Otitis media: viruses, bacteria, biofilms and vaccines

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ABSTRACT

- Otitis media typically presents as either acute otitis media (AOM), with symptoms including fever, otalgia, otorrhoea or irritability and short duration; or as otitis media with effusion (OME), which is often asymptomatic and characterised by accumulation of fluid in the middle ear.
- Diagnostic certainty of otitis media is challenging, given the young age of patients and variability of symptoms.
- Otitis media predominantly occurs as coincident to viral upper respiratory tract infections and/or bacterial infections.
- Common viruses that cause upper respiratory tract infection are frequently associated with AOM and new-onset OME. These include respiratory syncytial virus, rhinovirus, adenovirus, parainfluenza and coronavirus.
- Predominant bacteria that cause otitis media are *Streptococcus pneumoniae, Moraxella catarrhalis*, and non-typeable *Haemophilus influenzae*.
- Antibiotic therapy does not significantly benefit most patients with AOM, but long-term prophylactic antibiotic therapy can reduce the risk of otitis media recurrence among children at high risk.
- In Australia, 84% of AOM is treated with antibiotic therapy, which contributes to development of antibiotic resistance.
- Vaccine development is a key future direction for reducing the world burden of otitis media, but requires polymicrobial formulation and ongoing monitoring and modification to ensure sustained reduction in disease burden.

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Otitis media is inflammation of the middle ear, and may present as either acute otitis media (AOM) or otitis media with effusion (OME). AOM exhibits rapid-onset middle ear effusion and signs and symptoms of middle ear inflammation, including fever, otalgia, otorrhoea, or irritability,[1] whereas OME is middle ear effusion in the absence of symptoms of acute infection.[2]

On the basis of likelihood of presentation for treatment in non-Indigenous children, AOM may be over-reported by 22%–50%,[3,4] whereas OME maybe under-reported. In a recent prospective study, it was demonstrated that the frequency of AOM episodes is age related, with a mean of 1.97 episodes occurring each year among 6–11-month-olds, 1.67 among 12–23-month-olds, and 1.07 among 24–35-months-olds.[5] For older children, the frequency of new-onset OME is greater than that of AOM. In a cohort of 242 children aged 1.0–8.6 years, the frequency of new-onset OME was fourfold greater than that of AOM;[6] this finding is consistent with previous data, which indicated that 50% of OME cases occurred subsequent to AOM.[7]

Diagnostic challenge

Otitis media and upper respiratory tract infections (URTIs) share many common symptoms and are often coincident, increasing the variability of diagnosis for AOM. AOM is temporally associated with URTIs or cold-like illnesses in 50%–70% of all new AOM cases,[8,9] and between 29% and 61% of all cases of URTI may develop into otitis media.[5,10,11] In clinical practice, URTI symptoms and either rhinitis or cough were observed by French general practitioners in almost 90% of patients with suspected or diagnosed AOM.[3]

Otitis media can be difficult to confirm, as otoscopic observation of tympanic membrane changes — including bulging, erythema or opacity of the tympanic membrane[12,13] — are not always characteristic of AOM. Successful otoscopic examination of very young, distressed children, often on repeated occasions, further increases the diagnostic challenge for clinicians, as the peak incidence for AOM is between 6 and 18 months of age.[14,15]

The uncertainty of clinical diagnosis of AOM is demonstrated by a study in the United States, in which GPs stated certainty of their diagnosis for AOM in only 58% of cases among infants, 66% of cases among toddlers, and 73% of cases among older children.[16] Furthermore, although 78% of diagnoses of AOM in children 1–4 years of age were shown to be consistent between the GP and otolaryngologists, up to one-third of the incorrect diagnoses were identified as normal ears by the otolaryngologists.[3]

Otitis media: a polymicrobial disease

Otitis media is a multifactorial disease[17] with an extensive causal basis, including demographic, social, environmental, immunological and microbial risk factors.[18] The development and growth of the eustachian tube in the first 2 years favours episodes of tubal blockage, often exacerbated by pollutants, allergies and viral infections.[19] Abnormality of the eustachian tube is a contributing factor to children's susceptibility to recurrent episodes of AOM and OME.[20] Equalisation of middle ear pressure by reopening the eustachian tube insufflates nasopharyngeal bacteria into the tympanic cavity. It is important to note that clinically healthy middle ears may also contain bacteria or evidence of bacterial biofilms,[21] possibly resulting from transfer from the nasopharynx during normal events, such as sniffing.[22] Otitis media occurs when viruses and bacteria evade the host mucociliary and immune responses and inflammation is established within the middle ear. Complex interactions occur between the otopathogens that are thought to modify the colonisation dynamics of the nasopharynx and increase susceptibility for otitis media.

