**INTRODUCTION**

Streptococcus pneumoniae (S. pneumoniae) is the most common etiologic agent of acute otitis media (AOM). Pneumococcal meningitis and other sequelae, such as acute exacerbation of chronic otitis media, are severe complications of AOM. In Italy: 3 doses of PCV in the first year of life, 7 years of age, and at 15 years of age, respectively, are regarded as the minimum number of doses for the acquisition of immunity against pneumococcal carriage and disease, with a duration of 5 years after the last dose. In the pre-vaccine era, the incidence of invasive pneumococcal disease (IPD) was high, and its effects were most evident among children younger than 5 years of age. The introduction of PCV into the vaccination schedule has dramatically reduced the number of pneumococcal cases, especially those due to serotypes included in the vaccine (V). However, serotype replacement has become evident, mainly due to serotypes 19A, 19F, and 3.

**METHODS**

- **Inclusion**: children with AOM associated with STMP consecutively seen between April 1, 2015, and March 31, 2016
- **Exclusion**: children with tympanosclerosis tubae, cranial facial abnormalities or chronic TBM; 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 swelling within 12 hours of the STMP
- **MPE sample**: collected from near the perforation using an extra-oral flexible wire made (STMP) transport pneumococcal medium, Copan, Brescia, Italy. Only positive patients were considered for serotyping
- **Recruitment of study patients**: a history of 2 episodes of AOMint the previous 6 months or 24 years
- **Pneumococcal immunization schedule**: 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 vaccinated were considered the children who had completed the vaccine schedule whereas not fully vaccinated those who had not completed the recommended schedule
- **Bacterial genomic DNA was extracted from the swabs using a NuSens™ automated extraction system and tested for the multidrug–A-encoding and -C-encoding genes using real-time PCR**: the level of detection of the tests in 10 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 M13R–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/nuccore) of each serotype. cpsA (GenBank FJ244259.1), cpsC (GenBank DQ421016.1), cpsD (GenBank AM368951.1), cpsE (GenBank AY864651.1), cpsF (GenBank BC154334.1), cpsG (GenBank JQ157799.1), cpsH (GenBank EV004378.1), cpsI (GenBank EU294461.1), cpsJ (GenBank EU294462.1), cpsK (GenBank GQ815921.1), cpsL (GenBank JX419672.1), cpsM (GenBank EU294463.1), cpsN (GenBank EU294467.1), cpsO (GenBank JX419673.1), cpsP (GenBank JX419674.1), cpsQ (GenBank JX419675.1), cpsR (GenBank JX419676.1), cpsS (GenBank JX419677.1), cpsT (GenBank JX419678.1), cpsU (GenBank JX419680.1), cpsV (GenBank JX419681.1), cpsW (GenBank JX419682.1), cpsX (GenBank JX419683.1), cpsY (GenBank JX419684.1), cpsZ (GenBank JX419685.1)

**RESULTS**

A total of 177 children were enrolled (Table 1). S. pneumoniae was identified in the MEF of 48 (27.1%) subjects. The prevalence was significantly higher in children ≤4 years of age (p=0.004, 29.2%) compared with children ≥5 years of age (19.3%) (Figure 1). Among the S. pneumoniae-positive cases, 23/48 (48.9%) and 13/48 (26.9%) in the groups of children ≤4 years of age and ≥5 years of age, respectively, were fully vaccinated with PCV13. Of children 2-5 years old, 6/10 (60%) had received PCV7 and 5/10 (50%) had received PCV13. All but one child (<2 years old) had received the PCV vaccine available when they were the first year of life. Serotyping of S. pneumoniae revealed that in most of the cases (ST74, 77, 71, ST not included in PCV15 were present, independent of age and the previous history of recurrent AOM (ST74, 77, 71, ST not included in PCV15 were present, independent of age and the previous history of recurrent AOM. Among the 19 not well-vaccinated children, 3/19 were positive for pneumococcal ST: 3, 6 and 15A/1 (each).

**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 were not vaccinated with the PCV13 and that, after the introduction of PCV13 in the Italian AOM study group, the disease was significantly reduced in comparison with previous results. However, the incidence of pneumococcal disease has continued to be high, and the use of new and atypical serotypes has been reported. This might be explained by the fact that this study, in contrast to others, was carried out in a pediatric setting where the incidence of pneumococcal disease is lower and with a high rate of PCV13 coverage in children younger than 5 years. Our data further confirm the importance of periodical monitoring of pneumococcal ST as a replacement phenomena and the need for new vaccines with greater protective activity.