Significant increase in the prevalence of erythromycin-resistant, clindamycin- and miocamycin-susceptible (M phenotype) *Streptococcus pyogenes* in Spain


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In 1998 we conducted a multicentre study in Spain on the susceptibility of *Streptococcus pyogenes* isolates to different 14-, 15- and 16-membered macrolides and clindamycin, in which the number of strains examined was proportional to the number of inhabitants in each geographical area. The aim of the present work was to re-examine the antimicrobial susceptibility of *S. pyogenes* in 2001, using the same methodology and centres as in 1998, to determine the different susceptibility phenotypes to macrolides–lincosamides, and to compare the results from the 2 years by statistical tests. A total of 529 unique isolates of *S. pyogenes*, collected in 21 laboratories, were studied. Throat swabs provided 417 isolates (78.8%), and the remaining 112 were from other sources. Four hundred and thirty-five (82.2%) were isolated from children and 94 (17.8%) from adults. One hundred and fifty-seven (29.7%) of the isolates were resistant to erythromycin and azithromycin, whereas resistance to miocamycin, a 16-membered macrolide, was 1.5%. The prevalence of resistance to clindamycin was 1.3%. The majority (98.7%) of the 157 erythromycin-resistant strains presented the M phenotype. When we compared the results obtained in 1998 and 2001, we observed a statistically significant increase in resistance to erythromycin and azithromycin (\(P = 0.02, \chi^2\) test), but not to clindamycin or miocamycin (\(P = 0.47, \chi^2\) test with Yates’ correction). The significant increase in the prevalence of resistance to some macrolides of *S. pyogenes* in Spain underscores the need for continuous surveillance of antimicrobial resistance in this species.

Keywords: antimicrobial resistance, group A streptococci, erythromycin resistance, M phenotype

Introduction

Group A streptococci, or *Streptococcus pyogenes*, is by far the most common cause of acute bacterial pharyngitis, accounting for ~15–30% of cases in children, and 5–10% in adults.1 This bacterium also causes diseases such as impetigo, erysipelas and, less frequently, severe invasive diseases.

Group A streptococci are still susceptible to penicillin and other \(\beta\)-lactam antibiotics,2 but alternative options are limited. Resistance to macrolides, advocated for group A streptococcal infections primarily in cases of \(\beta\)-lactam allergy or intolerance, has been reported from an increasing number of countries in recent years.3–9

In 1998 we performed a multicentre study in Spain on the susceptibility of group A streptococci isolates to penicillin G, several macrolides and clindamycin.10 The aim of the present work was to study the antimicrobial susceptibility of group A streptococci in Spain in 2001, using the same methodology and centres as in 1998. The results from the two years were compared by statistical tests. Additionally, we tested the antimicrobial susceptibility to six oral cephalosporins used in primary healthcare in our country.
Materials and methods

Bacterial strains

A total of 529 unique isolates of *S. pyogenes* collected in 21 laboratories in Spain, from January to September 2001, were used. The country was arbitrarily divided into 21 geographical areas. The sample size was proportionally stratified according to the number of inhabitants of each area, with a ratio of approximately one strain/80,000 inhabitants. Throat swab samples provided 417 isolates (78.8%), and the remaining 112 were from other sources. Four hundred and thirty-five (82.2%) were isolated from children and 94 (17.8%) from adults. Identification was made by standard criteria. Strains were frozen in skimmed milk at −40°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the agar dilution method, according to NCCLS guidelines. Antibiotics were obtained, as standard reference powders of known potency, from Sigma Chemical Co., St Louis, MO, USA (penicillin G, cefaclor, cefuroxime, erythromycin and clindamycin), Pfizer Inc., New York, NY, USA (azithromycin), Merck, Barcelona, Spain (cefixime), Ely Lilly, Indianapolis, IN, USA (cefprozil), Schering Plough, Kenilworth, NJ, USA (ceftibuten), Sankyo Pharma, Munich, Germany (cefdodoxime) and Menarini, Barcelona, Spain (diacetil-midekamycin: miocamycin). The range of interpretative categories for each antibiotic was that recommended by NCCLS in the 2001 supplement. The MIC breakpoint for miocamycin resistance was >4 mg/L, as defined by the Comité de l’Antibiogramme de la Societé Française de Microbiologie. *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 were used as quality control strains. All susceptibility tests were carried out in the same laboratory to avoid inter-laboratory variation in the results.

