Antimicrobial Susceptibility of Beta Haemolytic Streptococci Isolated from Paediatric Patients with Pharyngoamigdalitis

Roberto Rivera Sánchez1,2, Rocío Flores Paz1, Carlos Parra Mendez1 and Myriam Arriaga Alba1*
1Hospital Juarez de Mexico, Laboratorio de Investigacion en Microbiologia Direccicon de Investigacion y Ensenanza Av, Mexico
2Clinica Medicina Familiar, ISSSTE IZTAPALAPA II Av San Lorenzo 278, Cerro de la Estrella delegacion Iztapalapa CP 09860 Mexico DF, Mexico
*Corresponding author: Myriam Arriaga Alba, Hospital Juarez de Mexico, Laboratorio de Investigacion en Microbiologia Direccicon de Investigacion y Ensenanza Av. Instituto Politecnico 5160 – Magdalena de la Salinas, delegacion Gustavo A Madero CP 07760 Mexico, Mexico, Tel: 57477560 (ext.) 7475; E-mail: arriaga_alba@yahoo.com

Abstract

Introduction: The beta hemolytic Streptococci (SBH), particularly the group A (pyogenes) is the leading bacterial cause of sore throat that occurs primarily in the paediatric population.

Objective: To evaluate the antimicrobial susceptibility of beta hemolytic Streptococcus from paediatric patients with pharyngitis, and infer the type of macrolide resistance mechanism.

Material and methods: A total of 335 beta-hemolytic Streptococcus species (304 in group A, 26 G, 4 and 1, Groups C and F respectively) from paediatric patients (age range of 0 –14 years) were determined in sore throat antimicrobial susceptibility to penicillin, ceftixime, erythromycin, and clindamycin, by the agar diffusion method. The phenotypes of macrolide resistance were characterized by the double diffusion test disk.

Results: All species were susceptible to penicillin, and clindamycin. Streptococcus pyogenes 10.5%, and 30.8% of group G Streptococcus, were resistant to erythromycin and all belonged to phenotype M.

Conclusion: It is advisable to conduct regular screening tests to monitor possible changes in the prevalence of macrolides resistance.

Keywords: Streptococcus; Paediatric pharyngitis; Erythromycin; Macrolides

Introduction

Beta-haemolytic streptococci (SBH), especially those of group A (pyogenes), are the main etiological agents of pharyngomigdalitis affecting paediatric patients. They are medical important as the may produce rheumatic fever, post-streptococcal glomerulonephritis. Another Lancefield group streptococci such as C and G, has not yet been well described as aetiological causes of pharyngitis its pathogenicity importance is still controversial [1,2].

Despite SBH has been reported to be widely sensitive to Penicillin, macrolids has been frequently employed, especially for those patients sensitive to penicillin and beta-Lactamic antibiotics. Unfortunately, macrolide antibiotic resistance among S. pyogenes has been increasing all over the world [3-8]. Streptococcus pyogenes may be resistance to macrolides by two mechanisms of action: The first one is due to gen mefA, and confers resistant to macrolides of 14 or 15 carbons but not to those of 16, lincomsides y estreptogramines B. Strains having this phenotype are known as phenotype M [9,10]. The second one is due to a mutation on gen erm resulting on an structural change of ribosomal 50s unity. By the present, two gens of this family has been described, ermB y el erm (A), subclasses' erm (TR), Codifying for methylases [11,12]. They are also resistant to a macrolides, lincomsides and streptogramines B. This cross resistance is known as phenotype MLSB, expressed constitutively (MLSc) or inducible (MLSi ). The main of this study was to evaluate the antibiotic susceptibility of beta-haemolytic Streptococci isolated from paediatric patients and elucidate the mechanism of antibiotic resistance observed among them.

Material and Methods

A total of 335 isolates of Beta-haemolytic Streptococci (SBH) were obtained from paediatric patients attending a first level clinic of medical attention in Mexico City. Patient’s ages were from 0 to 14 years old, having a diagnostic of pharyngomigdalitis. All patients presented through pain, fever, difficulty swallowing, tonsilopharingeal erythym, and head each. All the microbiological studies were performed at the clinical laboratory with a medical order necessary to patients’ treatment at the first attention level.

They were identified with conventional microbiological method; beta-haemolisis was observed on blood agar prepared with defibrinated blood sheep at 5%, Gram stain, catalase test, Voges-Proskauer and bacitracin 0.04UI (Oxoid, Hampshire, England) sensitivity employing the disk diffusion test. All streptococci Lancefield groups were classified as groups A, B, C, D, F, G (Sliex Strepto-Kit Bio-Meraux , Lyon France).
Antimicrobial sensitivity

It was determinate by the disk diffusion method, according to the Clinical Laboratory standard institute norms (CLSI) [13]. Penicillin, ceftriaxone, erythromycin, clindamycin (Oxoid, Hampshire, England) and moxifloxacin (Bayer diagnostics). Staphylococcus aureus ATCC25923 and Streptococcus pneumoniae ATCC49619, were employed as reference strain for internal quality control.

