

Throat Carriage Rate and Antimicrobial Resistance of *Streptococcus pyogenes* In Rural Children in Argentina

Gastón Delpech¹, Mónica Sparo^{1,2}, Beatriz Baldaccini¹, Gisela Pourcel¹, Sabina Lissarrague², Leonardo García Allende³

¹School of Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires, Olavarría; ²Hospital Ramón Santamarina, Tandil; ³Center of Biochemical Studies, Tandil, Argentina

Objectives: The aim of this study was to determine the prevalence of asymptomatic carriers of group A β -hemolytic streptococci (GAS) in children living in a rural community and to investigate the association between episodes of acute pharyngitis and carrier status.

Methods: Throat swabs were collected from September to November 2013 among children 5-13 years of age from a rural community (Maria Ignacia-Vela, Argentina). The phenotypic characterization of isolates was performed by conventional tests. Antimicrobial susceptibility was assayed for penicillin, tetracycline, chloramphenicol, erythromycin, and clindamycin (disk diffusion). The minimum inhibitory concentration was determined for penicillin, cefotaxime, tetracycline, and erythromycin.

Results: The carriage of β -hemolytic streptococci was detected in 18.1% of participants, with *Streptococcus pyogenes* in 18 participants followed by *S. dysgalactiae* ssp. *equisimilis* in 5. The highest proportion of GAS was found in 8 to 10-year-old children. No significant association between the number of episodes of acute pharyngitis suffered in the last year and the carrier state was detected ($p > 0.05$). Tetracycline resistance (55.5%) and macrolide-resistant phenotypes (11.1%) were observed. Resistance to penicillin, cefotaxime, or chloramphenicol was not expressed in any streptococcal isolate.

Conclusions: The present study demonstrated significant throat carriage of GAS and the presence of group C streptococci (*S. dysgalactiae* ssp. *equisimilis*) in an Argentinian rural population. These results point out the need for continuous surveillance of GAS and non-GAS carriage as well as of antimicrobial resistance in highly susceptible populations, such as school-aged rural children. An extended surveillance program including school-aged children from different cities should be considered to estimate the prevalence of GAS carriage in Argentina.

Key words: *Streptococcus pyogenes*, Prevalence, Pharyngitis, Rural population, Argentina

INTRODUCTION

Streptococcus pyogenes (group A β -hemolytic streptococci, GAS) is a re-emerging pathogen worldwide and one of the

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Corresponding author: Mónica Sparo, PhD
1406 Gral Paz, Tandil 7000, Argentina

Tel: +54-249-442-2011, Fax: +54-249-442-8797

E-mail: monicasparo@gmail.com

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biggest challenges for public health. Most commonly, GAS infections are observed in the pharynx. Children between five and seven years of age are more susceptible, with seasonal patterns in occurrence. Human skin and mucous membranes are their only known natural reservoirs. Transmission occurs through asymptomatic nasopharyngeal carriers [1]. The clinical manifestations range from superficial to invasive disease, which may present with clinical manifestations associated with toxin production and high mortality rates [2].

GAS remains sensitive to penicillin, the antimicrobial of choice in the empirical treatment of streptococcal infections. Macrolides are a therapeutic alternative for patients allergic to

penicillin. Macrolide resistance develops due to their use in clinical practice, and has become a worldwide emerging problem [3]. One of the mechanisms of macrolide resistance is inducible (i) or constitutive (c) dimethylation of 23S ribosomal RNA (the MLS_B phenotype). Additionally, the existence of an active efflux mechanism (M phenotype) has been described. The most common mechanism of tetracycline resistance is ribosomal protection. Chloramphenicol resistance occurs through drug inactivation by acetylation [4]. In Argentina, resistant penicillin strains have not been detected, and a prevalence of tetracycline resistance lower than 10% has been reported. Furthermore, in Argentina, an increase in erythromycin resistance has been observed, with the M phenotype as the predominant mechanism [5].

The asymptomatic carriage of GAS in the population of school-aged children is a frequent and dynamic event. Colonized children can eliminate GAS, remain carriers, or develop an infectious disease. Although colonization usually is harmless for pediatric carriers, they could transmit GAS to others. Throat carriage has gained great importance due to the re-emergence of severe diseases produced by this bacterium [6].

