respectively, were found (Table 1).

In summary, a high incidence of erythromycin resistance in *S. pyogenes* was found in this study. In most of the resistant isolates, the efflux pump *mef* mechanism, which affects the 14C-macrolides, but not the lincosamides or streptogramin B, was demonstrated. In the two *S. pyogenes* isolates with the MLS_B resistance phenotype, a new resistance mechanism could be involved and further experiments are being carried out to elucidate the resistance mechanism in these isolates. These results have clinical implications in relation to the establishment of therapeutical options in streptococcal infections.

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