

Letter to the Editor

Low macrolide resistance in *Streptococcus pyogenes* in Southern Argentina

Sir,

In recent years, macrolide resistance in *Streptococcus pyogenes* has increased, with great variability over different areas [1–4] and has been associated with increased macrolide use [5,6]. Two main mechanisms for macrolide resistance have been identified: target site modification and drug efflux [7,8].

In Argentina, although prevalence of erythromycin resistance in *S. pyogenes* is still low, it has increased significantly in recent years according to data from densely populated areas.

The aim of this study was to evaluate the dynamics of macrolide resistance in *S. pyogenes* in Bariloche, a small city (circa 100,000 inhabitants) in Northern Patagonia, and to determine the phenotypes and genetic mechanisms of macrolide-lincosamide resistance.

A total of 1068 consecutive *S. pyogenes* strains were collected during 2000–2003 in the clinical microbiology laboratories of the three main medical institutions of Bariloche. Four hundred and forty-four strains were obtained from the city's public hospital and 624 originated from two private hospitals. Of these, 78.6% of isolates were obtained from children (2–16 years old) and 21.4% from adults. Most isolates (1022) were recovered from throat swabs but 44 came from other clinical samples.

Strains were identified using standard procedures: β haemolysis, bacitracin sensitivity, and pyrrolidonyl arylamidase activity.

Macrolide-resistant strains were initially identified by the disk diffusion method on Mueller–Hinton agar supplemented with 5% defibrinated sheep blood (Biomérieux), using a 15 μ g erythromycin disks and 2 μ g clindamycin disks as recommended in the NCCLS guidelines. Penicillin sensitivity was also tested for all strains using an agar diffusion method and 10 units disks. The resistant phenotypes of erythromycin resistant strains were determined by the double-disk test using erythromycin and clindamycin disks separated by 15–20 mm [9]. The MICs of erythromycin and clindamycin were determined for all the macrolide resistant isolates by the Etest method (Biodisk AB, Solna, Sweden).

PCR-based detection of resistance genes was performed as described by Martinez et al. [4]. The primers used to detect

ermA, *ermB*, *ermC*, *ermTR* and *mefA* in *S. pyogenes* have been previously described [10].

Susceptibility testing showed that 26 of 1068 (2.4%) *S. pyogenes* isolates were resistant to erythromycin (MICs 2 to 256 mg/l; Table 1). All resistant strains came from outpatients. Resistance rates in adults were 3.5% and in children, 2.1% (difference not statistically significant; $\chi^2=0.867$, $P=0.35$).

All three different macrolide resistance phenotypes were found: 22 strains (84.6%) expressed the M phenotype, two (7.7%) expressed the constitutive MLS_B phenotype and two (7.7%) the inducible MLS_B phenotype. PCR-based detection of resistance genes showed that all strains showing the M phenotype harboured the *mefA* gene while *ermTR* genes were found in both strains expressing the inducible MLS_B phenotype; *ermB* genes were present in strains with constitutive MLS_B phenotype (Table 1). The M phenotype was the predominant resistance phenotype as shown previously by other investigators in Argentina and South America [3,4]. All *S. pyogenes* studied were susceptible to penicillin.

Resistance values rose significantly from 0.59% during the first year of the study to 5.02% at the end ($\chi^2=5.35$, $P=0.02$). Furthermore, most of the resistant strains isolated during 2003, the last year of study, originated from private institutions where resistance was 6.57%, compared with 1.61% in the public hospital (Table 2). Although these differences were not statistically significant ($\chi^2=3.42$, $P=0.06$), the incidence of resistance in isolates from private hospitals seems to be increasing at a greater rate than that in the population attending the public hospital. While public institutions are attended by social classes with low or no income, private

Table 1
Distribution of MICs of macrolides, phenotypes and genotypes for 26 resistant *Streptococcus pyogenes* isolates

Phenotype	Genotype	<i>n</i>	Antimicrobial agent	MIC (mg/l) Range
M	<i>mefA</i>	22	Erythromycin	2–24
			Clindamycin	0.019–0.125
iMLS _B	<i>ermTR</i>	2	Erythromycin	2–16
			Clindamycin	0.094–0.25
cMLS _B	<i>ermB</i>	2	Erythromycin	>256
			Clindamycin	>256

Table 2
Annual distribution of erythromycin resistance in private and public health institutions

Year	Private		Public		Global	
	Total <i>n</i>	Resistant strains <i>n</i> (%)	Total <i>n</i>	Resistant strains <i>n</i> (%)	Total <i>n</i>	Resistant strains <i>n</i> (%)
2000	–	–	169	1 (0.59)	169	1 (0.59)
2001	141	0 (0)	82	0 (0)	223	0 (0)
2002	209	4 (1.91)	69	1 (1.45)	278	5 (1.80)
2003	274	18 (6.57)	124	2 (1.61)	398	20 (5.02)

hospitals are attended by a wealthier population. The former is typically provided of antibiotics by the institution, which includes a limited use of macrolides. The latter instead, probably consume larger amounts of macrolides especially after the recent introduction of new types of macrolides with improved pharmacokinetic properties.

In conclusion, macrolide resistance in *S. pyogenes* in Bariloche is still low compared with other geographical areas of Argentina with a higher population density, and many other countries. Nevertheless, increase in resistance was statistically significant during the study period. This dynamic pattern and the abundant evidence on increasing resistance in streptococci related to increase macrolide use, suggest that surveillance should be continued and that a careful usage of macrolide antibiotics would be advisable.

Acknowledgements

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