

## Antimicrobial resistance in $\beta$ -hemolytic streptococci in Argentina

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All  $\beta$ -hemolytic streptococci studied in Argentina were susceptible to penicillin. Resistance to erythromycin in *Streptococcus pyogenes* oscillates between 0.5 to 12% in different regions of our country and it is mainly associated to phenotype M and serotype M12. In *Streptococcus agalactiae* we found a very high rate of tetracycline resistance (72.4%) but approximately 5% of erythromycin resistance. Highly-aminoglycoside resistant isolates of *S. agalactiae* and *Streptococcus dysgalactiae* subsp. *equisimilis* have been described in different studies.

**Keywords:** antimicrobial resistance, gram-positive,  $\beta$ -hemolytic, streptococci

### 1. Resistance to $\beta$ -lactam antibiotics and aminoglycosides

Penicillin (PEN) resistance was not yet demonstrated in A, C or G beta-hemolytic streptococci. On the other hand, in the literature, there are two reports of *Streptococcus agalactiae* isolates that were nonsusceptible to PEN [1, 2].

An Argentinian multicenter study concerning invasive infections caused by beta-hemolytic group A, B, C and G streptococci was performed between October 1998 and March 1999 [3, 4]. All beta-hemolytic streptococci included in this study were susceptible in vitro to PEN, cloramphenicol (CMP) and ceftriaxone (CRO). However, PEN tolerance, a phenomenon of questioned significance, was detected in  $\beta$ -hemolytic streptococci, including Argentinian isolates of *S. agalactiae* [5, 6]. Time-killing curves showed the in vitro enhanced killing activity of beta-lactams when an aminoglycoside (AG) was added [6]. Although either PEN or ampicillin are the antibiotics of choice for *S. agalactiae* infections, the combined use of a beta-lactam plus an AG was suggested by other authors for treating *S. agalactiae* neonatal sepsis, meningitis and endocarditis [7]. As was described for enterococci, such combination could achieve a bactericidal synergy in the absence of an enzymatic mechanism of resistance to AG. In enterococci, the detection of high-level aminoglycoside resistance can predict failure in the bactericidal synergy [8], however, nothing was established for *S. agalactiae* though one highly gentamicin (GEN) resistant and several streptomycin (STR) resistant strains have been described [9, 10]. High-level resistance to AG has been also described in a few isolates of *Streptococcus pyogenes* and *Streptococcus dysgalactiae* subsp. *equisimilis* [11, 12]. One anomalous moderately-highly AG resistant strain was described by us in *S. agalactiae* [3]. In spite of harboring the bifunctional 6'-acetyltransferase-2''-phosphotransferase enzyme, such strain behaved as it was a GEN susceptible strain considering breakpoints established for enterococci (MIC = 128  $\mu$ g/ml). However it cannot be synergically killed by the combination PEN + GEN. Moreover, according to the specificity of the enzyme, PEN + kanamycin (KAN) and PEN + amikacin (AKN) were combinations equally inactive. MICs of KAN and AKN could predict their failure, but only by the agar dilution method or by the use of hipercharged KAN disks (1000  $\mu$ g) currently used for the identification of anaerobic bacteria., changing the current breakpoints

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used for enterococci (no zone should be indicative of resistance and zones  $\geq 17$  mm should be considered susceptible) [3].

High-level AG resistance was due to the presence of *aac(6')-aph(2'')*, *aph(3')-IIIa* and *aadE* in our population of all gram-positive cocci. In one group G *S. dysgalactiae* subsp. *equisimilis* isolate, the spreading of *aac(6')-aph(2'')* was due to Tn4001 present in the  $\alpha$ -form, which has 2 IS256 flanking the *aac(6')-aph(2'')* gene in opposite directions [13].

## 2. Resistance to tetracycline and chloramphenicol

Resistance to tetracycline (TET) and chloramphenicol (CMP) has been observed among groups A, C and G beta-hemolytic streptococci [14, 15], however there is little available data concerning the epidemiology and antimicrobial susceptibility of invasive beta-hemolytic streptococci from Latin American countries.

In our studies lower rates of TET-R (7.3%) were observed in *S. pyogenes* than in *S. dysgalactiae* subsp. *equisimilis* (40.7%). All TET-R isolates were associated with the presence of the *tetM* gene [13]. High percentages (72.4%) of TET-resistant *Streptococcus agalactiae* were detected in one Argentinian multicenter study [3]. These results were similar to those recorded in several studies performed in different countries and in different times: Anthony et al. in the USA, 85.3% in 1975 [16], Traub and Leonhard in Germany, 74.5% in 1997 [17], de Azavedo et al. in Canada, 82 - 87% in 2001 [18], De Mouy et al. in France, 88.1% in 2001 [19], Betriú et al. in Spain, 89% in 1994 [5], Melin et al. in Belgium, 74.2-86.7%, in 2000 [20], and Fujita et al. in Japan, 71.9% in 1985 [21].

Thirty two of the TET-R isolates of *S. agalactiae* harbored only the *tetM* gene. All of them were also resistant to MIN. Three TET-R isolates harbored only the *tetO* gene, being one susceptible, one intermediately-susceptible and one resistant to MIN. Three TET-R and MIN-R isolates harbored both the *tetO* as well as the *tetM* gene. One of the isolates, that was TET-R but MIN-S did not show any of the tested mechanisms [13].

