Genetic and phenotypic characterization of resistance to macrolides in Streptococcus pyogenes from Argentina

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Abstract

Five hundred and seventy-eight strains of group A streptococci (GAS) isolated mostly from paediatric pharyngeal swabs were tested to evaluate their susceptibility to erythromycin. Resistant strains were then tested for their MICs to erythromycin and clindamycin, their phenotype of resistance to macrolides-lincosamides-streptogramins (MLSB) and for the presence of macrolide resistance genes. The rate of resistance to erythromycin was 8.2%. Constitutive, inducible and M phenotypes of resistance were detected in 2.1, 2.1 and 95.8% of resistant strains, respectively. All M phenotypes harboured the mefA gene, whereas constitutive and inducible phenotypes had ermB and ermTR genes, respectively.

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1. Introduction

Streptococcus pyogenes (Lancefield group A Streptococcus, GAS) is one of the most common human pathogens, being the responsible for the majority of cases of sore throat in paediatric patients [1]. Even after 50 years of use, penicillin remains the antibiotic of choice in the treatment of GAS infection, since S. pyogenes is still exquisitely sensitive to β-lactams.

In patients allergic to β-lactams, macrolides are an alternative for treatment of GAS infection. GAS resistance to erythromycin has been first described in the UK shortly after the introduction of the antibiotic into clinical practice [2]. After this and till the early 1970s, when higher rates were reported in Japan [3,4], erythromycin-resistant GAS strains were only occasionally isolated [5].

The newer erythromycin derivatives are being preferentially used for treatment of GAS pharyngitis in community medicine and empirical chemotherapy of respiratory tract infections because of their clinical efficacy, good tissue penetration and pharmacokinetics, allowing less frequent dosing [6]. Meanwhile, over the past few years, increased rates of erythromycin resistance have been reported for GAS in several countries [7–17]. A positive association between macrolides use and increase in resistance was reported in Finland [18]. Furthermore, a significant reduction in the frequency of resistance was reported after an active reduction in prescription of macrolides for outpatient therapy [19].

Up to now there are two known mechanisms of macrolide resistance in GAS [20]. Methylation of 23S rRNA due to ermB or ermTR-encoded methylase results in the inability of all macrolides, lincosamides and streptogramin B to bind to their target site in the 50S ribosomal subunit.
2. Materials and methods

2.1. Bacterial strains

A total of 568 non-related isolates of S. pyogenes recovered from eight institutions (listed in acknowledgements) were studied at the Faculty of Pharmacy and Biochemistry, University of Buenos Aires. All but one (coming from ear secretion) were from paediatric pharyngeal swabs.

Strains were identified according to Facklam [28] and visualized with an UV transiluminator. PCR positive controls, kindly provided by B.M. Willey (Mount Sinai Hospital, Toronto, Canada) were used for the ermA, ermTR and mefA genes. Erythromycin-sensitive GAS strains were used as negative PCR controls. Amplification of DNA from the positive controls with the corresponding primers yielded PCR products of the expected size: 639, 540 and 348 bp for ermA, ermTR and mefA, respectively [12].

2.2. Susceptibility testing

MICs of erythromycin and clindamycin (Sigma, St. Louis, MO) were performed by an agar dilution method according to the National Committee for Clinical Laboratory Standards guidelines [29] using Mueller Hinton agar plates supplemented with 5% sheep blood. The plates were incubated overnight at 35 °C (with 5% CO2 if needed).

2.3. Phenotypic detection of resistance mechanisms

The resistance phenotypes of erythromycin-resistant GAS were determined by the double disk test, with erythromycin (15 μg) and clindamycin (2 μg) disks separated by 10 mm as previously described [30]. Blunting of the clindamycin inhibition zone near to the erythromycin disk indicated an inducible type of MLSB resistance (iMLS B) and resistance to both erythromycin and clindamycin indicated a constitutive type of MLSB resistance (cMLS B). Susceptibility to clindamycin with no blunting indicated the M resistance phenotype.

