Introduction

During the 1990s, some European countries such as Finland, Italy, and Spain experienced an increase in the prevalence of resistance to macrolides in group A streptococci (GAS) with great variability over time, and even from area to area in the same country. Resistance rates recently reached 17% in Finland, 16–55% in Italy depending on the area and 22–27% in Spain. Very little recent data dealing with the French macrolide resistance rate and the mechanisms of resistance are available.

Three distinctive phenotypes of resistance to the macrolide, lincosamide and streptogramin B antibiotics (MLS) are now recognized in GAS: (i) constitutive resistant phenotype (cMLS) and (ii) inducible resistant phenotype (iMLS), both related to ribosome methylation; and (iii) M phenotype, a new resistance pattern related to an efflux mechanism and characterized by susceptibility to lincosamides. Recently, Giovanetti et al. described three subtypes of iMLS strains based on their susceptibility profile to 16-membered macrolides, the phenotypic characteristics of these subtypes correlated relatively well with genotypes.

The objectives of this study were: (i) to assess the current prevalence of erythromycin and clarithromycin resistance in GAS isolated in French adults; and (ii) to determine the phenotypes of macrolide–lincosamide resistance and the genetic mechanisms employed in the resistant strains.

Materials and methods

Bacterial isolates

A total of 303 consecutive strains were collected during 1998–1999 from patients (12–40 years of age) enrolled in the clinical and microbiological evaluation of short course therapy with clarithromycin-modified release. Throat swabs were obtained by general practitioners at the enrolment visit. The majority of samples were collected from four different regions of France (281 of 303 strains): Pays de la Loire, Rhônes–Alpes–Bourgogne, Provence–Alpes–Côte d’Azur and Alsace–Lorraine. Twenty-two additional swabs were obtained in two different towns outside these four regions. Specimens were mailed the same day to the central microbiology laboratory (Laboratoire BIO VSM) using Amies transport medium (Bio-Rad, Marnes-La-Coquette, France). On the day of arrival, the swabs were plated on Columbia agar supplemented with 5% sheep blood,
colistin and nalidixic acid (bioMérieux, Marcy l’Étoile, France), and then incubated for a total of 48 h in anaerobicosis. Colonies of GAS were identified on the basis of β-haemolysis, Gram’s stain and agglutination with a commercial latex reagent (bioMérieux). Susceptibility testing was performed and strains were stored at −70°C for further MIC determination and the search for macrolide resistance genes after all isolates were collected.

**Susceptibility testing**

All strains were tested for susceptibility to erythromycin by a standard diffusion method using Mueller–Hinton agar supplemented with 5% sheep blood (Bio-Rad) and erythromycin discs (15 μg, Bio-Rad). MICs of clarithromycin (Abbott, Rungis, France) were determined by broth microdilution in accordance with the NCCLS method using Mueller–Hinton broth (Bio-Rad) supplemented with 2.5% lysed horse blood (bioMérieux) and incubation in ambient air for 20–24 h. A quality control strain of *Streptococcus pneumoniae* ATCC 49619 was introduced into each batch of experiments. An additional strain of *Streptococcus pyogenes* ATCC 19615 was used for the control of in-laboratory reproducibility.

Phenotypes of MLS resistance were identified further by a triple disc diffusion test derived from Giovanetti et al., using Mueller–Hinton broth (Bio-Rad) supplemented with 5% sheep blood and erythromycin (15 μg), clindamycin (2 μg) and spiramycin (100 μg), a 16-membered macrolide, discs (Bio-Rad). After 18–24 h of incubation in ambient air, the absence of inhibition around the three discs indicated constitutive resistance (cMLS phenotype), and susceptibility to clindamycin and spiramycin with no blunting indicated the M phenotype of resistance. Blunting of the clindamycin zone of inhibition indicated inducible resistance (iMLS phenotype). The three subtypes of inducible resistance were searched for using the 16-membered macrolide disc: (i) the iMLS-A subtype was characterized by the absence of any zone of inhibition around the spiramycin and erythromycin discs; (ii) the iMLS-B subtype was characterized by blunting of the spiramycin zone of inhibition with no zone of inhibition around the erythromycin disc; and (iii) the iMLS-C subtype was characterized by blunting of the spiramycin zone of inhibition with moderate reduction of the inhibition zone around the erythromycin disc.