Role of viruses

AOM is solely associated with viruses that cause URTI in up to 30% of cases.[23,24] The extent of this association has been recently strengthened by two 12-month prospective research studies of healthy children, incorporating comprehensive clinical examination and improved viral detection techniques.[5,25] US researchers reported that 97% of children aged 6 months to 3 years experienced one or more URTIs per year, with a mean of 5.4 infections over the 12-month study period, and nearly 10% of children aged under 3 years suffered more than 10 URTIs per year.[5] Otitis media was identified in 61% of URTIs reported in these children; AOM was present in almost one-third of these

Microbiology of acute otitis media

	Proportion of cases
Microbe*	
Bacteria only	35%–55%
Virus only	20%–30%
Bacteria and virus	28%–70%
Bacteria*	
Streptococcus pneumoniae	25%–50%
Haemophilus influenzae†	15%–30%
Moraxella catarrhalis	3%–20%
Group A streptococcus	1%–5%
Virus[5,24,25]	
Respiratory syncytial virus	41%–56%
Coronavirus	50%
Adenovirus	17%–46%
Rhinovirus	30%–44%
Influenza (all types)	23%–35%
Enterovirus	34%
Parainfluenza (all types)	33%

* Proportions reflect those reported in a number of studies.
† Predominantly non-typeable *H. influenzae*. ◆

children and OME was present in almost a quarter of these children. Children in the study experienced a mean of 1.7 episodes of AOM per child per year, and child age was the strongest predictor of AOM development after URTI, after controlling for sex and ethnicity.[5]

Both of the recent prospective studies of healthy children in the US confirmed that a range of respiratory viruses, causal for URTI, are highly coincident with new episodes of otitis media, with 20%–73% of AOM and new-onset OME cases being coincident with infection with one or more upper respiratory tract viruses.[5,25]

The viruses most commonly coincident with otitis media were adenovirus (70%), influenza virus types A and B (65.5%), respiratory syncytial virus (RSV; 63.2%), enterovirus (62.5%), coronavirus (55.6%), rhinovirus (55.6%) and parainfluenza virus (types 1, 2 and 3; 55.3%).[5] Interestingly, AOM and OME were associated with different viruses, with AOM most frequently associated with coronavirus (50%), RSV (47.4%) and adenovirus (46.5%), whereas new-onset OME occurred with influenza virus (34.5%) and enterovirus (34.4%).[5] US researchers also confirmed a high frequency of association of otitis media with rhinovirus (44%) and RSV (56%).[25] Among unwell children attending hospital for AOM, viruses common in URTI were detected in 35% of patients acutely ill with AOM. Among these children, the most commonly identified viruses (using viral culture) were RSV (41%), influenza (types A, B and C combined; 23%) and adenovirus (17%).[24]

Improved viral detection has also resulted in identification of a broader range of respiratory viruses, including human metapneumonia virus,[26] coronavirus (HCoV-NL63),[27] and bocavirus,[28] as causal for either URTIs or otitis media, although their relative contribution to otitis media is yet to be determined. Caution regarding these viruses and their potential association with otitis media and URTIs is necessary, as these viruses can also be detected in children without symptoms,[8] or, in the case of rhinovirus, over a prolonged period.[10]

The failure to detect URTI symptoms in only 2%–3% of AOM episodes in otherwise healthy children may potentially indicate that the AOM arose through bacterial or other inflammatory pathogenesis. The association of OME and URTIs is reflected in the observation that only 0.6% of new-onset OME episodes were diagnosed 30 days before URTI establishment.[5]

The Box provides a summary of the contribution of viral infections to AOM.

