780

Impact of *Streptococcus pneumoniae* in Acute Otitis Media (AOM) with Spontaneous Tympanic Membrane Perforation (STMP): Serotype Distribution 4 Years After 13-Valent Pneumococcal Conjugate Vaccine Introduction

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INTRODUCTION

***Streptococcus pneumoniae* (*S. pneumoniae*) is the most common etiologic agent of acute otitis media (AOM).**
Serotypes (ST) included in the heptavalent pneumococcal conjugate vaccine (PCV7) were detected in the middle ear fluid (MEF) of most children with AOM, suggesting a potential role of this vaccine in reducing AOM incidence. After several years of PCV7 use, replacement of PCV7 ST by non-vaccine ST (particularly 19 A and 3) occurred and the incidence of pneumococcal AOM nearly returned to previous values. Because these ST were included in the 13-valent pneumococcal vaccine (PCV13), it was expected that administration of PCV13 would be effective in reducing pneumococcal AOM incidence.
The data regarding pneumococcal ST in AOM after PCV13 implementation are scarce.
We aimed to determine which pneumococcal ST could be detected in the MEF of children with AOM complicated by spontaneous tympanic membrane perforation (STMP) living in the greater Milan area, Italy, where since 2011 ≥90% of the children have received the PCV13 vaccine in the first year of life.

METHODS

- **Inclusion: children with AOM associated with STMP consecutively seen between April 1, 2015, and March 31, 2016**
- **Exclusion: children with tympanostomy tubes, craniofacial abnormalities or chronic TMP, immunodeficiency, dysmorphic or genetic syndromes, systemic or topical antibiotics in the previous 2 weeks**
- **Diagnosis of AOM with STMP: acute symptoms (i.e., fever, irritability or earache) lasting ≤3 days and otorrhea within 12 hours of the STMP.**
- **MEF sample: collected from very near the perforation using an extra-thin flexible wire swab (eNAT transport and preservation medium, Copan, Brescia, Italy). Only one swab was taken from each patient.**
- **Recurrent AOM definition: a history of ≥3 episodes of AOM in the previous 6 months or ≥4 in the previous year**

- **Pneumococcal immunization schedule in Italy: 3 doses of PCV in the first year of life. PCV7 was used from September 2002 to November 2010 and was later replaced by PCV13. Fully vaccinates were considered the children who had completed the vaccine schedule whereas not fully vaccinated those who had started but not completed the vaccine schedule.**

- **Bacterial genomic DNA was extracted from the swabs using a NucliSENS easyMAG automated extraction system and tested for the autolysin-A-encoding and wzg (cpsA) genes of *S. pneumoniae* using real-time PCR.**

- **The level of detection of the test was 16 genome copies, and each sample was tested in triplicate and considered positive if at least two of the three tests revealed the presence of both genes. The real-time PCR-negative specimens were also tested for the presence of an RNase P-encoding gene to exclude PCR inhibition and DNA extraction failure. All of the positive cases were serotyped using primers and probes designed on the basis of the GenBank database sequences (www.ncbi.nlm.nih.gov) of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C/D, 7 F, 8, 9 V, 10A/B, 11A/D, 12A/B/F, 14, 16F, 17F, 18C, 19A, 19 F, 20, 21, 22A/F, 23A, 23B, 23F, 24A/B/F, 29, 33A/F, 35B, 35F, and 38 and were synthesized by TIB Molbiol (Genoa, Italy).**

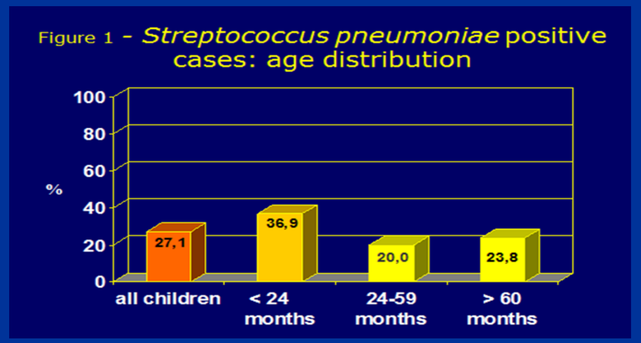
- **All the statistical analyses were performed using R software, version 3.2.2.**