**Determination of erythromycin resistance genes**

Erythromycin-resistant isolates were screened for the usual genes of macrolide resistance in GAS. The *ermB*, *ermTR* and *mefA* genes were detected by PCR amplification as described previously. The following primers were used: 5'-CGA GTG AAA AAG TAC TCA ACC-3' and 5'-GGC GTG TTT CAT TGC TGG AGT-3' to detect *ermB*; 5'-GCA TGA CAT AAA CCT TCA-3' and 5'-AGG TTA TAA TGA AAG AGA-3' to detect *ermTR*; and 5'-AGT ATC ATT AAT CAC TAG TGC-3' and 5'-TTC TTC TGG TAC TAA AAG TGG-3' to detect *mefA* (Genset, Paris, France). Amplification was performed in a DNA thermal cycler (no. 9600; Perkin-Elmer Cetus, Norwalk, CT, USA) programmed for one cycle of denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for 1 min. Amplification products were run through 2% agarose gels. Gels were stained with ethidium bromide and the DNA bands were visualized with a UV transilluminator.

*S. pyogenes* 02C 1061, *S. pyogenes* 02C 1110 and *S. pyogenes* 02C 1064 (kindly provided by J. Sutcliffe) were used as positive PCR controls for the *ermB*, *ermTR* and *mefA* genes, respectively. Amplification of DNA from the positive controls with the corresponding primers yielded PCR products of the expected sizes (616, 348 and 206 bp for *ermB*, *ermTR* and *mefA*, respectively).

**Results**

The overall rate of resistance to both erythromycin and clarithromycin (strains of intermediate susceptibility plus resistant strains) was 9.6% (29 of 303 strains). The prevalence of the three phenotypes was as follows: cMLS phenotype, 4.3% (13 of 29 strains); iMLS phenotype, 2% (six of 29 strains); and M phenotype, 3.3% (10 of 29 strains). All isolates of iMLS phenotype exhibited only a slight reduction in the zones of inhibition around the erythromycin disc (diameters of 17–20 mm), suggesting a low level of resistance to this antibiotic.

The Table summarizes MICs of clarithromycin in relation to the susceptibility of isolates to erythromycin and the phenotypes of resistance. MICs of the iMLS isolates were lower than those of the M phenotype isolates, and cMLS isolates were of high-level resistance.

PCR amplification for the 29 resistant strains (Table) showed that all cMLS, iMLS and M phenotype isolates harboured *ermB*, *ermTR* and *mefA* genes, respectively. No strain harboured more than one gene simultaneously.

The prevalence of erythromycin-resistant strains was not statistically different (*Student’s χ² test, P = 0.11*) in the four main regions investigated: six resistant strains out of a total of 105 strains (5.7%) in Pays de la Loire, seven out of 55 strains (12.7%) in Rhônes–Alpes–Bourgogne, 10 out of 57 strains (17.5%) in Provence–Alpes–Côte d’Azur and six out of 64 strains (9.4%) in Alsace–Lorraine.

**Discussion**

The 9.6% nationwide resistance rate of GAS to erythromycin and clarithromycin obtained in this study is similar to...
The M phenotype is predominant amongst strains isolated in European countries such as Spain, some areas of Italy and Finland, which have high rates of erythromycin resistance. The absence of inducible phenotype of resistance in the strains isolated from children remains questionable.

Phenotypic characteristics of these iMLS isolates together with low MICs of clarithromycin are consistent with the description of the iMLS-C phenotype recently made by Giovanetti et al. However, the ermTR gene alone was not detected in our iMLS isolates, whereas none of their strains harboured this phenotype. The absence of inducible phenotype of resistance in the strains isolated from children remains questionable.

The type of macrolide prescribed predominantly in each country, e.g. short- versus long-acting macrolides, or C-14 versus C-15 and C-16 macrolides, could impact on the prevalence of resistance and the distribution of genotypes. Such a hypothesis warrants further investigation.

The present study confirms that the macrolide resistance rate in France seems stabilized at ~10% amongst GAS isolates. With this relatively low resistance rate compared with those of other European countries, erythromycin and clarithromycin still remain an alternative to penicillin G for the treatment of streptococcal pharyngo-tonsillitis in France. However, continued surveillance of macrolide resistance in GAS is advisable.

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### References


### Table

<table>
<thead>
<tr>
<th>Erythromycin phenotype</th>
<th>No. of strains</th>
<th>Clarithromycin MIC (mg/L)</th>
<th>Genes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>Susceptible</td>
<td>274</td>
<td>0.016</td>
<td>0.032</td>
</tr>
<tr>
<td>cMLS</td>
<td>13</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>iMLS</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>M</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

ND, not determined; NA, not applicable.

<sup>a</sup>cMLS, constitutive phenotype of resistance to the macrolide, lincosamide and streptogramin B antibiotics; iMLS, inducible phenotype.


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