Antimicrobial susceptibility of *Streptococcus agalactiae* isolated from pregnant women in Misiones, Argentina

M. Quiroga¹, E. Pegels¹, P. Oviedo¹, M. Laczeski¹, M. Vergara¹


*Streptococcus agalactiae* or Group B *Streptococcus* (GBS) is the leading cause of neonatal infections. The aims of this study were to determine the susceptibility patterns of GBS infection in Misiones, Argentina. To this end, 3125 pregnant women were studied between 2004 and 2010, and 293 GBS strains were identified. A total of 96 GBS strains were randomly selected for in vitro susceptibility testing. No resistance to penicillin, ampicillin, quinupristin-dalfopristin, and vancomycin was found. High-level resistance to gentamicin was not detected in any of the isolates. The rate of resistance to erythromycin (11.6%) was higher than that reported previously in Argentina. The identification of resistant strains in this study suggests that these agents should be used with caution in the prophylaxis or treatment of GBS infection. The knowledge of the most prevalent phenotypes in our region is essential to carry out appropriate surveillance and appropriate procedures for the control and prevention of GBS infection.

**Keywords:** Group B streptococci; antimicrobial susceptibility; macrolides; phenotype; pregnant women.

### 1. Introduction

Group B *Streptococcus* (GBS) continues to be an important cause of infection and of significant morbidity and mortality in newborns and pregnant and non-pregnant women. Infants acquire GBS in utero by the ascending route through ruptured or intact membranes or during the birth process [1].

With the specific objective to prevent the early onset of GBS disease in the neonate, the Centre for Disease Control and Prevention (CDC) [2] recommended two strategies to identify mothers colonized with GBS. The strategies consist in identifying important maternal risk factors (previous child with invasive GBS disease, GBS bacteriuria during this pregnancy, delivery at <37 weeks’ gestation, intrapartum temperature ≥ 38°C, membrane rupture ≥ 18 hours) or carrying out routine cultures of anogenital swabs in pregnant women at 35-37 weeks of gestation.

If colonization is detected, an antibiotic prophylaxis during delivery is recommended, since it may effectively prevent transmission of GBS to the newborn. In most cases, this prophylaxis results in a significant decrease in invasive neonatal GBS infections [3].

In our country, the search for GBS in all pregnant women with gestational age between weeks 35 and 37, who either present or not risk factors, has been obligatory by Law Nº 26369 for the entire nation since April 2008 (http://test.e-legis-ar.msal.gov.ar - 2008).

Penicillin G or ampicillin is still the drug of choice for prevention of perinatal GBS disease and erythromycin and clindamycin are recommended as treatment for women who are penicillin-intolerant.

The widespread use of antibiotics carries the potential for emergence of antibiotic resistance. Recent reports of the increasing incidence of macrolide and clindamycin resistance in different countries have raised concerns about the possibility of inadequate prophylaxis or treatment with these antibiotics.

The purpose of this study was to determine the susceptibility patterns of GBS in a population of pregnant women in Misiones, a province situated in the northeastern of Argentina, which limits with Paraguay and Brazil.

### 2. Materials and methods

A total of 3125 pregnant women between 35 and 37 weeks of gestation were enrolled in the study between 2004 and 2010. Vaginal-rectal swabs were collected without speculum and then placed in Stuart transport medium (Ventura Transystem- Medica-Tec Argentina) and transported to the laboratory at room temperature for microbiology analysis. Swabs were incubated in a selective Todd-Hewitt broth containing colistin (10 µg/ml) and nalidixic acid (15 µg/ml) (Laboratorios Britania-Argentina) for 18-24 hours before subculture onto sheep blood agar plates (Agar Columbia-Laboratorios Britania Argentina) for 18-24 hours at 37 °C with 5% CO₂. If GBS was not identified after the incubation for 18-24 hours, the blood agar plates were reincubated and examined at 48 hours to identify suspected organisms.

GBS strains were identified by biochemical standard methods. GBS identification was completed by group B-specific latex agglutination (Phadebact Strept B Test- ETC International-Bactus AB-Sweden). Serotypes were detected using Strep-B Latex (Statens Serum Institut, Denmark) for the identification of the capsular polysaccharide antigens Ia, Ib, II, III, IV, V, VI, VII, VIII and IX, according to the manufacturer’s instructions.
A total of 96 GBS strains, randomly selected for in vitro susceptibility testing to penicillin G, ampicillin, tetracycline, levofloxacin, gatifloxacin, quinupristin-dalfopristin, vancomycin, were tested using microdilution panels (MicroScan, Dade Behring Inc, USA).

