undertaken by PFGE (n = 186) and MLST (n = 73). Genetic Clustering was performed by PFGE; one strain from each cluster was randomly chosen to be further characterised by MLST (with selection of a greater number of isolates for larger PFGE clusters). Linage assignment was performed by e-BURST analysis.
Results: The combination of PFGE and MLST data, permitted to identify the genetic lineages of the majority of macrolide resistant isolates. We found 41 Sequence Types (ST) integrated in 16 clonal complexes (CC) and 4 singletons. In this study, 24 STs were also found elsewhere and 17 were described only in Portugal including 13 novel STs. Seven Pneumococcal Molecular Epidemiology Network (PMEN) clones were found and 10 STs were Single Locus Variant of PMEN clones. In the period 1994–1998, were found different CCs, with the following putative founders: ST156, ST15, ST90, ST63, ST81, ST315, and ST97. In the following years (1999–2004), in which was observed an increase of macrolide resistance, we identified the main previous CCs, and the emergence of new ones, with the following putative founders: ST230, ST177, ST717, ST193, ST176, ST90, ST180, ST191, ST88 and ST271. It was also observed the emergence of new single genetic lineages such as: ST2360, ST2357 and ST2359. However, in the period 2002–2004 the ST180 and ST191 disappeared and the number of isolates from the ST315 decreased significantly. Among the CCs, which emerged within the macrolide resistance, 5 were described as susceptible in the earlier years; 2 were described internationally in resistant strains and 3 were described in both susceptible and resistant strains.
Conclusion: Our results suggest that macrolide resistance among pneumococci increased in Portugal due to the expansion of clonal complexes (which appeared before the emergence of macrolide), and due to the acquisition of resistance by susceptible circulating clones and to the import of international resistant clones.

P996 Pneumococcal invasive isolates of non-vaccine serotypes in Italy, 1999–2003 (pre-vaccine era)

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Objectives: The introduction of the 7-valent conjugate vaccine (PCV7) for children has caused a dramatic decrease of pneumococcal infections due to vaccine serotypes (VS) and a relative increase of infections due to vaccine-related (VRS) or non-vaccine serotypes (NVS). The aim of this study was to characterise invasive pneumococci belonging to serotypes non included in PCV7 before the implementation of this vaccine in Italy.
Methods: All pneumococcal invasive isolates recovered from children and adults in an Italian nation-wide surveillance in 1999–2003 were studied. All isolates were serotyped and examined for susceptibility to penicillin, cefotaxime, erythromycin, clindamycin, tetracycline and chloramphenicol following the CLSI standard procedures. Pulsed-Field Gel Electrophoresis and Multilocus Sequence Typing (MLST) were used to define clonal groups among penicillin non-susceptible (PNSSP) and/or multi-drug resistant isolates and selected susceptible isolates. The sequence types (ST) were related to those reported in the MLST website (www.mlst.net) and described in the Pneumococcal Molecular Epidemiology Network (PMEN) as international clones.
Results: Among 790 invasive isolates, mostly obtained from adult patients, 392 (49.6%) belonged to VS; the other group included isolates belonging to VRS (93 isolates), to NVS (297 isolates) or that were non-typable (8 isolates). Among these isolates, 19 (5.7%) were PNSSP, all in the intermediate range, and 63 (15.8%) were erythromycin-resistant. The most prevalent serotypes, were 6A, and 19A among VRS and 1, 3, 7F, 8, 10A, 11A, 12F, 15B/C, 20 and 22F among NVS. Among the PNSSP the most frequent serotypes were 19A and 35F (5 isolates each). Several PMEN clones were identified, such as Sweden15A-25/ST63, Greece21–30/ST193, Netherlands3–31/ST180, Netherlands8–33/ST53, Netherlands15B-37/ST199, Netherlands7F-39/ST191 and Sweden1–40/ST304. Some cases of capsular switching were detected. Some isolates belonged to clonal groups that had not been previously identified, such as serotype 15B/C isolates (ST1577) and serotype 6A isolates (ST675 and ST1833).
Conclusions: In the pre-vaccine era, serotypes not contained in PCV7 (VRS or NVS) show a lower rate of penicillin or erythromycin resistance than VS. Both international clones and newly-described clones were detected and capsular switching was observed. Future monitoring of serotypes and clones in the vaccine era is of paramount importance.

P997 Population structure of Spanish mef-PCR positive *Streptococcus pneumoniae* clinical isolates

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Objectives: To determine the population structure of a collection of mef-PCR positive *Streptococcus pneumoniae* clinical isolates recovered in different Spanish hospitals. Prevalence of associated erythromycin resistance determinants and phenotypes were also screened.
Methods: A PCR assay was performed to identify erythromycin resistance genes (ermB or mef) in a collection of 712 clinical isolates recovered in Spain from 1999 through 2003. All mef-PCR positive *S. pneumoniae* strains (n = 46) were selected for further population structure analysis (serotyping with Neufeld's Quellung method, PFGE with SmaI digestion, and MLST with the standard 7 housekeeping loci scheme). Susceptibility testing was performed by the standard microdilution technique (CLSI) and resistance phenotypes by diffusion assay using commercial erythromycin, clindamycin, and rokitamycin disks. A multiplex-PCR was designed to distinguish between mef(A) and mef(E). msr(D) determinant (mel gene) was also detected by PCR.
Results: Resistance values among 46 selected mef positive isolates were: penicillin, 67.3% (intermediate + resistant), clindamycin, 52.2%, and tetracycline 56.5%. No telithromycin resistance was found (MIC range, 0.03–1 mg/L) but one isolate was resistant to levofloxacin (MIC, 8 mg/L). Interestingly, 4 mef positive isolates (8.7%) showed erythromycin MICs in the susceptible range (0.12 mg/L), but increased $\geqslant$32 mg/L endowing the M phenotype when plated on increased concentrations of erythromycin. The M and MLSB phenotypes in erythromycin resistant isolates were observed in 45.2% and 54.72% of isolates, respectively. The presence of both erm and mef determinants was found in 50% of isolates. All mef determinants belonged to mef(E) subclass and all isolates presented the msr(D) gene. Serotype distribution was as follows: 14, 21.7%; 19F, 19.5%; 19A, 15.2%; 6B, 6.5%; 9V 6.5%; and others, 30.6%. Most isolates belonging to the same serotype showed similar PFGE patterns. England^14–9 and Taiwan^19F-14 multiresistant clonal complexes were represented within mef positive isolates and Spain^23F-1, Poland^6B-20, Sweden^15A-25 and Spain 6B among erm and mef positive isolates.
Conclusions: A high proportion of *S. pneumoniae* isolates harbouring the mef gene in our collection also presents the ermB determinant. Nearly all of them displayed the MLSB phenotype. A complex population structure was found in our mef positive *S. pneumoniae* collection.

P998 Antimicrobial resistance patterns and genotypes of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* ssp. *dysgalactiae* from bovine mastitis in Portuguese dairy farms

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Objectives: To evaluate the profiles of antimicrobial (ATB) resistance and genotypes of the contagious bovine mastitis pathogen *Streptococcus agalactiae* (GBS) and of *Streptococcus dysgalactiae* ssp. *dysgalactiae* (GCS), which is considered to be either a contagious or an environmental bovine pathogen.
Methods: Among 459 milk samples collected during 2002/2003 from mastitis quarters of 377 bovines in 11 Portuguese herds, 13.9% of the bacteria were GBS and 3.7% were GCS. A total of 32 GBS and 17 GCS of these field isolates were studied. ATB resistance was evaluated by disk diffusion against macrolides-M (erythromycin-E),