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

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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, ceftriaxone, 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 pharyngoamigdalitis 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 Penicilin, macrolids has been frequently employed, especially for those patients sensible 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 resistence to macrolides by two mechanisms of action: The first one is due to gen *mef A*, and confers resistant to macrolides of 14 or 15 carbons but not to those of 16, lincosamides 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, lincosamides 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 pharyngoamigdalitis. All patients presented through pain, fever, difficulty swallowing, tonsilopharingeal erythem, 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 (Slidex Strepto-Kit Bio-Meraux , Lyon France).

Antimicrobial sensitivity

It was determinate by the disk diffusion method, according ling 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 a128.0 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 35°C con 5% de CO_2 results were interpreted according ling to recommendations of CLSI (National Committee for Clinical Laboratory Standards, (2012) [13]. Recommended values for erythromycin were ≤0.25 µg/ml (susceptible), ≥1 µg / ml (resistant).

Moxifloxacin (Bayer diagnostics), 5 µg/disc was evaluated against all isolates, according to CLIS manual [13]. In order to know the inhibition diameter sizes and know if there was a significant statistical difference according ling to those recommended by the fabricant, a t-student was performed (P<0.05). The recommended values were (sensible ≥18 mm, intermediate 16-17 mm, resistant ≤15 mm).

Macrolid resistant phenotypes

The double disc diffusion method was employed for this purpose. The tested strain was poured on 5% blood sheep agar as described above on antibiotic susceptibility section. Then erythromycin 15 µg and clindamycin 2 µg disks were placed at a 12 mm distance. Inhibition zones shapes were evaluated, a flattening inhibition zone on clindamycin discs and an absence or inhibition zone in both antibiotics was evaluated. An M phenotype was considered when a flattened inhibition zone is observed between clindamicine and erythromycin disks [9].

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 respectably. 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 among 10.5% of *Streptococcus pyogenes* and 30.8 % of group G *Streptococcus* (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).

Phenotype

The proximal flattening of inhibition zones around clindamycin and the sensibility to clindamycin without any alteration of the inhibition zone and resistance to erythromycin, was considered as an efflux antibiotic resistance mechanism (Phenotype M). The obtained results are presented on Table 1.

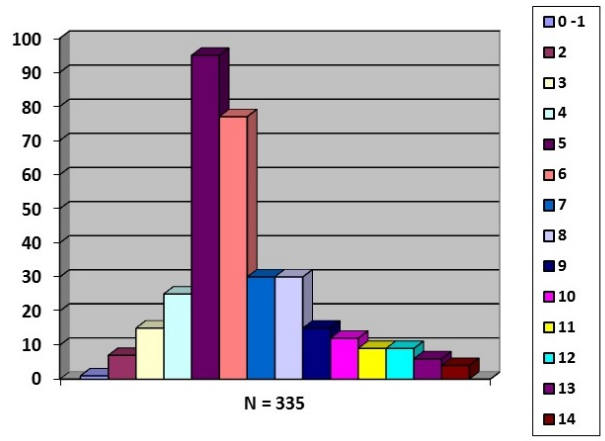

Figure 1: Prevalence of BHS from age (0 to 14 years old)

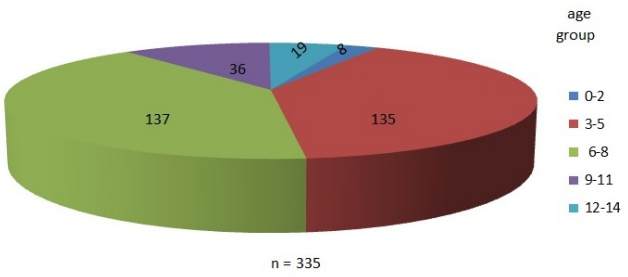

Figure 2: Distribution of BHS on different age ranges

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.

On the present work it was not observed any penicillin resistance among SBH, so it is recommended that this antibiotic should be continue to be considered the first election treatment for pharyngitis

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 intermediated 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.

Streptococcus ß- hemolíticus	n= 335	ß- lactàmic	Quinolones	Erytrhomycin	Clindamycin	Fenotype
A (pyogenes)	304	100 % (304)	100% (304)	89.5% (272)	100% (304)	M
G	26	100% (26)	100% (26)	69,2% (18)	100% (26)	M
C	4	100% (4)	100% (4)	100% (4)	100% (4)	
F	1	100% (1)	100% (1)	100% (1)	100% (1)	

Table 1: Percentage of suseptibility of beta haemolytic Streptococcus

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%), Polonium (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, Polonium and France, the genes *ermB*, *ermA* and subclass *erm (TR)* were respectively the main resistant mechanism.

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