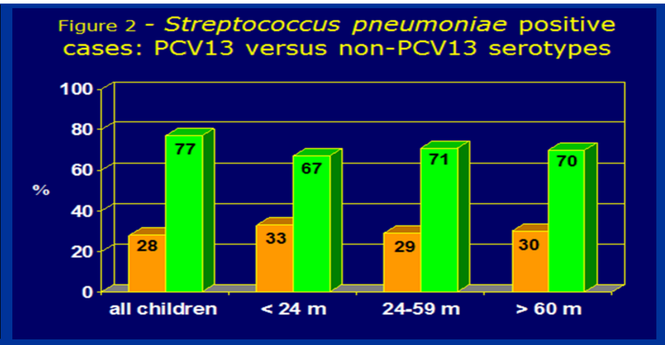
RESULTS

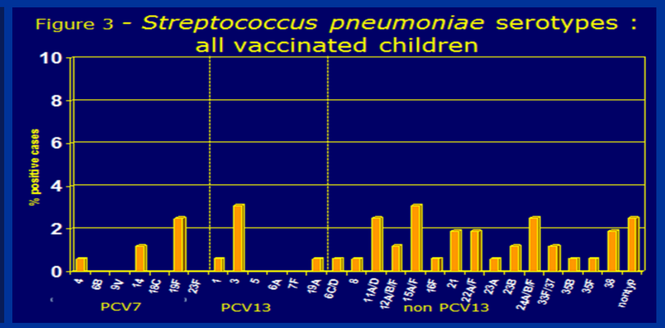
A total of 177 children were enrolled (Table 1).
***S. pneu*moniae was identified in the MEF of 48 (27.1%) subjects. The prevalence was higher in younger patients than in older patients (figure 1).**
Among the *S. pneumoniae*–positive cases, 23/24 (95.8%) and 13/14 (92.9%) in the groups of children <2 years and 2-4 years old, respectively, were fully vaccinated with PCV13. Of children ≥ 5 years old, 6/10 (60%) had received PCV7 and 3/10 (30%) had received PCV13. All but one child (<2 years old) had received the PCV vaccine available when they were in the first year of life.
Serotyping of *S. pneumoniae* revealed that in most of the cases (37/48, 77.1%) ST not included in PCV13 were present, independent of age and the previous history of recurrent AOM: 13 (68.4%) in children with single AOM and 24 (82.8%) in children with recurrent AOM (p=0.30) (figure 2).
ST 15A/F, 11 A/D, and 24A/B/F were the most common. ST included in PCV13 (mainly 1, 3 and 19F) were identified in 8 (33.3%) children <2 years old, 4 (28.6%) children 2-4 years old and 3 (30%) children ≥5 years old (figures 4 - 6). Among the 15 not vaccinated children, 3 (20%) were positive for pneumococcal ST: 3, 8 and 15A/F (one each).

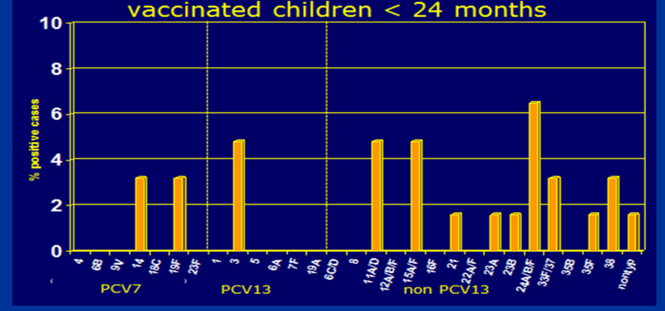
Table 1 - STUDY POPULATION

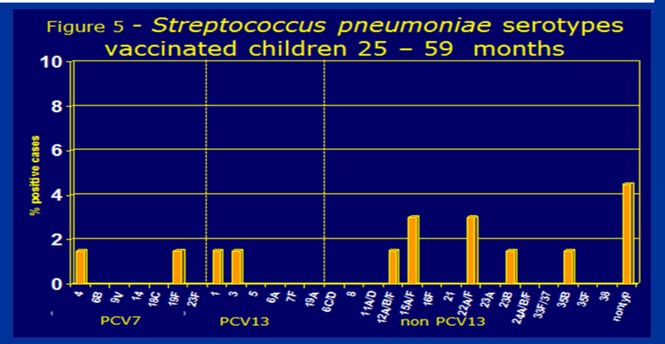
	Children N = 177 (%)
Sex (males)	103 (58.2)
Age (group)	
< 24 months	65 (36.7)
25 - 59 months	70 (39.6)
≥ 60 months	42 (23.7)
mean age ± SD (years(	3.5 ± 2.7
History of rAOM	100 (56.5)
PCV 7 or PCV 13	162 (91.5)

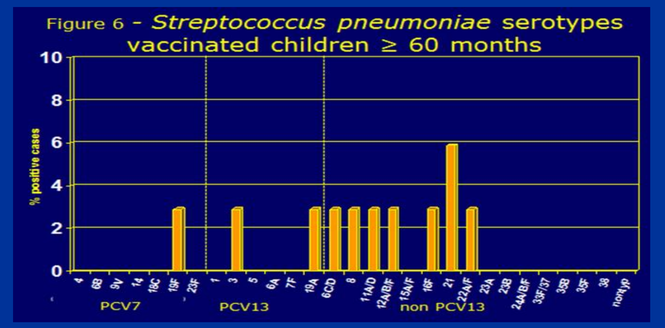












CONCLUSIONS

This study confirms that *S. pneumoniae* is one of the pathogens more commonly associated with AOM even when it is complicated by STMP and shows that, despite full immunization with PCV13, children can develop AOM due to ST included in the vaccine they receive. Five years following the introduction of PCV13 in greater Milan in Italy, most of the pneumococcal cases of AOM with STMP are associated with ST not included in the vaccine. Although there are no data indicating which ST were associated with AOM in the pre-vaccine era, studies on IPD and carriage in Italy showed that vaccine-related ST were those most frequently detected in pre-vaccine era. This finding suggests that a significant replacement phenomenon has developed, reducing the potential effect of PCV13 on AOM incidence.
The prevalence of AOM with STMP due to non-PCV13 ST in this study is the highest ever reported. This might be explained by the fact that this study, in contrast to the others, was carried out several years after the introduction of PCV13 and enrolled children living in an area with very high PCV13 vaccination coverage. For its effects to be fully evident, replacement requires a long duration of vaccine use and high vaccination coverage.
Our data further confirm the importance of periodical monitoring of pneumococcal ST circulation after introduction of PCV with a defined number of ST, in order to evaluate replacement phenomenon and the need for new vaccines with greater protective activity.