Trends in Macrolide Resistance for *Streptococcus pyogenes, Streptococcus agalactiae* and *Streptococcus pneumoniae* and its Association with Social Clustering in Argentina

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Abstract: The aim of this work was to study and compare macrolide resistance patterns among *Streptococcus pneumoniae, Streptococcus agalactiae* and *Streptococcus pyogenes*, to describe the phenotypes of macrolide-lincosamide resistance and to further investigate associations between macrolide resistance and social clustering. Susceptibility data were obtained from 4 clinical microbiology laboratories in Bariloche, Argentina for the period 2002- 2008. Patients were differentiated in two population clusters according to the institution they attended (public or private). A total of 4310 strains were studied: 2615 *S. pyogenes,* 995 *S. agalactiae* and 700 isolates of *S.pneumoniae.* Erythromycin resistance rates over the study period were 1.5% for *S. pyogenes*, 7.8% for *S. agalactiae* and 11.3% for *S. pneumoniae.* For the complete study group, these values differed significantly among species (contingency table χ^2 = 164.52, p<0.0001). Resistance in *S.pyogenes* and *S. pneumoniae* showed irregular trends whilst *S. agalactiae* showed an increasing tendency during the whole period appearing to be a better indicator of the trends in macrolide resistance. A dual character MLS_B phenotype of both inducible and constitutive resistance was observed in *S. agalactiae* only. Resistance in isolates from the population attending private institutions was significantly higher than those attending the public hospital (*S. pyogenes*, (1.9 vs. 0.6, χ^2 = 5.851, p<0.025); *S. pneumoniae*, 19.1 vs. 7.0 (χ^2 = 21.98, p<0.001), and. *S. agalactiae*, 12.4 vs. 3.8 (χ^2= 24.5, p<0.001)). In conclusion, macrolides resistance rates during 2002-2008 were significantly different for *S. pyogenes, S. agalactiae* and *S.pneumoniae*. We also found significant variations in macrolide resistance levels in different population groups.

Keywords: Erythromycin, streptococci, resistance- trends.

INTRODUCTION

Increasing antimicrobial resistance is becoming a serious international problem in both hospital and community settings [1, 2]. Many factors are probably involved in the emergence and spread of antibiotic resistance, but antibiotic consumption seems to be the main driving force [3-5].

Macrolides are frequently used for the treatment of community-acquired respiratory tract infections and are the first alternative choice for those patients that are hypersensitive to penicillin. Since the introduction of the newer, long-acting macrolides, resistance to these drugs in streptococci has increased worldwide. In addition, resistance rates vary in different geographic areas both among countries and among regions within countries suggesting that resistance surveillance must be undertaken both nationally and locally [3, 6-11].

Macrolide resistance in Streptococci is mediated by two major mechanisms: methylation of the ribosomal macrolide target site which results in resistance to 14-, 15- and 16-membered macrolide, lincosamides and streptogramin B (MLS_B phenotype), and drug efflux which results in resistance to 14- and 15-membered macrolide resistance only (M phenotype) [12]. The prevalence of these two major mechanisms among macrolide resistant streptococci varies in different species and geographically.

In Argentina, although prevalence of erythromycin resistance in streptococci is still low, it has increased significantly in recent years in most regions [9, 13-15]. In previous work carried out in Bariloche we found low overall resistance rates for *S. pyogenes*, but a significant rise in resistance in one particular population group [9]. In that report, we found that isolates originated from groups attending public hospital, showed very little resistance while there was an increase in resistance in isolates from a more affluent group attending private hospitals. Association between antibiotic resistance and social grouping has been previously described in Wales although their results showed higher resistance values associated with deprivation [16].

The aim of this study was to describe erythromycin resistance patterns among different species of streptococci, *S. pneumoniae, S. agalactiae* and *S. pyogenes,* in Bariloche,

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Table 1. **Erythromycin and Clindamycin Resistance in *S. pyogenes, S. pneumoniae* and *S. agalactiae* for the Period January 2002 to December 2008**

	S. pyogenes		S. pneumoniae		S. agalactiae	
	Public	**Private**	**Public**	**Private**	**Public**	**Private**
Erythromycin Resistance (%)	**0.6**	**1.9**	**7.0**	**19.1**	**3.8**	**12.4**
Clindamycin Resistance (%)	**0.2**	**0.5**	**2.6**	**8.3**	**3.0**	**11.1**
Total tested	**820**	**1795**	**454**	**246**	**529**	**466**

Argentina during the period 2002-2008. We describe the phenotypes of macrolide-lincosamide resistance and further investigate associations between macrolides resistance and social clustering.