Role of bacteria

Bacterial co-infection with upper respiratory tract viruses, rather than viral or bacterial pathogenesis alone, predominates and is reported to range from 28% to 70% in the middle ear and nasopharynx.[23,24,29] The three most commonly recovered bacteria associated with otitis media are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*, which are all commensal within the nasopharynx; most H. influenzae isolated is nontypeable.[29-31]

In a hospital-based study, bacteria were cultured in up to 90% of nasopharyngeal secretions and 43% of middle ear fluid obtained from patients with AOM. *S. pneumoniae, H. influenzae* and *M. catarrhalis* were identified in 57%, 52% and 56% of nasopharyngeal secretions respectively, and less frequently in middle ear fluid (22%, 21% and 4% respectively).[24] Non-cultivable forms of *S. pneumoniae* and *H. influenzae* can also stimulate an immune response and result in OME,[32] and can occur in up to 36% of nasopharyngeal secretion samples.[33] Nasopharyngeal colonisation with *S. pneumoniae, H. influenzae* and *M. catarrhalis* and early onset of otitis media are closely correlated.[33-35] The nasopharynges of Indigenous Australian children, who are at high risk of otitis media, are colonised by these bacteria and by *Staphylococcus aureus* by 3 weeks of age.[36] For children at low risk of otitis media, first episodes of AOM, involving *S. pneumoniae*, *H. influenzae* and *M. catarrhalis,* peak in the first year of life, and AOM from all causes has a peak incidence between 6 and 12 months of age, as determined from culture of middle ear fluid.[37] In contrast, Indigenous Australian children at high risk of otitis media experience high rates of AOM between 3 and 6 weeks of age, with tympanic membrane perforation occurring in about two of 10 children in the study.[36] Overall, nine of 10 Indigenous children aged 6–30 months had clinical signs of otitis media, and tympanic membrane perforation had occurred in four of 10 children by 18 months of age.[36] Unfortunately, among these older children, AOM is often asymptomatic until discharge from the ear is visible.[38-40]

Otitis media has a high rate of recurrence, with three or more episodes of AOM reported among 50% of children aged 3 years; this rises to 65% for children aged 5 years of age, whereas OME recurs in 50% of children within 24 months.[14] For Indigenous children at high risk of otitis media, the rate of recurrence of AOM and OME is better described as persistent AOM. For these children, despite antibiotic treatment, AOM episodes do not typically present as acute in onset or short in duration. Indeed, persistent suppuration remained present for 77% of children for up to 14 days after initial diagnosis.[41]

In the first few years of life, about 20% of cases of AOM do not respond to antibiotic therapy,[42,43] and among such children, AOM may continue to either persist or recur.[42] There is ongoing controversy as to whether this ongoing AOM results from persist-

ence of the original infection or establishment of new infection; however, it has been reported that new infections may cause up to 54% of recurrent AOM episodes within 1 month of antibiotic treatment, whereas bacterial relapse of the original infection comprises about 28% of all cases.[43]

Early clinical recurrence of AOM within 3 weeks of initial treatment is associated with nasopharyngeal carriage of *S. pneumoniae*,[44] although *H. influenzae* is clearly associated with recurrent AOM[37] and was the most prevalent pathogen (42%) observed in both bilateral and unilateral otitis media.[45]

Extensive geographical variation has been observed in bacterial carriage[46] and disease,[47] as well as the pneumococcal serotypes[48] and the relative proportions of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* that are responsible for otitis media.[45,49,50] This variation adds to the complexity of developing an efficacious vaccine against otitis media.

The proportion of AOM cases attributable to bacteria is summarised in the Box.

Biofilms

Bacterial biofilms are microbial communities that attach to the mucosal surface and produce their own three-dimensional structures covered in an exopolysaccharide matrix. Biofilms are involved with a number of otolaryngological conditions.[51] Pneumococcal biofilms have been visualised in 92% of middle ear mucosal biopsy samples from children symptomatic for OME,[52] and *H. influenzae* isolates obtained from patients with recurrent AOM form biofilms in vitro.[53] It is important to recognise that middle ear biofilms may be present in up to 9% of healthy ears[54] without provoking symptoms.