To identify antibiotic resistance phenotypes, discs containing erythromycin (15 μg) or clindamycin (2 μg) were used; different phenotypes of macrolide–lincosamide–streptogramin (MLS) resistance were recognized, in accordance with the description of Seppälä *et al.*

Macrolide resistance gene identification

Twenty-eight erythromycin-resistant strains were selected, 20 with the M phenotype (one per laboratory), and all those with the *MSB* phenotype. The MLS resistance mechanism was determined by PCR amplification of *erm* genes, using degenerate *erm* primers, as well as specific primers for *erm*(*A*) subclass *erm*(TR), and *erm*(B).* The conditions used in each case were those recommended by the authors. The efflux pump mechanism was determined by PCR, using primers and specific conditions for amplification of *erm(A)* genes. Positive and negative controls from our collection were used in all cases.

Statistical analysis

χ² test and χ² with Yates’ correction were used. A two-tailed *P* value of ≤0.05 was considered significant.

Results

MIC ranges, MICs at which 50% (MIC₅₀) and 90% (MIC₉₀) of the isolates were inhibited and the percentage of susceptible strains are given in Table 1. In addition, MIC₅₀, MIC₉₀ and the percentage of susceptible strains from our previous study are given in Table 1 to facilitate comparison of the two sets of data.

Penicillin remained remarkably active, with an MIC₉₀ of 0.015 mg/L. As stated in table 2H of the NCCLS document, we considered 100% susceptibility to all cephalosporins tested except cefixime, as this antibiotic does not figure in the list of antibiotics to which streptococcal isolates susceptible to penicillin can be considered susceptible. However, important differences in MIC₉₀ were appreciable between the different cephalosporins.

One hundred and fifty-seven (29.7%) of the isolates were resistant to erythromycin (MIC breakpoint 1 mg/L). The resistance to both 14- and 15-membered macrolides tested was 29.7%, whereas to miocamycin (a 16-membered macrolide) resistance was 1.5%. The prevalence of resistance to clindamycin was 1.3%.

The different phenotypes of susceptibility to macrolides–lincosamides were as follows: 98.6% of the 157 erythromycin-resistant strains were susceptible to clindamycin and miocamycin, and induction with erythromycin did not modify the susceptibility to the latter antibiotics; these strains were designated as having the M phenotype. Seven isolates were resistant to erythromycin, azithromycin, miocamycin and clindamycin, which indicates a constitutive type of resistance. One erythromycin-resistant strain was susceptible to clindamycin, but it showed an inducible type of resistance.

All 20 strains with the M phenotype when assayed by PCR showed the presence of the *erm(A)* gene responsible for the efflux system. Three of the eight strains with the *MSB* phenotype had the *erm(B)* gene and five had the *erm(A)* subclass *erm*(TR).

When we compared the results obtained in 1998 and 2001 we observed a statistically significant increase in resistance to erythromycin and azithromycin (*P* = 0.02, χ² test, but not to clindamycin or miocamycin (*P* = 0.47, χ² test with Yates’ correction).
Increase in resistance in \textit{S. pyogenes} in Spain

Table 1. \textit{In vitro} susceptibilities of 529 recent \textit{S. pyogenes} strains to penicillin G, clindamycin, three macrolides and six oral cephalosporins

\begin{tabular}{lcccccc}
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Antibiotic & MIC range (mg/L) & 50\% & 90\% & \%S$^b$ & 50\% & 90\% & \%S$^b$ \\
\hline
Penicillin G & $\leq$0.008–0.03 & $\leq$0.008 & 0.015 & 100 & $\leq$0.008 & 0.015 & 100 \\
Erythromycin & $\leq$0.12–16 & $\leq$0.12 & 8 & 70.1 & $\leq$0.06 & 8 & 76.5 \\
Azithromycin & $\leq$0.12–16 & $\leq$0.12 & 8 & 70.3 & $\leq$0.25 & 16 & 76.5 \\
Miocamycin & 0.25–16 & 0.5 & 1 & 98.5 & 0.5 & 1 & 99.0 \\
Clindamycin & $\leq$0.12–16 & $\leq$0.12 & $\leq$0.12 & 98.5 & $\leq$0.06 & 0.12 & 99.2 \\
Cefuroxime & $\leq$0.008–0.06 & 0.015 & 0.03 & 100 & \\
Cefprozil & $\leq$0.008–0.03 & 0.03 & 0.03 & 100 & \\
Cefpodoxime & 0.015–0.06 & 0.015 & 0.03 & 100 & \\
Cefixime & 0.06–0.5 & 0.12 & 0.25 & – & \\
Cefaclor & 0.12–1 & 0.25 & 0.25 & 100 & \\
Ceftibuten & 0.12–1 & 0.5 & 0.5 & 100 & \\
\hline
\end{tabular}

Additionally, data for penicillin G, clindamycin and the three macrolides from our previous study with 486 strains were included.

