Microbiology of Spontaneous Othorrhea in Italian Children with Acute Otitis Media

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Acute otitis media (AOM) is a common disease of infants and young children. In the first three years of life almost all of the children experience at least one episode of this disease and about 50% of them suffers from recurrent episodes [1]. AOM is mainly a bacterial disease and *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes* are the pathogens that play the major etiologic role [2]. In the majority of cases, even the most severe, the tympanic membrane, despite very hyperemic and bulging, remains intact. However, in a number of children, spontaneous tympanic membrane perforation occurs and middle ear fluid passes into the external ear canal [3]. Because in most of the cases perforation repairs spontaneously within few days without any further clinical problems, it is considered a mild AOM complication. The most recent guidelines for the diagnosis and management of AOM do not consider AOM with othorrea a special disease with peculiar characteristics. Only in some cases spontaneous perforation is indicated as a sign of severity leading to the systematic antibiotic prescription to avoid further clinical problems [4-6]. Moreover, its prevention, in children with recurrent AOM episodes, is significantly more difficult than the prevention of uncomplicated cases [7,8]. Knowledge of the etiology of AOM with othorrea may be useful to plan adequate therapeutic and preventive measures for children, including pneumococcal vaccination, especially for those with recurrent AOM.

We evaluated the etiology of AOM complicated by spontaneous tympanic membrane perforation in children living in Milan, Italy, in a one year period. where since 2011 more than 90% of the children in the first year of life received PCV13 vaccine.

Patients and methods

Middle ear fluid specimens taken from children with AOM with spontaneous perforation consecutively seen between April 1, 2015, and March 31, 2016 were evaluated. Children with tympanostomy tubes, craniofacial abnormalities or chronic middle ear conditions (including chronic tympanic membrane perforation), primary or secondary immunodeficiency, dysmorphic or genetic syndromes, who had received antibiotics in the previous 2 weeks and those receiving topical (i.e., in the ear and/or the nose) antibiotic treatments were excluded.

The diagnosis of AOM with othorrea was based on acute symptoms (i.e., fever, irritability or earache) lasting ≤3 days plus otorrhea within 12 hours of the perforation and was confirmed by a validated otoscopist (PM). Middle ear fluid was obtained after the bulk of the otorrhea fluid had been removed and the ear canal had been cleansed with a dry cotton swab. Under direct otoscopic visualization, the remaining fluid was collected from very near to the perforation using an extra-thin flexible wire swab (Copan eNAT transport and preservation medium, Brescia, Italy). If both tympanic membranes had perforated, only one was considered and one swab was taken from each patient. All of the swabs were processed within 4 hours of collection.

The patients' clinical history were reviewed, and their demographic and clinical data were entered into a computerized database. The children who, at the time of enrolment, had a history of ≥3 episodes of AOM in the previous 6 months, or ≥4 in the previous year were considered as having recurrent AOM. The patients' pneumococcal vaccination status was established by consulting the official vaccination chart issued by the Vaccination Service of Lombardia Region. The pneumococcal immunization schedule recommended by the Italian Ministry of Health includes three doses of PCV in the first year of life; PCV7 was used from September 2002 to November 2010 and was later substituted by PCV13. The study was approved by the Ethics Committee of Milan Area B, Italy, and written informed consent was obtained from the patients' parents or legal guardians.

Bacterial genomic DNA was extracted by clinical specimens by semi-automatic DNA extraction system. The presence of *S. pneumoniae* (and its serotypes), *H. influenzae*, *M. catarrhalis, S. pyogenes* (group A beta hemolyticus Streptococcus - GAS) was evaluated by means of real time PCR using specific oligos-probe sets.

Results

A total of 177 children (103, 58.2%, males; mean age ± standard deviation, 3.5 ± 2.7 years) was enrolled. Of them, 65 (36.7%) were <2 years old, 70 (39.6%) 2-4 years, and 42 (23.7%) ≥5 years. A history of recurrent AOM was present in 100 (56.5%) of the children (**Table 1**).

Table 1. Study population.

	Nº children = (%) **177**
Sex (males)	103 (58.2)
Age (groups)	
< 2 years	65 (36.7)
2-4 years	70 (39.6)
> 5 years	42 (23.7)
History of recurrent AOM	100 (56.5)
PCV7 or PCV13	162 (91.5)

Middle ear fluid samples were positive in 144 (81.3%) children and negative in 33 (18.6%).*S. pneumoniae* was identified in 48 (27.1%) subjects, *nontypeable H.influenzae* in 90 (50.8%), *M.catarrhalis* in 46 (26.0%) and *group A streptococcus* (GAS) in 62 (35.0%). **Table 2**

Table 2. Microbiology of 177 Italian children with othorrea (April 2015 to March 2016)

S. Pneumoniae	27.1%
H. Influenzae	50.8%
M. Catarrhalis	26.0%
Group A Streptococcus GAS	35.0%

The comparison of the results with those of our previous study [9], which evaluated the etiology of othorrea with traditional culture is reported in **Figure 1**