Materials and Methodology

Susceptibility data for *S. pyogenes, S. agalactiae* and *S. pneumoniae* were obtained from clinical samples routinely collected in Bariloche , a city located in Northern Patagonia, Argentina, between January 2002 and December 2008. Data were available from 4 clinical microbiology laboratories, three private and one public, which together serve more than 80 % of the city population.

Strains were identified using standard manual procedures. Macrolides susceptibility was tested by the disk diffusion method on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (Laboratorio Argentino, Buenos Aires, Argentina), using 15μg erythromycin disks and 2 μg clindamycin disks (Laboratorios Britania, Buenos Aires, Argentina) according to CLSI guidelines. The phenotypes of erythromycin resistant strains were determined by the double-disk test with erythromycin and clindamycin disks separated by 15-20 mm [12].

Two different patient groups were observed according to the institutions they attended. The patients that attend the public hospital mostly lack health insurance and commonly have low incomes. The other group corresponds to patients with medical insurance which can be assigned to a more affluent population.

Differences in the prevalence of resistance between different groups were assessed by the χ^2 test.

RESULTS

During the study period 4310 strains of three different species of *Streptococci* were collected and analyzed. The city's public hospital provided 1803 strains whilst 2507 originated from private hospitals.

A total of 2615 *S. pyogenes* isolates were obtained. The majority (96.8%) were isolated from throat swabs, 1.3% from soft tissue samples and the remaining 1.9% from other samples. The mean age of the patients from which these isolates were obtained was 12.8 years (range: 0 to 78). Nine hundred and ninety five isolates of *S. agalactiae* were obtained from the following samples: rectal-vaginal swabs (46.8%), vaginal swabs (30.7%), urine (15.2%), blood (1.6%) and other sites (5.7%). For this species the patients

mean age was 30.5 years (range: 8 to 89). Finally, 700 isolates of *S. pneumoniae* were obtained from: sputum (59.2%), bronco-alveolar lavage (3.9%), pleural fluid (0.9%), blood (24%), CSF (0.6%) and other sites (11.4%). The mean age for these patients was 47.65 (range: 0 to 92).

The global rates of resistance to erythromycin over the complete study period were 1.5% for *S. pyogenes*, 7.8% for *S. agalactiae* and 11.3% for *S. pneumoniae*. For the complete study group, resistance differed significantly among species (contingency table $\chi^2 = 164.52$, p<0.0001). These differences were also observed when analysing samples obtained from public ($\chi^2 = 44.19$, p<0.0001) and private institutions ($\chi^2 = 188.31$, p<0.0001) separately.

The resistant rates for erythromycin and clindamycin, for the different species and patients groups are described in Table **1**.

Annual resistance rates for *S. pyogenes* showed a significant increase in 2002 compared with data from previous studies (0.6% in 2000, and 0.0% in 2001) with a peak in 2003 and a sharp decline in 2004 (Fig. **1**) [9]. The resistant isolates of this peak (2003) originated mostly from private laboratories (90.5%) and belonged mainly (85.7%) to the M phenotype.
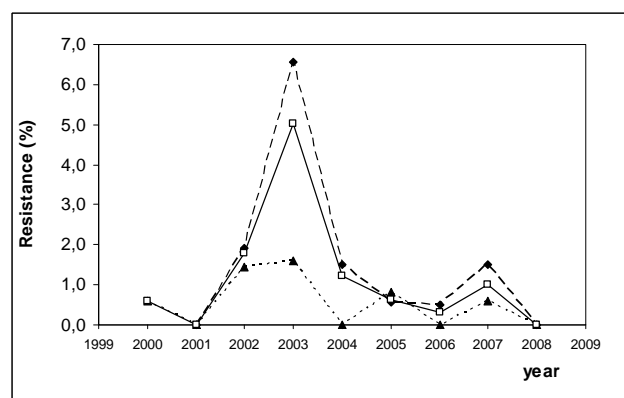

Fig. (1). Erythromycin resistance in *S.pyogenes*. Filled triangles correspond to values for public hospital, filled diamonds for private hospitals and open squares for the total population.

