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Microbial profiling does not differentiate between childhood recurrent acute otitis media and chronic otitis media with effusion

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ABSTRACT

Objectives: Otitis media (OM) is one of the most frequent diseases of childhood, with a minority of children suffering from recurrent acute otitis media (rAOM) or chronic otitis media with effusion (COME), both of which are associated with significant morbidity. We investigated whether the microbiological profiling could be used to differentiate between these two conditions.
Methods: Children up to five years of age, with rAOM (n = 45) or COME (n = 129) and scheduled for tympanostomy tube insertion were enrolled in a prospective study between 2008 and 2009. Middle ear fluids (n = 119) and nasopharyngeal samples (n = 173) were collected during surgery for bacterial culture and PCR analysis to identify *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, and to detect 15 distinct respiratory viruses.
Results: The occurrence of bacterial and viral pathogens in middle ear fluids did not significantly differ between patients suffering from rAOM and COME. In both patient cohorts, *H. influenzae* and rhinovirus were the predominant pathogens in the middle ear and nasopharynx. Nasopharyngeal carriage with two or three bacterial pathogens was associated with the presence of bacteria in middle ear fluid (P = 0.04). The great majority of the bacteria isolated from middle ear fluid were genetically identical to nasopharyngeal isolates from the same patient.
Conclusions: Based on these results, we propose that the common perception that rAOM is associated with recurrent episodes of microbiologically mediated AOM, whereas COME is generally a sterile inflammation, should be reconsidered.

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1. Introduction

Otitis media (OM) is one of the most frequent diseases during childhood and the most common reason for young children to visit a physician. In many countries, it is the most common reason for children to receive antibiotics or to undergo surgery [1–3].

OM is a common denominator for a variety of middle ear diseases that can be divided into various categories, including acute otitis media (AOM) and otitis media with effusion (OME) [4]. AOM is defined as the presence of middle ear effusion accompanied by signs of acute inflammation of the middle ear, such as otalgia, otorrhea, fever, and malaise or irritability of the child [3,5]. OME, on the other hand, can either develop as a sequel to AOM, or develop de novo, the primary symptom being hearing loss due to the presence of middle ear fluid in the middle ear cavity (but in the absence of signs of acute inflammation) [6]. Albeit often self-limiting, in 10–20% of the cases OM can result in chronic OME (COME) or recurrent AOM (rAOM) disease [7].

Although there is currently no universal standard for OM management, several options are available to the clinician, namely: watchful waiting, antibiotic treatment, adenoidectomy, ventilation tube insertion, or vaccination. AOM management generally involves adequate analgesics with an optional observation period for 48–72 h [5]. Thereafter, antibiotic treatment or, in the case of recurrent infections, antibiotic prophylaxis or ventilation tube insertion are considered [5,8,9]. In contrast, for OME disease, medical intervention is appropriate only if persistent

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clinical benefits can be achieved in the absence of spontaneous resolution. Therefore, healthy children with OME are observed for at least 3 months before medical intervention is considered, in which case surgical treatment involving the insertion of ventilation tubes or adenoidectomy is feasible [3,10]. Vaccination against OM diseases is currently very limited, being directed against very few bacterial OM pathogens, and only one of the viral OM pathogens, i.e. the influenza vaccine. The heptavalent pneumococcal conjugate vaccine is primarily directed against pneumococcal invasive disease, whereas the introduction of vaccination only reduced the overall OM incidence by 6–9% due to serotype or pathogen replacement [11]. However, herd immunity may induce a further decline in OM incidence, in line with nasopharyngeal colonization studies 3 years after the introduction of the vaccine [12,13].

A key element in the pathogenesis of OM is the nasopharynx, as this niche is the reservoir for bacterial pathogens involved in middle ear infections [4]. Colonization of the nasopharynx with *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* (the three most important bacterial pathogens associated with OM disease) at an early stage, has been shown to predispose children for development of rAOM [14]. In addition, OM-prone children have increased carriage rates of these bacterial pathogens compared to healthy controls [15,16]. In AOM, *S. pneumoniae* is the most frequently detected pathogen in middle ear fluid, followed by non-typeable *H. influenzae* and *M. catarrhalis* [17–19]. However, *H. influenzae* tends to predominate in COME, followed in a lesser extent by *S. pneumoniae* and *M. catarrhalis* [20]. In general, bacteria have been found less frequently in the middle ear of children suffering from COME compared to AOM (approximately 30% versus 70%, respectively) [17].

In addition, many studies over the past decades have shown a close association between AOM and respiratory viral infections [21–24]. Viral upper respiratory tract infections (URI) predispose children to AOM, as infection may cause Eustachian tube dysfunction, and facilitate an increase in adherence of bacteria to epithelial cells, resulting in a rise in bacterial colonization of the nasopharynx and a modulation of the host's immune function [25–27]. Nevertheless, studies describing specific associations between respiratory viruses and bacteria in rAOM and COME are scarce.