$^a$MIC required to inhibit 50\% and 90\% of the tested isolates.

$^b$Susceptibility rates.

\section*{Discussion}

Antibiotic resistance is a public health problem. Because of the continuous evolution of antibiotic resistance, regular monitoring of this phenomenon appears to be necessary to counter the difficulty, and to improve guidelines for empirical antibiotic therapy, which must consider the most probable microorganisms and their antibiotic susceptibilities.

In spite of the extensive use of penicillins and other $\beta$-lactam antibiotics, all our strains remain susceptible to penicillin G and the cephalosporins tested, as is the case in other parts of the world.\cite{2}

In recent years, an important increase in the prevalence of resistance to erythromycin in group A streptococci has been reported in some countries.\cite{3,4,5,6,7,8,9} However, in other countries, such as France or some parts of the USA, the situation appears stable.\cite{20,21} Even in Spain, in a recent study the rates of macrolide resistance were \textasciitilde20\%, and, as in our study, the M phenotype was clearly predominant among erythromycin-resistant isolates.\cite{22} However, when the authors compared their results with those obtained before by the same group\cite{23} a decrease was observed, from 27\% to 20\%. Yet, interestingly, centres in northern Spain (Santander, San Sebastián and Bilbao) had the lowest rates of erythromycin resistance. However, in their first study, two of 21 centres in northern Spain were included,\cite{23} whereas four of 17 were included in their last.\cite{22} The authors concluded that this is likely to have biased the mean rate.\cite{22}

The design of our surveillance study, based on the collection of strains per population, avoids the bias resulting from some laboratories contributing a greater number of strains than that which corresponds to the population assigned. Additionally, we obtained the strains from the same laboratories as in the 1998 survey. For these reasons, we think that the significant increase in the prevalence of resistance to erythromycin and azithromycin documented in our study are data of great value.

In recent years, macrolides have been used more and more as empirical therapy for respiratory tract infections. A temporal factor has already been reported in Spain, in which antibiotic consumption over time correlates well with the evolution of macrolide resistance in \textit{S. pyogenes},\cite{24} as has occurred in other countries, such as Slovenia\cite{6} and Finland.\cite{25}

In a study by García-Rey \textit{et al.}\cite{26} that analysed differences between consumption of macrolides and rates of erythromycin resistance among \textit{S. pyogenes} in Spain, it was shown that the total consumption of macrolides, but not the consumption of a specific group of macrolides, presented a significant correlation with the prevalence of resistance.

Miocamycin (the 16-membered macrolide tested) and clindamycin retained full activity against strains with the M phenotype, the great majority of resistant strains, and it could therefore be an alternative for treatment, including empirical treatment in areas in which macrolide resistance is mediated by the \textit{mef(A)} gene.
Our group, and another Spanish group, have recently described a considerable number of individuals, both healthy and with pharyngitis, who carry viridans group streptococci with the M phenotype of resistance, due to the mef(A) gene in their pharyngeal flora.\(^2\)\(^7\)\(^8\) It is reasonable to suppose that the resistance gene could be transmitted to respiratory pathogens that are genetically similar to viridans group streptococci, such as \textit{S. pyogenes}. Viridans group streptococci may thus be a reservoir for the dissemination of mef(A) to other species that share the same habitat, such as \textit{S. pyogenes}.

Currently, many microbiology laboratories do not perform antimicrobial susceptibility tests on group A streptococcal strains. As macrolide resistance in group A streptococci has emerged in recent years in different parts of the world, sometimes suddenly,\(^4\) we recommend that laboratories test the susceptibility of group A streptococci isolates to erythromycin and clindamycin, and, based on the results, predict the pattern of susceptibility to all macrolides and lincosamides.

In a recent study,\(^2\)\(^9\) an association between erythromycin resistance and cell invasiveness in \textit{S. pyogenes} was demonstrated. The significant increase in Spain of strains of this species with the M phenotype is cause for serious concern. Strains combining erythromycin resistance with the ability to enter human respiratory tract cells may enable them to escape both β-lactams, by virtue of intracellular location, and several macrolides, by virtue of resistance. However, the 16-membered macrolides, and clindamycin, remain active and could be an alternative.

The significant increase in the prevalence of resistance to some macrolides of group A streptococci in Spain underscores the need for continuous surveillance of antimicrobial resistance in \textit{S. pyogenes}. Changes in susceptibility need to be documented, to aid physicians in choosing the most appropriate patient therapy, and to help in designing prescription guidelines.

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**References**


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