The isolates were considered susceptible or resistant according to the MIC breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) [4].

The MIC of gentamicin (Bagó, Argentina) was determined by the agar dilution method according to the guidelines of the CLSI. High-level resistance to gentamicin was defined as a MIC of ≥500 µg/ml.

A total of 112 strains with an erythromycin-resistant phenotype were determined by the double-disk diffusion method with disks containing erythromycin (15 µg) and clindamycin (2 µg) from Laboratorios Britannia, Argentina, on sheep blood agar plates (Agar Mueller -Hinton – Biokar, France) for 18-24 hours at 37 ºC with 5% CO₂.

Different phenotypes of macrolide-lincosamide-streptogramin B (MLSₐ) resistance were recognized in accordance with the description of Seppälä et al. [5] and CLSI recommendations [4]. Inducible clindamycin resistance by erythromycin was detected by a blunting of the clindamycin zone closest to the erythromycin disk, giving the appearance of a “D” (phenotype iMLSₐ). Resistance to clindamycin (confirmed by the agar dilution method) with no blunting of the clindamycin inhibition zone indicated constitutive resistance (cMLSₐ).

The M phenotype was characterized by susceptibility to clindamycin with no blunting of the inhibition zone around the clindamycin disk.

3. Results

All GBS were susceptible to penicillin G, ampicillin, quinupristin-dalfopristin, and vancomycin.

Of the isolates examined, 88.5 % were susceptible to clindamycin, 85.4 % to erythromycin, 59.4 % to tetracycline, 99.0% to gatifloxacin and 95.8 % to levofloxacin.

The rank order of susceptibility for GBS for the quinolones was: gatifloxacin (99.0 %) > levofloxacin (95.8%).

The gentamicin MIC ranged from 2 to 256 µg/ml (MIC₉₀ = 64 µg/ml and MIC₅₀= 8 µg/ml). High-level resistance to gentamicin was not detected in any of the isolates.

Among the 112 isolates, 1.8 % was resistant to clindamycin and 11.6% to erythromycin. Among the erythromycin-resistant isolates, nine displayed the cMLSₐ resistance phenotype, two the iMLSₐ resistance phenotype, and two the M resistance phenotype. Six of the thirteen erythromycin-resistant strains belonged to serotype Ia, four to serotype III, two to serotype II and one to serotype V.

4. Discussion

The antibiotics tested in this study were those that are considered to have potential clinical utility for the treatment and prophylaxis of streptococcal infections and, therefore, have susceptibility breakpoints recommended by the CLSI [4].

GBS is sensitive to many antimicrobial agents, especially β-lactam antibiotics. Penicillin G and ampicillin are the antimicrobial agents extensively used against SGB. Penicillin G continues to be the antibiotic of choice for intrapartum prophylaxis for GBS-colonized pregnant women because of its effective transplacental passage, its low cost and broad spectrum of action directed at streptococcal infection [6].

In this study neither penicillin-resistant nor intermediate penicillin-susceptible GBS strains were found, as it was reported by different authors [7-10].

Our results show that all strains of GBS remain susceptible to penicillin or ampicillin.

Some authors have described in vitro studies where the rate of killing of GBS by these antibiotics is relatively slow as compared with that of other streptococcal pathogens. This has led some authors to recommend dual therapy using a penicillin plus gentamicin for severe GBS-associated infection [11].

This approach requires the pathogen to be susceptible to both penicillin and aminoglycoside antibiotics. High-level resistance to aminoglycoside indicates that the isolate will not be affected synergistically by the combination of both penicillin and aminoglycoside antibiotics.

None of the strains tested in this study showed high-level resistance to gentamicin.

We detected high percentages of tetracycline-resistant GBS. Our results are similar to those previously reported in Argentina [12] and other countries [13].

Resistance to quinolones has only recently been described for GBS [14]. In our study, we identified 1.0 % of the isolates resistant to gatifloxacin and levofloxacin, respectively. This rate is in agreement with the data published by Gordon et al. [15].

Erythromycin and clindamycin are recommended as treatment for women who are penicillin-intolerant.