For *S. pneumoniae* erythromycin resistance rates increased from 6.0% in 2002 to 7.2% in 2008 but variations were observed during the study period. Erythromycin resistance rose abruptly from 9% in 2004 to 17.5% in 2005 and 20% in 2006, and fell again to 8.5% in 2007. In this case, the increased resistance values seen during this peak

occurred both in private and public laboratories and 62.5% and 75% of the resistant isolates (respectively) showed the M phenotype (Fig. **2**).
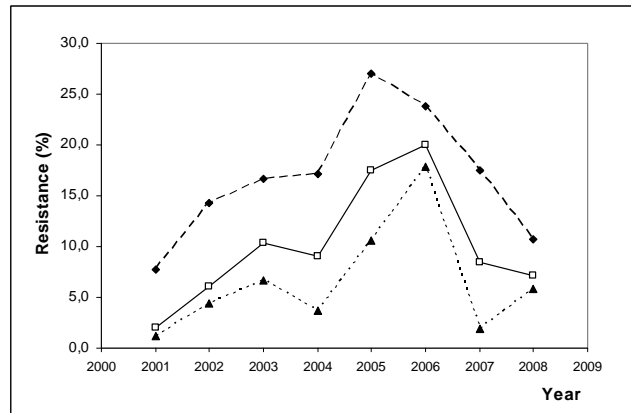

Fig. (2). Erythromycin resistance in *S.pneumoniae*. Filled triangles correspond to values for public hospital, filled diamonds for private hospitals and open squares for the total population.

Resistance for *S. agalactiae* rose from 1.2% in 2002 to 10,7% in 2008 and we noted a steady increasing tendency in resistance during the whole period ($R^2 = 0.77$; p<0.009) with a rapid increase only at the beginning of the study period . This pattern was observed both in strains isolated at private and public institutions (0% in 2002 to 13.2% in 2008, and 2.0% in 2002 to 7.9% en 2008 respectively) (Fig. **3**).
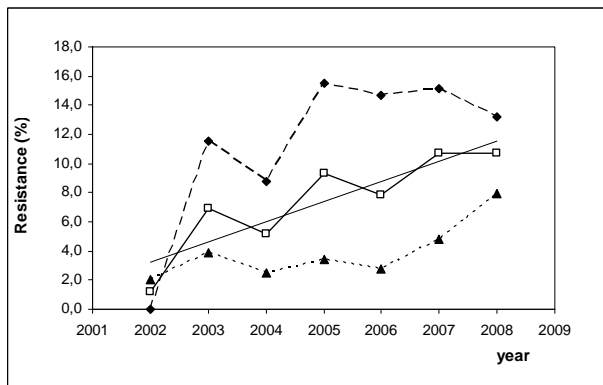

Fig. (3). Erythromycin resistance in *agalactiae*. Filled triangles correspond to values for public hospital, filled diamonds for private hospitals and open squares for the total population. Full line indicates a linear regression line corresponding to data for the total population (r^2=0.77; p<0.009).

During the study period, the incidence of macrolides resistance in isolates from private hospitals was higher than those obtained in isolates from public hospital. These differences were significant for the three species, *S. pyogenes*, ($\chi^2 = 5.851$, p<0.025), *S. pneumoniae*, ($\chi^2 = 21.98$, p<0.001), and. *S. agalactiae*, ($\chi^2 = 24.5$, p<0.001) (Fig. **4**).