2.4. PCR-based detection of resistance genes

The primers used to detect ermA, ermB, ermC, ermTR and mefA in S. pyogenes were those previously described by Suncliffe et al. [31]. DNA amplification was performed as follows: a single colony from a 24 h blood agar plate was resuspended in 20 μl of milliQ water and heated for 10 min at 100 °C in a Biomera T-Gradient thermocycler (Gottingen, Germany). Then a mix containing 50 mM KCL, 10 mM Tris–HCl pH 9.0, 2.5 mM MgCl2, 0.1% Triton X-100, 0.01% gelatin, 0.2 mM deoxynucleotide triphosphate, 2 pmol of each primer and 0.6 U of Taq polymerase (Biotools, Madrid, Spain) was added to yield a final volume of 25 μl. Amplification was performed by 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 54 °C for 1 min and extension at 72 °C for 30 s, followed by an extension step at 72 °C for 10 min. Amplicons were run through 1.5% agarose gels, stained with ethidium bromide and visualized with an UV transilluminator. PCR positive controls, kindly provided by B.M. Willey (Mount Sinai Hospital, Toronto, Canada) were used for the ermA, ermTR and mefA genes. Erythromycin-sensitive GAS strains were used as negative PCR controls. Amplification of DNA from the positive controls with the corresponding primers yielded PCR products of the expected size: 639, 540 and 348 bp for ermA, ermTR and mefA, respectively [12].

3. Results and discussion

Of 568 GAS strains studied, 60 were resistant to erythromycin, with inhibition zone diameters between 6 and 14 mm. When further investigated for their erythromycin MIC by the agar dilution method, only 47 were viable, and all were erythromycin resistant (MIC > 1 mg/l).

By the double disk test, 45 isolates (95.7%) were assigned to the M phenotype, one isolate was constitutively resistant showing the cMLS8 phenotype (2.1%) and another single isolate was inducibly resistant, expressing the iMLS8B phenotype (2.1%).

All of the M phenotype isolates had a slightly higher than published resistance level to erythromycin (see Table 1), while clindamycin MICs values were in good agreement.
with reported data from different countries and were the same as those for the erythromycin-susceptible strains. 

The mefA gene was present in all of the strains showing the M-resistance phenotype. 

The isolate expressing a cMLS B phenotype was highly resistant to both erythromycin and clindamycin (MICs > 128 mg/l), and its characterization was confirmed genotypically by the presence of the ermB gene.

The inducible phenotype isolate harbouring the ermTR gene (now considered a variant of ermTR) [32,33], had MIC values similar to those reported previously. 

Genes coding for both resistance mechanisms were not found in the same SGA strain. No amplification was detected in any of the strains when primers specific for ermC or ermC were used in PCR. 

Of the Argentinian GAS isolates studied, 8.27% were resistant to erythromycin using the agar dilution method. 

When compared with some European countries, our resistance rate remained relatively low, and hopefully, stabilized, suggesting that erythromycin and clindamycin remain alternatives for the treatment of SGA infections. 

Careful usage of macrolide antibiotics and continued surveillance of resistance rate is advisable.

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References


Table 1 

Distribution of phenotypes, macrolide susceptibility ranges and phenotypes of resistant SGA

<table>
<thead>
<tr>
<th>Resistance phenotype</th>
<th>No. of isolates</th>
<th>%</th>
<th>Antibiotic</th>
<th>MIC (mg/l)</th>
<th>50%</th>
<th>90%</th>
<th>Range</th>
<th>Resistance genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>45</td>
<td>95.7</td>
<td>Erythromycin</td>
<td>32</td>
<td>64</td>
<td>8-64</td>
<td>mefA</td>
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</tr>
<tr>
<td>ErmB</td>
<td>1</td>
<td>2.1</td>
<td>Clindamycin</td>
<td>0.125</td>
<td>0.25</td>
<td>0.032-0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ErmC</td>
<td>1</td>
<td>2.1</td>
<td>Clindamycin</td>
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<td>0.5</td>
<td>0.125-0.5</td>
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