The minimum inhibitory concentration (MIC) was evaluated to confirm erythromycin resistant (Sigma Chemical Co., St Louis Mo ) strains, was performed by dilution method on Muller-Hinton agar (BBL, México) supplemented with 5% (vol/vol) with 5% of defibrinated blood sheep. The antibiotic was incorporated on culture medium employing log 2 from 0.125 to 128.0 µg/ml.

Bacterial strains was prepared with a turbidity value of a 0.5 of Mc Farland, giving an inocule of de 10^4 ufc/ ml. The tester strains were evaluated suspending them on sterile isotonic saline solution to a 0.5 Mc Farland value. Then evaluated strains were disposed employing a Steer replicator. Plates were incubated from 18 - 24 hrs a 35⁰C con 5% de CO₂

Results

The most frequently observed group B streptococci were group A (pyogenes) 304 of 335, 26 were group G, 4 and 1 were group C and F respectively. Most of them were isolated from male patient 214, and 121 were from female patients. The Voges Proskauer test was negative in all the 335 isolates. The main prevalence of SBH was observed among children 5 to 6 year’s old (174) (Figure 1). Whereas the most susceptible to infections were children from 3- 5 (135) and 6-8 years old (137) (Figure 2).

All the evaluated SBH were sensible to penicillin, ceftriaxone and clindamycin, as observed in Table 1. Resistance to erythromycin was observed in the strain with MIC > 1 µg / ml.

All the 335 Streptococci evaluated with moxifloxacin presented a mean of inhibition zone 22mm, which is higher to dose recommended for the fabricant (18mm). Nevertheless there was not any significant statistical (p = 0.5390).

Discussion

In the present study 31 different haemolytic Streptococci were isolated, being classified as C, G (forming great size colonies), and F Lancefield groups causing pharyngitis diagnosed on bases of clinical features and symptoms. Never mind, the aetiological role of these SBH, remains to be further evaluated, as they are not still well defined. James et al. [1] have associated group C SBH with exudative pharyngitis. Baquero et al. [2] also identified these species on its research work. So it is recommended the performance of further prospective studies on the association of SBH in these patients.
in children, but it must be taking into account some cases of allergic problems and or unexpected resistance problem, it is important to have another therapeutic choice.

None of the 335 isolated strains from Mexican children showed resistance or intermeditated value to moxifloxacin, suggesting that these strains are susceptible to this quinolone, being it a suitable therapeutic alternative for patients allergic to penicillin or resistant to macrolides. It must be considered that the employment of quinolones in paediatric patients as they may be toxic for their articulations.

The results or high sensitivity to quinolones differ from other reports who have demonstrated the presence of parC and gyr A genes on S. pyogenes, conferring them resistance to quinolones [14,15], these results implicates that geographic region is important on the distribution of SBH having these genes.

### Table 1: Percentage of susceptibility of beta haemolytic Streptococcus

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n= 335</th>
<th>β-lactamic</th>
<th>Quinolones</th>
<th>Erythromycin</th>
<th>Clindamycin</th>
<th>Fenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (pyogenes)</td>
<td>304</td>
<td>100% (304)</td>
<td>100% (304)</td>
<td>89.5% (272)</td>
<td>100% (304)</td>
<td>M</td>
</tr>
<tr>
<td>G</td>
<td>26</td>
<td>100% (26)</td>
<td>100% (26)</td>
<td>69.2% (18)</td>
<td>100% (26)</td>
<td>M</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>100% (4)</td>
<td>100% (4)</td>
<td>100% (4)</td>
<td>100% (4)</td>
<td>M</td>
</tr>
<tr>
<td>F</td>
<td>1</td>
<td>100% (1)</td>
<td>100% (1)</td>
<td>100% (1)</td>
<td>100% (1)</td>
<td>M</td>
</tr>
</tbody>
</table>

The resistance to macrolides observed in the present work (11.05% of 335) was similar to other geographic groups who has been reported among S. pyogenes, France (22.4%), Spain (29.7%), Germany (14%), Greece (38%), Poland (12%) and Italy (31.3%) (3-8). In North America this frequency is lower, United States (6.8%) Canada (4.6%) [16,17]. In Asiatic region it seems to be higher, a study in Korea reported that 63.3% of SBH had the phenotype (MSLB) resistant to macrolides-lincomicine-streptogramine, 23.9% had the M phenotype and 12.8% had the inducible MLSB [18]. The observed resistance to erythromycin when observed by individual groups was 10.5% of SBHGA and 30.8% for group G. This result was due probably for the employment of macrolides against another pathogen bacterium, like S. pneumonia [19]. So it should be advisable to employ macrolides for pharyngitis problems to allergic patients after performing microbiological studies and avoid the empiric antibiotics therapeutic measures for these patients.

In the present work the phenotype M due to gen mef (A) was the only observed resistance mechanism, none of the studied strains had iMLSB, CMLSB.

These results suggest that a selective and clone resistance to erythromycin has been presented among this population. These results are similar to those found in Spain [4], Germany [5], Greece [6], Canada [20] and Chile [21] were the phenotype M was predominant. In contrast, in Italy, United States, Poland and France, the genes ermB, ermA and subclass erm (TR) were respectively the main resistant mechanism.

### References