Four different phenotypes were identified by the double-disk test. Resistance to erythromycin with blunting of the clindamycin zone of inhibition on the side of the erythromycin disk indicated an inducible MLS$_B$ phenotype (i MLS$_B$), resistance to both erythromycin and clindamycin indicated a constitutive MLS$_B$ phenotype (c MLS$_B$), and

resistance to erythromycin and susceptibility to clindamycin with no blunting, indicated an M phenotype. A dual character MLS$_B$ phenotype of both inducible and constitutive resistance was observed in *S. agalactiae* only. It showed resistance to erythromycin with blunting of the clindamycin zone of inhibition on the side of the erythromycin, such as in the i MLS$_B$, but slight bacterial growth inside the D shaped clindamycin inhibition zone is observed. This phenotype has been recently described in *Staphylococcus aureus* and denominated fMLS$_B$ [17]. The phenotype distribution for each species can be seen in Fig. (**5**). The M phenotype was the predominant resistance phenotype in *S.pyogenes* (76.3%) and S. pneumoniae (60.8%) but less frequent in *S.agalactiae* (12.8%). The cMLS$_B$ phenothype was second in frequency for the three species: *S.pyogenes* (13.2%), *S. pneumoniae* (31.6%) and *S.agalactiae* (30.8%). The inducible MLS$_B$ phenotype (i MLS$_B$) was the predominant phenotype in *S.agalactiae*, and represented only 10.5% and 7.6% of *S. pyogenes* of *S. pneumoniae* resistant isolates respectively. Finally, the f MLS$_B$ phenotype was only observed in *S.agalactiae* (17.9%).
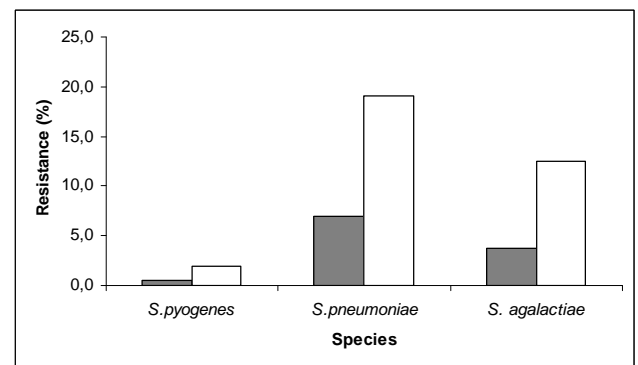

Fig. (4). Erythromycin resistance in *S. pyogenes, S. agalactiae* and *S.pneumoniae* for public hospital (grey bars) and private hospitals (white bars) during the period 2002-2008.
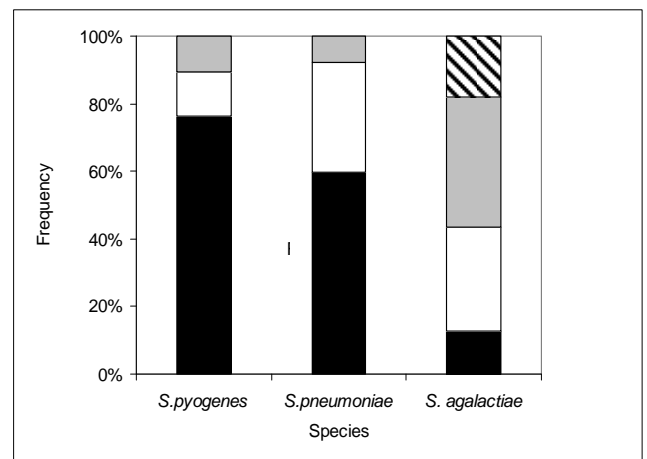

Fig. (5). Erythromycin resistance phenotype distribution in *S. pyogenes , S. pneumoniae and S. agalactiae* during the period 2002-2008. Full bars correspond to M phenotype, white bars correspond to c MLS$_B$ phenotype, grey bars correspond to i MLS$_B$ phenotype and hatched bars correspond to f MLS$_B$ phenotype.

DISCUSSION

The prevalence of erythromycin resistance in *S. pyogenes, S. agalactiae* and *S. pneumoniae* for the whole study period was low. However, resistance values were significantly different for the three species with higher values for *S. pneumoniae*. The observed differences in resistance among species may be explained by different factors such as patient age and gender, sites of isolation or even co-selection of resistance in multirresistant *S. pneumoniae*. A detailed analysis of these factors was not considered here and deserves specific designed studies.

The distribution of resistance phenotypes was also different for these three species, with a higher prevalence of the M phenotype in *S. pyogenes* and of the different expressions of the MLS_B phenotype in *S. agalactiae*. On the other hand, *S. pneumoniae* showed a similar distribution of the two main resistance mechanisms. We observed a different f MLS_B phenotype for *S. agalactiae*, similar to that first described in *S. aureus* [17] which is characterized by both constitutive and inducible expression of MLS_B at once.