Worldwide, each year, approximately 15% of school-aged children are estimated to have GAS pharyngitis [7]. The County Health System of Tandil (Buenos Aires Province, Argentina) reported a significantly greater prevalence in the rural area of María Ignacia-Vela. Rural pediatric carriage may constitute a GAS reservoir associated with increases of acute pharyngitis episodes.

The aim of this study was to determine the prevalence of asymptomatic carriers of GAS in children living in a rural community and to investigate the association between episodes of acute pharyngitis and carrier status.

METHODS

Study Design (Observational, Prospective, and Transversal Research)

Collection and initial processing of samples

Throat swabs were collected from September to November 2013 among children from the rural community of María Ignacia-Vela, Tandil County, Argentina.

Briefings with parents or guardians were carried out and written informed consent was obtained. This research was approved by the Ethics Committee, Hospital de Clínicas, Argentina.

Children between 5 and 13 years of age, without acute

pharyngitis, who had not received antimicrobials for four weeks and who had not been hospitalized for 3-month prior to obtaining the samples were included as participants.

The appropriate ethical regulations (Declaration of Helsinki and Argentinian laws) were applied. Results were analyzed in an anonymous fashion, ensuring the confidentiality of the information that was obtained.

A total of 138 participants were considered, but after several meetings with the goal of including as much of the pediatric population as possible, nine children were eliminated because the parents did not agree to participate and two refused to be included. A total of 127 children 5-13 years of age, with a mean age of 10.40 ± 3.19 years, were included. Participants were separated into three age groups. Group I included children 5-7 years of age; group II, children 8-10 years of age; and group III, children 11-13 years of age. Children with GAS in their throats without signs or symptoms of acute tonsillitis at the time of examination were considered to be asymptomatic carriers.

Throat swab samples were inoculated onto Columbia agar with 5% sheep blood and incubated at 35°C in 5% carbon dioxide for 24 hours.

Phenotypic characterization of bacterial isolates

The phenotypic characterization of bacterial isolates to the species level was carried out by conventional tests and the detection of Lancefield-group antigens (BioMérieux, Buenos Aires, Argentina), following the manufacturer's instructions [2]. The quality controls were *S. pyogenes* ATCC 19615 and *S. agalactiae* ATCC 13813.

The VITEK 2[®] Compact automated system (BioMérieux) was used for the validation of the phenotypic characterization.

Each isolate was assigned a co-relative alphanumeric identification code that included the sampling date. An electronic record was maintained in an Excel[™] file.

Antimicrobial susceptibility in vitro tests

Qualitative antimicrobial susceptibility was investigated by the disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) [8]. The antimicrobial disks used were penicillin (10 IU), tetracycline (30 µg), and chloramphenicol (30 µg).

For detection of macrolide and lincosamide resistance phenotypes, the double-disk method was used, placing erythromycin (15 µg) and clindamycin (2 µg) disks closely together, at 15 mm from edge to edge, in the same Mueller-Hinton agar

Table 1. Prevalence of β -hemolytic streptococci among children 5-13 years of age in the rural community of Maria Ignacia-Vela, Tandil, Argentina

Throat culture	n	%
GAS	18	14.2
Group C	5	3.9
Total (n=127)	23	18.1

GAS, group A β -hemolytic streptococci (*Streptococcus pyogenes*); Group C, *S. dysgalactiae* ssp. *equisimilis*.

plate with 5% sheep blood [8].

Quantitative susceptibility was studied by the agar dilution method, according to the recommendations of CLSI [8]. The minimum inhibitory concentration (MIC) for penicillin, cefotaxime, tetracycline, and erythromycin were determined. When an isolate expressed resistance to more than two antimicrobial groups, it was considered to be multidrug resistant.

Statistical Analysis

Data analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Descriptive measures (mean \pm standard deviation) were used for quantitative variables, and frequencies were used to present qualitative variables. The chi-square test was used for verifying the existence of associations. Odds ratios (ORs) with 95% confidence intervals were used for risk quantification, and the *t*-test for independent samples was used to compare mean values. The *p*-values <0.05 were considered to indicate statistical significance.

