

# GENOTYPES OF MACROLIDE-RESISTANT *S.pneumoniae* (Spn) ISOLATED FROM ARGENTINEAN PEDIATRIC PATIENTS WITH ACUTE OTITIS MEDIA (AOM)

V. REIJTMAN<sup>1</sup>, P. GAGETTI<sup>2</sup>, D. FACCONI<sup>2</sup>, S. FOSSATI<sup>3</sup>, P. SOMMERFLECK<sup>4</sup>, C. HERNÁNDEZ<sup>1</sup>, P. BERNALDEZ<sup>4</sup>, H. LOPARDO<sup>1</sup>, A. CORSO<sup>2</sup>  
<sup>1</sup>Servicio de Microbiología and <sup>4</sup>Otorrinolaringología, Hospital de Pediatría "Prof. Dr. J. P. Garrahan"; <sup>2</sup>Servicio Antimicrobianos and <sup>3</sup>Bacteriología Clínica, INEI ANLIS "Dr. Carlos G. Malbrán"; Buenos Aires, Argentina. e-mail: vreijtm@anlis.gov.ar

## Background

Macrolide-resistant *Streptococcus pneumoniae* (Spn) emerged in Argentina in 1995 and now it can be found in 26% in invasive infections in <5 years old. Acute otitis media (AOM) is the most common disease caused by Spn and one of the most frequent diagnoses in children <2 years old.

## Objetives

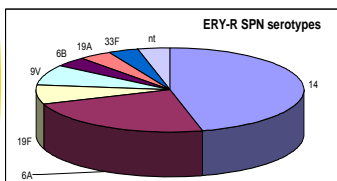
- TO DETERMINE THE PREVALENCE OF *ermB* (RIBOSOMAL METHYLASE) AND *mefA* (EFFLUX PUMP) GENES IN MACROLIDE RESISTANT SPN ISOLATES FROM AOM.
- TO DETERMINE THEIR GENETIC RELATEDNESS.

## Materials and methods

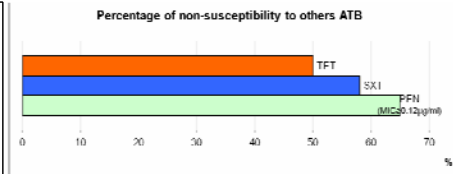
324 immunocompetent children with a first episode of AOM were studied between May 2009 and August 2010. Serotypes were determined by Quellung and MICs by the agar dilution procedure (interpreted according to CLSI). Phenotypes of macrolides resistance were determined by using erythromycin (ERY) (15 µg) and clindamycin (CLI) (2 µg) disks as previously described: M-phenotype (ERY-resistant and CLI-susceptible); cMLS<sub>B</sub> (ERY and CLI resistant) and iMLS<sub>B</sub> (ERY-resistant with induction to CLI). PCR to detect *ermB* and *mefA* genes was performed under standard conditions. Clonal relationship was established by *Sma*I-PFGE and MLST was performed in dominant clones (<http://www.mlst.net/>).

## Results

- ✓ One hundred and twenty six Spn were isolated by tympanocentesis [126 (39%)].
- ✓ Twenty six of them [26 (20.6%) ] were resistant to erythromycin (ERY-R).

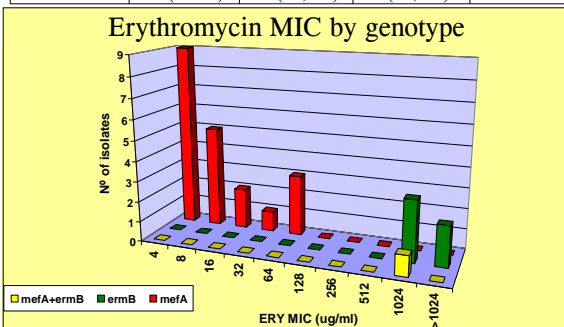


SEROTYPE OF ERY-R Spn	PERCENTAGE
14	46,2
6A	23,1
19F	7,7
9V	7,7
6B	3,8
19A	3,8
33F	3,8
nt: non- typable	3,8



