

**XIX Lancefield International Symposium on Streptococci and
Streptococcal Diseases
November 9-12, 2014 – Paseo La Plaza, Buenos Aires - Argentina**

4) Genotypes of macrolide-resistant *Streptococcus pneumoniae* isolates from invasive disease among adult patients in Argentina.

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Background and aims: *S. pneumoniae* (Spn) is a major cause of pneumonia and meningitis in adult patients worldwide. Macrolide-resistant Spn emerged in Argentina in 1995 and nowadays represent the 14% of the isolates in adult patients (WHONET Argentina Network 2012). The aim of this study was the molecular characterization of macrolide-resistant Spn isolated from adult patients with invasive pneumococcal disease (IPD) and included the prevalence of *ermB* genes (ribosomal methylase) and *mefA* genes (efflux pump) and the clonal relationship between isolates.

Methods: Strains were serotyped by Quellung reaction and MICs determined by agar dilution (CLSI 2013). Double disk diffusion assays using erythromycin (15 µg) and clindamycin (2 µg) disks were performed in order to evaluate the inducible or constitutive expression of the MLSB-phenotype. Presence of macrolide/lincosamides resistance genes *ermB*, *mefA* and *lnuB* were identified by PCR and clonal relationship by Smal-PFGE.

Results: Between 2010 and 2012 a total of 116 Spn isolated from sterile fluids were collected in adults >18 years from 27 hospitals in Argentina (7 provinces and Buenos Aires City). Percentage of non-susceptibility to antibiotics was: penicillin 19%/0% for meningitis/non-meningitis break-points, cefotaxime 3.4%/2.6% for meningitis/non-meningitis break-points, amoxicillin 1.7%, meropenem 5.2%, erythromycin 13.8%, tetracycline 9.5%, trimethoprim-sulfamethoxazole 34.5%. All strains were susceptible to rifampin, levofloxacin, chloramphenicol and vancomycin. From a total of 116 isolates, 16 (13.8%) were erythromycin resistant (ERY-R) and were selected for further studies. Serotypes of ERY-R SPN were 14 (n=6), 6A (n=2), 19A (n=2), 19F (n=1), 9V (n=1), 1 (n=1) and NT (n=3). Nine isolates (56.3%) carried the *mefA* gene (ERY MIC >16 mg/L), 6 (37.5%) carried *ermB* gene (ERY MIC >16 mg/L), and 1 (6.3%) the dual mechanism *ermB* + *mefA* genes (ERY MIC >16 mg/L). All the isolates with *ermB* gene showed cMLSB phenotype. The *lnuB* gene was not detected in any of the isolates. All serotype 14 isolates carried the *mefA* gene. A total of 11 clonal types were identified by PFGE: clone A (n:3), clone B (n:2), clone C (n:2), and one isolate of each clonal type D to K. Clone A and B isolates were serotype 14 and has the *mefA* gene, and clone C isolates were NT serotype and harbour the *ermB* gene.

Conclusions: Macrolide resistance among adult patients with invasive pneumococcal disease was 13.8%, mainly due to the presence of MefA efflux pump and different clonal types. Studies to determine the MLST of dominant clones A, B and C are ongoing.