An interesting discrepancy in the resistance trends for these species was observed. *S. agalactiae* showed a clear increasing tendency during the whole period in the two study groups with no sharp changes. In addition, the distribution of the resistant phenotypes in this species was diverse. On the other hand, both *S. pyogenes* and *S. pneumoniae* showed irregular trends. Resistance in *S. pyogenes* was very low except during 2002 and 2003. The abrupt increase in resistance in these two years occurred mainly in one of the studied populations and resistant isolates possessed mostly the M phenotype. In both populations studied, resistance in *S. pneumoniae* showed a slow increasing tendency during the first years but increased sharply during 2005 and 2006, with abrupt decline in 2007. During these two years, prevalence of the M phenotype was higher than that obtained for the complete study period. Although clonality of resistant isolates was not examined here, the occurrence of these peaks for both *S. pyogenes* and *S. pneumoniae* are probably due to fluctuations in the occurrence of specific resistant clones.

The dissemination of dominant clones of community acquired pathogens such as *S. pyogenes* and *S. pneumoniae* has been frequently observed and probably explains the lack of a clear trend in resistance observed for these two species in the present study [18, 19]. *S. agalactiae* isolates originate mostly from screening samples or from sites of endogenous infections where the spread of resistant clones is probably rare and has not been described. These isolates seem to be a more heterogeneous population and could probably be a better indicator of the tendency of macrolides resistance in our community.

In Bariloche, where antimicrobial restriction policies for macrolides are non-existent, the increasing resistance trend observed in *S. agalactiae* suggests that the reduction in resistance for *S. pneumoniae* and *S. pyogenes* may be the result of a natural decline of resistant clones and not a consequence of a diminishing selection pressure.

Because antibiotic resistance is an ongoing issue in the treatment of infectious diseases, much effort is devoted to the development of policies that restrict antimicrobial use in the community. In a recent article, Enne reviews the results of studies on resistance control in the community after a prescribing restriction policy [20]. In particular, in Finland, where good results were obtained for *S. pyogenes* when restricting macrolides usage, Enne considered the possibility that the observed reduction in resistance may have been due to the natural decline of the prevalent resistant clone rather than as a direct consequence of the antimicrobial restriction policies. Our results suggest that monitoring of macrolide resistance patterns after any intervention might be better accomplished by studying related species, such as *S agalactiae*, where clonal dissemination appears to be less common.

A remarkable finding of our work is the observation of higher resistance values noted for isolates obtained from the population attending private hospitals of Bariloche for all three *Streptococcus* species studied. This could be linked to differences in macrolide consumption in the studied groups. Private hospitals are more frequently attended by a more affluent population that can afford buying antibiotics and therefore could conceivably consume greater amounts of macrolides, especially after the recent introduction of new types of macrolides with greater pharmacokinetic advantages. On the other hand, public hospitals often provide their patients with antibiotics but rarely offer macrolides (Head Pharmacist at Hospital Ramon Carrillo, Bariloche, pers.com.). Pharmacy records and/or antibiotic utilization could not be tallied in this study but may warrant future investigation. Our results are thus opposite to those obtained by Howard *et al.* (2001), who found deprivation associated with higher amoxicillin resistance values in *Escherichia coli* from urinary tract infection.[16] Our findings suggest that social clustering might have diverse implications in relation to antibiotic consumption for different geographical areas while emphasizing how antibiotic consumption may relate to resistance in very complex ways.

In conclusion, resistance to macrolides in streptococci in Bariloche is still low, however significant differences between the studied species were observed. We also describe for the first time in *Streptococcus agalactiae*, a different phenotype, the f MLS_B, similar to that previously described in *S. aureus* with both constitutive and inducible characteristics. *S. agalactiae* appears to be a better indicator of the trend of macrolide resistance in this community and should be considered when macrolides restriction policies are to be applied and their results evaluated. Finally, we also describe significant variations in macrolide resistance levels for diverse population groups of the same geographic area, which may be associated with differences between antibiotic consumption patterns in different social groups in Argentina. However the contribution of other factors to these temporal patterns is difficult to assess and therefore requires further studies.

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