ATB	% OF NON-SUSCEPTIBILITY
TET: tetracycline	50
SXT: trimethoprim-sulfamethoxazole	57,7
Penicillin MIC 0,12-1 µg/ml	65,4
Penicillin MIC ≥2 µg/ml	0
Amoxicillin MIC ≥4 µg/ml	0
Cefotaxime MIC ≥1 µg/ml	0
Chloramphenicol	0
Ofloxacin	0
Vancomycin	0

GENOTYPE	N° isolates	PHENOTYPES		
		M	cMLS <sub>B</sub>	iMLS <sub>B</sub>
<i>mefA</i>	20 (76,9%)	20 (100%)	0	0
<i>ermB</i>	5 (19,2%)	0	5 (83,3%)	0
<i>mefA+ermB</i>	1 (3,9%)	0	1 (16,7%)	0
TOTAL	26 (100%)	20 (76,9%)	6 (23,1%)	0

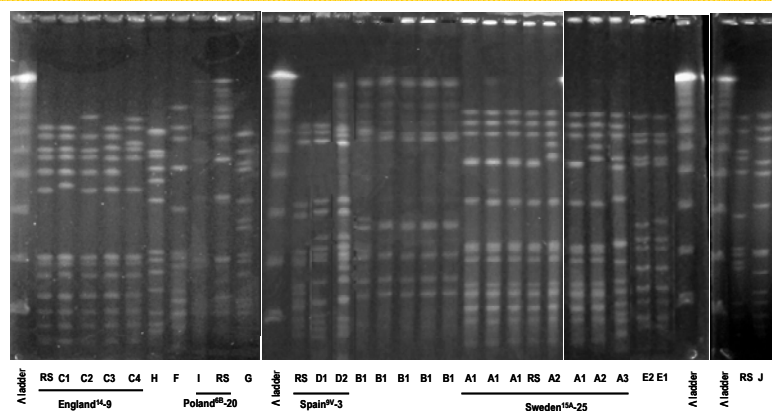


GENOTYPE	N° of isolates (%)	MIC range (µg/ml)
<i>mefA</i>	20 (76.9)	4 - 64
<i>ermB</i>	5 (19.2)	1024 - >1024
<i>mefA + ermB</i>	1 (3.9)	1024
TOTAL	26 (100)	4 - >1024

## *Sma*I-PFGE

<i>Sma</i> I PFGE	International clone by PFGE	ST	N° of isolates (%)	Serotype	Genotype
A	Sweden <sup>15A-25</sup>	782	7 (27)	14	<i>mefA</i> (n= 6) <i>mefA+ermB</i> (n=1)
B		473	6 (23)	6A	<i>mefA</i>
C	England <sup>14-9</sup>	9	4 (15,5)	14	<i>mefA</i>
D	Spain <sup>9V-3</sup>	162	2 (7,7)	9V	<i>mefA</i>
E			2 (7,7)	19F	<i>ermB</i>
F			1 (3,8)	33F	<i>ermB</i>
G			1 (3,8)	nt	<i>ermB</i>
H			1 (3,8)	19A	<i>mefA</i>
I	Poland <sup>6B-20</sup>	315	1 (3,8)	6B	<i>ermB</i>
J			1 (3,8)	14	<i>mefA</i>

✓ 80,8% (21/26) of the isolates were related to 5 clones: PFGE A to E. Four international clones were detected: Sweden<sup>15A-25</sup>/ST782 (SLV 63) (27%); England<sup>14-9</sup>/ST9 (15%); Spain<sup>9V-3</sup>/ST162 (SLV 156) (8%) and Poland<sup>6B-20</sup> / ST315 (4%).



## Conclusions

- More than 20 % of Spn from AOM were ERY-R, mainly due to the presence of *mefA* efflux pump.
- ERY-R Spn strains was associated to at least four international clones being Sweden<sup>15A-25</sup> and England<sup>14-9</sup> two of the most frequent.
- The understanding of the prevalence of different macrolide-resistant mechanisms would predict the efficacy of macrolides and lincosamides in pneumococcal AOM infections in our country.