However, recent reports of the increasing incidence of macrolide and clindamycin resistance in several countries, with some geographic variations [16], have raised concerns about the possibility of inadequate prophylaxis or treatment with these antibiotics as alternative agents in these patients.
We further found that resistance to erythromycin and clindamycin varied among geographic regions and was notably higher in California and Texas (32% and 23.4% respectively) [1].

However, in Europe, there are few data concerning this important increase in resistance to macrolides and lincosamides. In a study with strains obtained in Spain [16], a high percentage of resistance to macrolides and lincosamides, similar to that reported in the USA, was found.

Two resistance mechanisms have been described: modification by methylation of the antibiotic target, the ribosome, and the active efflux of the antibiotic across the membrane.

The most frequently macrolide resistance mechanism encountered in streptococci is target site modification by a methylase encoded by the erm gene, which leads to the inducible or constitutive expression of resistance phenotypes (iMLSB and cMLSB respectively).

The drug efflux by a membrane-bound protein encoded by a mef gene confers resistance to 14- and 15-membered macrolides, but susceptible to lincosamides and streptogramin B (M phenotype).

In this study, we report the prevalence and mechanisms of macrolide-lincosamide resistance in 112 GBS isolates.

Thirteen (11.6%) of the isolates were resistant to erythromycin and 2 (1.8%) to clindamycin.

This rate are comparable with data published by our study group [17][18] from GBS colonizing strains as well as with data from a Spanish multicenter study [10] from GBS colonizing strains and isolates from newborns diagnosed with early onset of the disease. In addition, this rate is in agreement with data reported by Lermontov Borges et al. (9.4% in Brazil [19]), but higher than those reported in Argentina by Garcia et al. (2.1% of GBS colonizing strains) [20], Lopardo et al. (5.2% of invasive isolates) [12], Di Bartolomeo et al. (1.74%) [21], Mollerach et al. (2.4% of invasive and colonizing isolates) [22], Perez et al. (6% for isolates from infectious processes) [23], Gonzalez et al. (5.9% in Mexico) [24], Simeso et al. [25] and Correa et al. [26] n Brazil, Martinez et al. (4%) in Chile [27], and lower than rates reported by Betriu et al. (17.4% in Spain) [16], Hsueh et al. (46% in Taiwan) [8] and Young Uh et al. (30% in Korea) [28].

These differences may be explained by the different policies in the use of antimicrobials in different regions.

All nine erythromycin-resistant isolates of GBS presented the cMLSb phenotype, which indicates concomitant constitutive resistance to clindamycin. This result is in agreement with the data published by Lopardo et al. [12] and Pérez et al. [23].

Among the erythromycin-resistant isolates, two expressed the iMLSB resistance phenotype and two the M resistance phenotype. However, some reports have shown that for β-hemolytic streptococci, the M phenotype is significantly more prevalent in the Americas [15].

The data of the iMLSB phenotype isolates and the risk that such organisms may become resistant to clindamycin suggest that laboratories should consider using the D test on GBS strains that are resistant to erythromycin but susceptible to clindamycin.

In this study, we found that six of the thirteen erythromycin-resistant strains belonged to serotype Ia, four to serotype III, two to serotype II and one to serotype V. Different studies have shown that serotype V is associated with erythromycin resistance and that this serotype has emerged as a cause of human disease with high propensity to acquire macrolide resistance and spread [29]. In our study, only one serotype V strain possessed erythromycin resistance.

Our results suggest that for women who are penicillin-intolerant the selection of an alternative prophylactic antibiotic should be guided by the contemporary antibiotic resistance patterns in the region.

The high resistance to erythromycin in Misiones justifies performing antibiotic susceptibility testing. In the absence of these data, erythromycin and clindamycin cannot be relied upon as an alternative antibiotics for prophylaxis and treatment of GBS infections.

The knowledge of the most prevalent phenotypes in our region is essential to carry out adequate surveillance and appropriate procedures for the control and prevention of GBS infection.

Our study is the first to correlate the distribution of macrolide and clindamycin resistance in Misiones, Argentina.

Acknowledgements This study was supported by a grant from Agencia de Investigación Científica y Tecnológica PICTO-UNaM No 36831 BID 1728/OC-AR. FONCYT. Ministerio de Ciencia, Tecnología e Innovación Productiva. Universidad Nacional de Misiones, Argentina.

References