## 3. Resistance to macrolides and lincosamides

Erythromycin (ERY) is regarded as an alternative to PEN in the prophylaxis or treatment of infections due to *S. agalactiae* in PEN-allergic patients.

The most commonly described macrolide-resistance mechanisms in gram-positive cocci are target-site modification [22] and efflux [23]. Target site modification is determined by the *erm* genes which codify a methylase that leads to the inducible or constitutive expression of the MLS<sub>B</sub> resistance phenotype [22], while efflux is mediated by *mefA* (subgroups *mefA* or *mefE*) gene in streptococci [23]. In the first instance, ERY and other 14- and 15-membered macrolides act as inducer agents while clindamycin (CLI), streptogramins B, and 16-membered macrolides become inactive when inducers are present. The constitutive MLS<sub>B</sub> phenotype is characterized by resistance to all macrolides, lincosamides and streptogramins B, even without the presence of an inducer [22]. Phenotype M, due to active efflux, was described as mediated by the *mefA* (subgroups *mefA* or *mefE*) gene in *S. agalactiae* [24] (2).

In our experience, ERY resistance was observed in four invasive isolates of *S. pyogenes* (5.9%) (*mefA* gene) and in 23% of pharyngeal isolates (23.5%). All these isolates showed the M phenotype. Only one ERY-R *S. dysgalactiae* subsp. *equisimilis* was isolated from invasive infections. It showed an inducible-methylase mediated mechanism (*ermTR* gene). No ERY-R isolates were found among pharyngeal group C or G streptococci [4].

ERY-R was detected in 5.2% of the invasive *S. agalactiae* [3]. This rate can be compared to other Argentinian studies [25, 26, 27] (Table 1), Japanese (3.1 - 3.0%) [21] and German (4.9%, 12%) data [17, 28], but it was higher than rates reported in Morocco [29] and lower than rates reported in Spain (8.0 - 14.7 - 18.0%) [5, 30], the USA (7.4%-16%) [10, 31, 32], France (18.0 - 21.4%) [19, 33], and Canada (18.0) [18]. Both M and cMLS<sub>B</sub> phenotypes were observed in two and one ERY-R isolates, respectively. Other authors highlighted the prevalence of MLS<sub>B</sub> phenotype over M phenotype in GBS [19, 29, 30, 32].

**Table 1** Erythromycin (ERY) and clindamycin (CLI) resistance in Argentinian isolates of *Streptococcus agalactiae*

Reference	N° of isolates	Year	ERY R %	CLI R%
Pérez [26]	66	2000-1	6	4.5
Truppia [25]	115	1999-2000	5.2	ND
DiBartolomeo [27]	87	2003-4	2.3	2.3
Our results [3]	58	1998-99	5.2	1.7

MLS<sub>B</sub> resistance is usually mediated by *ermAM/ermB* class genes in *Streptococcus* spp. [34, 35]. The *ermTR* gene was described for the first time in Finland by Kataja *et al.* in group A streptococci [35]. The nucleotide sequence of *ermTR* is 83% identical to that of *ermA* described in *Staphylococcus aureus* and coagulase-negative staphylococci and recently it was included among the *ermA* denomination [36]. In our bacterial population, one isolate of *Streptococcus pyogenes* that has an MLS<sub>B</sub> phenotype, harbored the *ermTR* gene.

MICs of AZI were slightly higher than those of ERY, and it appears to be unuseful to test both macrolides, since susceptibility to AZI can be predicted testing only ERY.

Serotype M12 was strongly associated with erythromycin resistance in *S. pyogenes* isolated from pharyngeal exudates in Argentina. [4]

**Table 2** Erythromycin (ERY) resistance in Argentinian isolates of *Streptococcus pyogenes*

City [Reference]	Year	N° of isolates	ERY R %	Phenotype M (%)
B.Aires [37]	1989	126	1.5	0
B.Aires [37]	1991-1994	247	0	0
Argentina [37]	1994	1,767	0.28	25%
Mendoza [37]	1995-96	135	11.1	100
Neuquén [39]	1997-98	251	12.0	100
B.Aires [37]	1999	884	6.6	94.4
B.Aires [37]	2000	742	9.9	100
Cipolletti [38]	1999-2001	55	4.1	0
Bariloche [38]	2000-2001	395	0.5	50
Córdoba [38]	1999-2001	47	4.2	100
Mendoza [38]	1999-2001	544	5.5	100
Esquel [38]	2001	83	12.0	100

Rates of erythromycin resistance in *S. pyogenes* isolated in our pediatric hospital of Buenos Aires City are similar to results from other Argentinian cities as can be seen in table 2. In this table we also show the evolution of such resistance from 1989 to 2002 in our hospital. As well as other authors we found a positive correlation between antibiotic use and prevalence of resistance [37]. In other Argentinian multicenter study, the average of ERY-resistance rates was 6.7% (range 0.5 - 14.1%) [38]. Control of antimicrobial use should be performed to warrant the future effectiveness of macrolide antibiotics regarding the positive association between use and resistance.

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