Biofilms are hypothesised to cause chronic suppurative otitis media, and to explain the condition's resistance to antibiotic treatment.[55] Recent evidence has demonstrated that pneumococcal biofilms with a high biofilm-forming index exhibit greater resistance to azithromycin.[56] Effective eradication of biofilm infections requires killing the bacteria and the destruction of the matrix to minimise persistence of the viable organism.[57] Thus, persistent otitis media infections can arise from the failure to completely eradicate the original bacterial infection,[45] the presence of biofilms,[52] or intracellular bacterial infection of the middle ear mucosal cells, particularly mucus-secreting cells.[58]

Host response

The immune response in the middle ear to infection is characterised predominantly by an inflammatory response, which normally results in clearance of micro-organisms from the middle ear cavity in the acute phase. The polymicrobial nature of the disease explains,[17] at least in part but not entirely, why recurrent acute infection occurs. However, some children are at higher risk of recurrent and chronic disease, but the immune mechanisms important for protection against otitis media are poorly understood. Some studies have suggested that children who are prone to otitis media may have one of several immune perturbations, although none of these studies are conclusive with respect to causal linkage. It has also been suggested that the mucosal immune response may be down-regulated by persistent high nasopharyngeal carriage.[59]

Otitis media development is often preceded by a viral URTI, which may predispose children to secondary bacterial infections through mucosal epithelial damage, impaired mucociliary function and up-regulated inflammatory cytokine responses.[60]

Adenoid mucosa-associated lymphoid tissue aids the local immune protection against bacteria and viruses by local production of secretory antibodies that inhibit antigen uptake and block attachment and colonisation of microbes. Absence or lack of secretory IgA increases bacterial adherence to epithelia and bacterial colonisation of the nasopharynx.[61] Therefore, the observation that children who are prone to otitis media may have lower levels of IgA and certain IgG subclasses, particularly IgG2, is not unexpected.[62-65] However, there does not appear to be any overall deficit in the antibody response to routine paediatric vaccines.[66] An effective immune response to viral URTIs is dependent on the induction of a number of immunoregulatory cytokines, such as interleukin (IL)-2, IL-10, transforming growth factor β and allergy-associated cytokines, including IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor.[67] A recent study examining cytokine polymorphisms demonstrated that high-production IL-10 phenotypes were more frequent among children with new otitis media episodes coincident with RSV and rhinovirus infection, whereas low production of IL-6 and high production of tumour necrosis factor α (TNF-α) phenotypes contribute to otitis media risk during rhinovirus infection.[68] The association of certain genetic polymorphisms in *TNFA, IL6*, *IL10*, and *TLR4* (toll-like receptor 4) genotypes with increased susceptibility for otitis media[68-70] suggests that characteristics of the initial inflammatory response to infection may be crucial in setting the course for recurrent disease. Importantly, a number of environmental factors such as exposure to cigarette smoke and breastfeeding may further modify genetic risk.[69]

Management of otitis media and antimicrobial resistance

Australian therapeutic guidelines recommend antibiotics at initial consultation for infants under 6 months of age who are diagnosed with AOM and for all Indigenous children diagnosed with AOM.[71] However, given the high rate (80%) of self-resolving episodes and the minimal benefit of antibiotic treatment[72] for children who are not at high risk of developing complications, prudent use of antibiotics is proposed[73] to minimise the rate of development of antibiotic-resistant bacterial strains. Typically, a "wait and watch" approach using analgesia to reduce acute pain is recommended for children aged over 2 years who are at low risk,[74] as 90% of children with AOM treated with analgesia alone recover in a few days.[75] Middle ear effusion normally resolves within 7 days in 40% of cases and 75%–90% resolution occurs within 4 weeks.[6]

Unfortunately, in practice, despite an overall reduction (24%) in the use of antibiotics in Australian general practice between 1990–1991 and 2002–2003, the overall rate of antibiotic prescription for AOM in children increased, with antibiotics prescribed for 78% of cases in 1990–1991, rising to 84.4% in 2002–2003.[76]

The continued high rate of antibiotic prescription is not justifiable on the basis of prophylactic administration to reduce the prevalence of OME, as only marginal improvement (4%) has been observed in children at low risk.[77] Children at high risk of OME, such as Indigenous Australian children have benefited from prophylaxis, showing increased return of normal middle ear function (9.6%) and reduced risks of tympanic perforation (14%) and pneumococcal carriage (12%).[78] Over-prescription of antibiotics in the general community increases development of antibiotic-resistant *S. pneumoniae*. This was clearly demonstrated in a recent study,

which showed that antibiotic resistance was highly correlated with the use of antibiotics geographically across Europe.[79] Increasing antibacterial resistance has been demonstrated in all of the three most common bacterial otitis media otopathogens, *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*,[80] and indeed may negate the small vaccination protective effect against otitis media that has been observed with the pneumococcal conjugate vaccine.[81]