In this study, we investigated bacterial and viral colonization and infection in the middle ear and nasopharynx of children diagnosed with rAOM or COME. In particular, the contribution of 3 major bacterial pathogens (*S. pneumoniae*, *H. influenzae* and *M. catarrhalis*) in the absence or presence of 15 distinct respiratory viruses was studied in relation to rAOM and COME disease.

2. Materials and methods

2.1. Study design

A cohort of children up to five years of age who suffered from rAOM or COME was examined in this prospective clinical study. The cohort was enrolled at a secondary and a tertiary care hospital in Nijmegen, the Netherlands, from April 1st 2008 to July 1st 2009, and included children suffering from rAOM or COME who underwent surgery for the insertion of ventilation tubes. Recurrent AOM was defined as 3 or more episodes of AOM in the last 6 months or 4 episodes in the last 12 months [28]. The COME patient population consisted of children who had experienced a period of persistent OM with effusion lasting longer than 3 months. RAOM or COME diagnosis was made by an otolaryngologist in routine clinical practice based upon signs, symptoms, otoscopy and audiometry including tympanometry. Patients with a history of malignancy, organ transplantation or immune deficiency were excluded from participation, as well as patients with recent elective ear surgery or systemic infectious diseases. Children with AOM and/or fever ($T \geq 38\,^{\circ}C$) at the time of ventilation tube insertion were rescheduled for the procedure. Adenoidectomy performed in the same surgical setting was not considered to be an exclusion criterion. Patient characteristics and risk factors were acquired using a questionnaire, collected at the day of ventilation tube insertion. Ethical permission was obtained from the Committee on Research Involving Human Subjects in January 2008 (CMO 2007/239, international trial register number: NCT00847756).

2.2. Clinical samples

Per child, one middle ear fluid sample and one nasopharyngeal sample was collected. Middle ear fluid was collected during surgery using a middle ear fluid aspiration system (Kuijpers Instruments, Groesbeek, The Netherlands) [29], nasopharyngeal samples, taken through the nose, were obtained using a cotton wool swab (192C, Copan, Brescia, Italy). Middle ear fluid was suspended in 2 ml saline and divided into aliquots for bacterial culture, bacterial PCR, and viral multiplex PCR. The nasopharyngeal samples were used for bacterial culture and thereafter stored at $-80\,^{\circ}C$ in 1 ml of 80% glycerol for subsequent viral analysis.

2.3. Microbiology

Middle ear fluid and nasopharyngeal samples were cultured directly after collection according to standard laboratory procedures [14] to determine the presence of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. Thereafter, bacteria were stored at $-80\,^{\circ}C$ in appropriate media containing an additional 15% (*S. pneumoniae*, *H. influenzae*) or 50% glycerol (*M. catarrhalis*). The less frequently described otopathogens *Staphylococcus aureus*, *Streptococcus pyogenes*, *Haemophilus parainfluenzae*, *Pseudomonas aeruginosa* and *Alloicoccus otitidis* were also included in the analysis if detected during bacterial culture.

To further characterize the bacterial isolates, all *S. pneumoniae* and *H. influenzae* isolates were cultured overnight on, respectively, blood agar and chocolate agar plates at $37\,^{\circ}C$ supplemented with 5% CO_2. Pneumococcal isolates were serotyped using the Quellung reaction (Statens Serum Institute, Copenhagen, Denmark) and multiplex PCR as described previously [30]. *H. influenzae* isolates were serotyped using slide agglutination according to the manufacturer's instructions (Becton–Dickinson, Breda, The Netherlands) [31].

S. pneumoniae, *H. influenzae* or *M. catarrhalis* obtained from middle ear fluid, as well as the equivalent pathogen isolated from the nasopharynx of the same patient, were further analyzed using multilocus sequence typing (MLST). Genomic DNA was isolated using a Qiagen Genomic-tip 20/G Kit (Venlo, The Netherlands) according to the manufacturer's instructions. DNA amplification of the MLST loci and sequencing was performed as described previously [32–35]. Quantitative DNA analysis of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* by real-time PCR was performed as previously described. The respective genes chosen for bacterial quantification were the *S. pneumoniae ply* gene [36], *H. influenzae* 16S rRNA gene [37] and the *M. catarrhalis ompJ* gene [38].

2.4. Virology

Middle ear fluid and nasopharyngeal samples were analyzed by multiplex PCR as previously described [39]. Briefly, upon thawing, nucleic acids were extracted from each sample using the MagNA Pure LC System and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Applied Science, Almere, The Netherlands) according to manufacturer's instructions. A multiplex real-time PCR assay

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