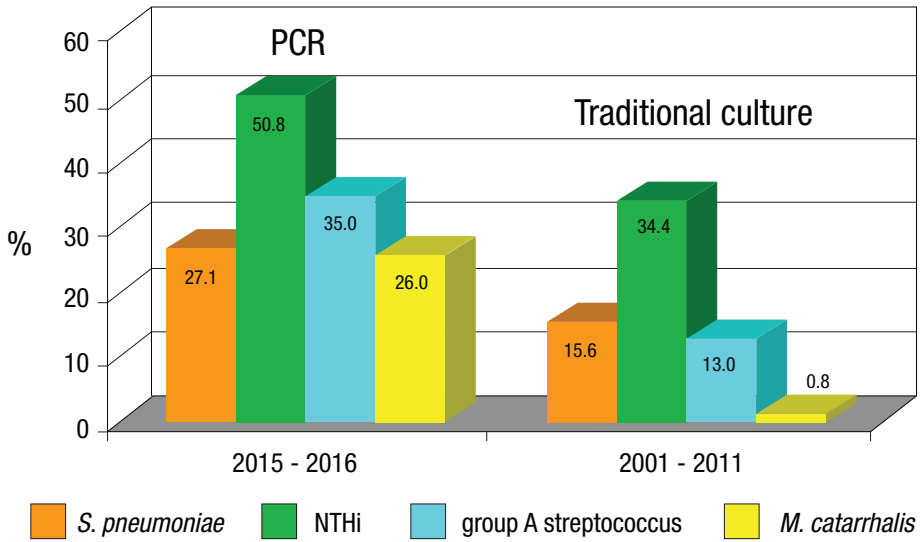

Figure 1. Etiology of otorrhea with traditional culture and PCR

A mixed etiology was detected in more than half of the children. The most common co-infection was nontypeable *H.influenzae* and *M.catarrhalis*.**Table 3**

Table 3. Mixed etiology

Nº of childrens with mixed etiology	76/144 (**52.8%**)
2 pathogens*	53 (36.8%)
3 pathogens	21 (14.6)
4 pathogens	2 (1.4%)

*most common: NTHi plus *M. catarrhalis*

Among the *S. pneumoniae* positive cases, 96% and 93% in the groups of children <2 years and 2-4 years old, respectively, resulted fully vaccinated with PCV13. Of children ≥5 years old, 60% had received PCV7 and 40% had received PCV13. All of them had received pneumococcal vaccine in the first year of life. The distribution of pneumococcal serotypes is shown in the next figure. In most of the cases serotypes not included in PCv13 were detected, independently of age and a history of recurrent AOM. **Figure 2**

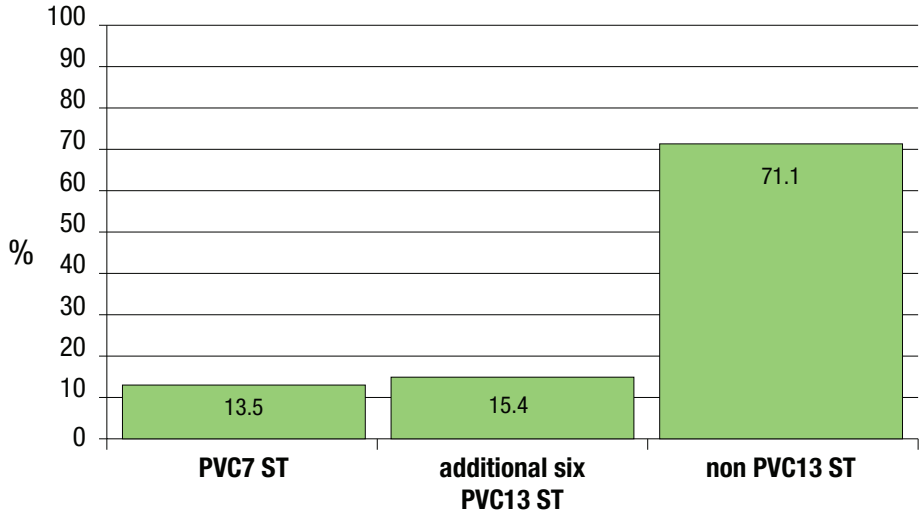

Figure 2. Distribution of pneumococcal serotypes

Comments

The etiology of AOM is changing in Italian children and this can have relevant implications for the daily practice.

Nontypeable *H.influenzae* is the predominant pathogen in acute otitis media associated with othorrea due to spontaneous perforation in Italian children. Group A beta hemolyticus streptococcus (GAS) is confirmed to be a main cause of spontaneous otorrhea. The role of *M.catharralis* is definitively emerging.

The role of *S.pneumoniae* is still relevant, despite full immunization with PCV13. After about 5 years since the introduction of PCV13 in great Milan in Italy, most of the pneumococcal cases of AOM with othorrea are associated with serotypes not included in the vaccine. Despite there are no data indicating which were the serotypes associated with AOM complicated by othorrea in the pre-vaccination period in this area, this finding suggests that a significant replacement phenomenon has developed, reducing the potential effect of PCV13 on AOM incidence.

The role of non-PCV13 serotypes as cause of pneumococcal disease has been already reported in studies regarding invasive pneumococcal disease and AOM. However, the prevalence of AOM with othorrea due to these serotypes found in this study is the highest ever reported. At this regard, our data are different from those reported by other authors that evidenced only a limited role of non-PCV13 serotypes as cause of AOM [10] and in contrast with those reported by Ben-Shimon *et al*. who found only a marginal, not statistically significant replacement phenomenon [11]. These differences might be explained by the fact that this study, contrarily to the others, was carried out several years after PCV13 introduction and enrolled children living in an area with very high PCV13 vaccination coverage. To be fully evident, replacement requires long time of vaccine use and high vaccination cover-

age [12]. In conclusion, our study suggests that the role of pathogens potentially able to produce beta lactamases should be taken into account when planning therapeutic strategies for AOM, and this includes debating the role of amoxicillin as first choice drug for AOM complicated by othorrea.

In addition, our study highlights the importance for a strict monitoring of pneumococcal distribution after PCV introduction in order to evaluate the real protection offered by the available vaccines and the need for different protection from pneumococcal AOM.

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