RESULTS

Group A β -hemolytic Streptococci (*Streptococcus pyogenes*) Prevalence

In this study, carriage of β -hemolytic streptococci was detected (18.1%) among 5 to 13-year-old children from a rural population. Table 1 shows the distribution of bacterial isolates according to the phenotypic characterization. GAS was the most prevalent type β -hemolytic streptococci found (n=18), followed by group C β -hemolytic streptococci (n=5).

The distribution of GAS carriers and non-carriers by age group is shown in Table 2.

The highest percentage of GAS was found in children from group II (55.6%), followed by participants from groups I (38.9%) and III (5.5%). GAS isolates were recovered from male (55.6%) and female (44.4%) children. In all groups, differences between female and male carriage were detected, with fe-

Table 2. Prevalence of group A β -hemolytic streptococci (*Streptococcus pyogenes*) by age group among children 5-13 years of age in the rural community of Maria Ignacia-Vela, Tandil, Argentina

Group	Age (y)	n _{nc}	n _c (f/m)	% _c
I	5-7	40	7 (4/3)	5.5
II	8-10	31	10 (4/6)	7.9
III	11-13	38	1 (0/1)	0.8
Total (n=127)		109	18	14.2

nc, non-carrier; c, carrier; f, female; m, male.

Table 3. Episodes of acute pharyngitis among children 5-13 years of age in the rural community of Maria Ignacia-Vela, Tandil, Argentina

Culture	Acute pharyngitis episodes	
	$\geq 2^1$	Total
GAS-negative	21 (19.3)	109
GAS-positive	4 (22.2)	18
Total	25 (19.7)	127

Values are presented as number (%).

GAS, group A β -hemolytic streptococci (*Streptococcus pyogenes*).

¹Number of episodes.

male predominance in group I (57.1%). Male patients positive for GAS carriage were more common than female carriers in groups II (60%) and III (100%).

In Table 3, the culture results are shown for GAS and number of patients with ≥ 2 episodes of acute pharyngitis.

In this study, no significant association was found between the number of episodes of acute pharyngitis suffered in the last year and carrier status ($p>0.05$). According to these results, carrier children had 1.1 times the risk of having ≥ 2 episodes of acute pharyngitis than those who did not carry GAS (OR, 1.1). It is important to note that most of the samples were not positive for GAS.

Antimicrobial Susceptibility

All GAS isolates were susceptible to cell-wall active agents, such as the β -lactam penicillin (MIC ≤ 0.06 μ g/mL) and a third-generation cephalosporin, cefotaxime (MIC ≤ 0.06 μ g/mL).

Chloramphenicol-resistant GAS were not recovered from participants. Nevertheless, resistance to three of the assayed antimicrobials was expressed among human *S. pyogenes* isolated from this rural population.

Tetracycline resistance was observed in 10 of the 18 (55.5%) studied GAS isolates. This resistance phenotype was the most prevalent among non-susceptible streptococci. Furthermore,

the MIC range for tetracycline was the widest, ranging from 1 to ≥ 16 $\mu\text{g/mL}$.

In 2 of the 18 (11.1%) samples of *S. pyogenes*, macrolide resistance phenotypes were detected.

GAS-007 expressed a cMLS_B phenotype, with high-level erythromycin resistance (MIC, 256 $\mu\text{g/mL}$) and resistance to clindamycin.

Erythromycin resistance (MIC, 8 $\mu\text{g/mL}$) and clindamycin susceptibility were observed in isolate GAS-018 (M phenotype).

DISCUSSION

In this study, 18.1% of the children were carriers of β -hemolytic streptococci, mostly characterized as GAS (14.2%), as well as *S. dysgalactiae* spp. *equisimilis* to a lesser extent (3.9%).

Carapetis et al. [7] showed that GAS carriage prevalence decreased with age, but in this study, the highest prevalence occurred in children 8-10 years of age (group II, 7.9%), and a significantly lower prevalence was found in older children (group III, 0.8%).