Vaccines, now and in the future

Otitis media is a polymicrobial disease, with four otopathogens predominating — *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and RSV. Hence, vaccine strategies should be initially directed at these microbes. For *S. pneumoniae*, the vaccine will need to include the major serotypes responsible for disease. Currently, three pneumococcal vaccines are available: a 7-valent polysaccharide conjugated vaccine that uses a non-toxic mutant of diphtheria toxin as a carrier protein (Prevenar, Wyeth, Sydney, NSW); a 10-valent polysaccharide conjugated vaccine that uses protein D as the main carrier protein (Synflorix, GlaxoSmithKline, Melbourne, Vic); and a 23-valent polysaccharide formulation (Pneumovax 23, Merck Sharp & Dohme, Sydney, NSW). All these vaccines were primarily developed for immunisation against invasive pneumococcal disease. Pneumovax 23 has been shown to be relatively efficacious (56%–81%) in preventing invasive pneumococcal disease in individuals over 2 years of age,[82] but has not been demonstrated to be efficacious for use in children against recurrent AOM when used as a booster to pneumococcal conjugate vaccination.[83,84] Immunisation with the 7-valent pneumococcal vaccine reduces the occurrence of AOM episodes by 6%–7%,[85] but performs better against AOM due to vaccine-type pneumococci — the reduction of episodes due to these serotypes is around 55%.[17,85] Of concern is the relative increase in the proportion of disease arising from non-vaccine serotypes of *S. pneumoniae*[86,87] and other bacterial pathogens.[37]

Newer vaccine formulations incorporating a greater number of *S. pneumoniae* serotypes, particularly one incorporating a protein antigen from *H. influenzae*, protein D (Synflorix), should improve vaccine efficacy against otitis media. Indeed, studies of an earlier vaccine formulation containing this protein, PCV11-*Hi*D, conferred protection against AOM caused by *H. influenzae* (approximately 35% of cases) in addition to that observed for the pneumococcal serotypes (approximately 53% of cases) present in the vaccine.[49]

Based on animal model studies,[88] the possibility of developing a tribacterial (*S. pneumoniae*, *H. influenzae* and *M. catarrhalis*) vaccine in the future is not beyond reality.[17] The inclusion of viral components in a future polymicrobial vaccine will come later.[17]

Experience from studies to date suggests that the microbial ecology of the nasopharynx may be altered by vaccination,[50,90] particularly as the microbes targeted are often part of the commensal flora. In addition, with the introduction of pneumococcal conjugate vaccines and the consequent decrease in vaccine serotypes — which were commonly resistant to antimicrobials — it is generally accepted that there has been an overall reduction in the rate of detection of antibiotic resistance.[90] However, there is some evidence of an increase in the antibiotic resistance of non-vaccine pneumococcal serotypes, which could reduce the overall impact of pneumococcal conjugate vaccination on antibiotic resistance.[81] Hence, it will be necessary to establish ongoing surveillance studies to monitor any changes in the otopathogen profile and to continue to prescribe antibiotics cautiously for treatment of otitis media.

Conclusion

Otitis media occurs frequently in young and very young children and results from infection of the middle ear by bacteria, viruses or both. Predominant bacteria causal for otitis media include *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, whereas viruses most commonly associated with otitis media include RSV, coronavirus, adenovirus and influenza virus.

The excessive use of antibiotics for the treatment of AOM in children who are not at risk of developing complications has contributed to the development of antimicrobial resistance. However, early and prophylactic antibiotic use is of benefit to children at high risk of developing complications from otitis media, such as Indigenous Australian children.

Future development of vaccines, including a greater number of pneumococcal serotypes and antigens from *H. influenzae* and *M. catarrhalis*, is needed. The development of therapeutic prevention strategies is complicated in that these bacteria may be able to evade antimicrobial therapy and host immune responses through the formation of biofilms and the capacity to reside intracellularly in middle ear mucosal cells. The development of protein-based vaccines with antigenic components from the three predominant causative bacteria and common upper respiratory tract viruses is a more distant possibility.

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