In the present study, a rate of global throat GAS carriage of 14.2% was found. Similarly, Takeuchi et al. [9], in a study of school-aged children, reported a rate of 12.2%. However, the Argentinian Society of Pediatrics established the prevalence to be around 5 to 7% [10]. According to Kreikemeyer et al. [11], carrier status, relapses, and recurrent infections by GAS can be explained by the same mechanism. Additionally, being a healthy carrier has been proposed to facilitate recurrent acute infections. Nevertheless, in the present study, no relationship was found between colonization and the number of episodes of acute pharyngitis suffered in the last year.

In this study, carriage prevalence was higher among children between 8 and 10 years of age (group II), followed by 5 to 7-year-old participants (group I), while a lower prevalence was detected for the age range of 11-13 years (group III). In Morocco, a pediatric and adult population study showed that one of the two peaks of GAS prevalence was found among participants with a similar age range to group III, unlike what was observed in this study [12]. In Argentina, a study of GAS frequency in the throats of symptomatic pediatric patients showed that children between 5 and 10 years old were positive at a higher rate than those aged 11-13 years [13].

Antimicrobial resistance is an important concern for many bacterial pathogens. Specifically, GAS can cause invasive infections, making it important to monitor the emergence of resis-

tant strains.

In GAS isolates, resistance to β -lactams such as penicillin and cefotaxime was not detected. In other countries, resistance to β -lactams has likewise not been observed. A multicenter study that included 25 institutions from eight European countries showed that all the investigated GAS isolates did not express resistance to penicillin or cefotaxime, in agreement with the results of this research [14].

In addition, resistance to chloramphenicol was not observed. In agreement with this result, previous Argentinian studies have not reported the recovery of chloramphenicol-resistant *S. pyogenes* [2,15]. In 188 streptococcal isolates recovered from children with pharyngitis in China, chloramphenicol resistance was not detected in any of the studied GAS isolates [16].

These GAS isolates from rural children presented a high prevalence of tetracycline resistance (55.5%). In an Argentinian multicenter study, a lower frequency of tetracycline resistance (7.3%) was observed among invasive and non-invasive *S. pyogenes* [2]. The prevalence of tetracycline-resistant GAS found in this study is significant, since previous reports have established tetracycline resistance as a relevant cofactor in the selection of erythromycin resistance [17,18]. A study in children from China reported a very high prevalence of tetracycline resistance among GAS isolates (92%), almost twice the frequency observed in this study [16].

In 1.1% of GAS isolates from the studied region, erythromycin resistance was observed, expressed as two different macrolide-resistant phenotypes, M and cMLS_B . In Chinese pediatric patients, high levels of resistance to erythromycin (94.0%) and clindamycin (96.9%) were detected. These frequencies were significantly higher than those reported in this study, indicating the predominance of the cMLS_B phenotype [19]. The MLS_B phenotype was also the most prevalent among GAS isolates from pediatric patients in 8 European countries, while MLS_B phenotypes as well as a lesser frequency of M phenotype strains were observed in French children [14,20].

In 2004, in Argentina, a study that included nationwide reports assessing antimicrobial resistance in GAS isolates concluded that there were two prevalent macrolide resistance phenotypes among circulating streptococcal strains, the M and cMLS_B phenotypes, although the M phenotype was not considered to be frequent [15].

The present study showed significant throat carriage of GAS and the presence of group C streptococci (*S. dysgalactiae* ssp. *equisimilis*) in an Argentinian rural pediatric population.

These results underscore the need for continuous surveillance of the carriage of GAS and other β -hemolytic streptococci, as well as of antimicrobial resistance in highly susceptible populations, such as school-aged rural children. An extended surveillance program including school-aged children from different cities should be considered to assess the situation in Argentina.

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CONFLICT OF INTEREST

The authors have no conflicts of interest associated with the material presented in this paper.

ORCID

Gastón Delpech <http://orcid.org/0000-0002-9109-7615>

Monica Sparo <http://orcid.org/0000-0002-0768-894X>

Beatriz Baldaccini <http://orcid.org/0000-0002-1539-6947>

Gisela Pourcel <http://orcid.org/0000-0002-5095-2160>

Sabina Lissarrague <http://orcid.org/0000-0002-4041-0931>

Leonardo García Allende <http://orcid.org/0000-0001-5237-2840>

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