

# Viral–Bacterial Interactions in Acute Otitis Media

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**Abstract** Acute otitis media (AOM) is a polymicrobial disease, which usually occurs as a complication of viral upper respiratory tract infection (URI). While respiratory viruses alone may cause viral AOM, they increase the risk of bacterial middle ear infection and worsen clinical outcomes of bacterial AOM. URI viruses alter Eustachian tube (ET) function via decreased mucociliary action, altered mucus secretion and increased expression of inflammatory mediators among other mechanisms. Transient reduction in protective functions of the ET allows colonizing bacteria of the nasopharynx to ascend into the middle ear and cause AOM. Advances in research help us to better understand the host responses to viral URI, the mechanisms of viral–bacterial interactions in the nasopharynx and the development of AOM. In this review, we present current knowledge regarding viral–bacterial interactions in the pathogenesis and clinical course of AOM. We focus on the common respiratory viruses and their established role in AOM.

**Keywords** Eustachian tube · Upper respiratory tract infection · Middle ear fluid · Respiratory viruses · Vaccine · Influenza · Adenovirus · Rhinovirus · Respiratory syncytial virus

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## Introduction

Acute otitis media (AOM) is the most common illness necessitating medical therapy for children younger than 5 years in the United States [1]. It is also one of the major reasons for antibiotic prescriptions for children in many countries. Evidence to date suggests that AOM may not be a pure bacterial disease, but rather a polymicrobial disease, in which both bacteria and respiratory viruses participate and interact in the disease pathogenesis [2, 3]. In most cases, viral upper respiratory tract infection (URI) occurs before and/or concurrently with AOM [4–6]. It has also been shown that about one-third of children with viral URI developed AOM within 4 weeks of its onset [7]. Using conventional and molecular diagnostics, respiratory viruses have been detected from the nasopharynx in the majority of cases and in up to 70 % of the middle ear fluid (MEF) from children presenting with AOM [8–11]. It is notable that tympanic membrane changes in AOM can be observed as early as the first day of symptomatic viral URI [12•].

Current evidence suggests that specific virus-bacterial interactions may be associated with a different risk of AOM development in different mechanisms [13•, 14•]. In this review, we sought to identify the current knowledge regarding viral–bacterial interactions in the pathogenesis and clinical course of AOM.

## Pathogenesis of Virus-induced Acute Otitis Media

The three most common bacterial pathogens of AOM (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*) colonize the nasopharynx from early infancy and are considered as part of the normal flora [15]. Like other non-pathogenic bacterial flora colonizing the nasopharynx, these pathogenic bacteria do not cause

symptoms until there are changes in the nasopharyngeal milieu. Viral URI plays a pivotal role in AOM pathogenesis by causing nasopharyngeal inflammation, changes in bacterial adherence properties and colonization, and Eustachian tube (ET) dysfunction. The ET is the natural barrier which prevents influx of colonizing bacteria from the nasopharynx to the middle ear cavity. Young children are susceptible to AOM not only because of immaturity of the systemic immunity but also from the lack of anatomic immunity of the ET [16].

During viral URI, inflammatory changes in the nasopharynx and the ET are induced and bacterial adherence and colonization increase. Influenza A virus (IAV), coronavirus NL63 and respiratory syncytial virus (RSV) augment bacterial adherence to epithelial cells [17–19]; IAV has also been shown to promote nasopharyngeal colonization of *S. pneumoniae* [20]. Viruses also modify host immune functions [21, 22], and interfere with antibiotic activity [23–25]. Viruses alter mucous property and diminish the normal mucociliary clearance of the coating epithelial cells by reducing the production of bactericidal substances in the nasopharynx, ET, and the middle ear cavity, and hence increase bacterial pathogenicity [26, 27]. Mucociliary changes of the ET lead to ET dysfunction and/or obstruction and negative middle ear pressure, which occurs more severely in young children, as documented in tympanometric measurements during URI [28]. This negative middle ear pressure facilitates the entrance of both pathogenic bacteria and viruses into the middle ear cavity causing middle ear inflammation, MEF accumulation, and signs and symptoms of AOM.

### Viral Upper Respiratory Tract Infection

Viral URI evolves following direct invasion of the mucosa coating the upper airway by viral agents, which undergo frequent changes in antigenicity, posing challenges to the immune defense system. Inoculation by the virus begins when secretions are transferred by touching a hand exposed to the pathogen to the nose or mouth or by directly inhaling respiratory droplets. Most viral URI symptoms result from the inflammatory response of the immune system to the invading pathogens. Local infection in the nasopharynx can easily spread to the adjacent organs, leading to sinusitis, laryngitis, tracheobronchitis, pneumonia, and AOM in particular [29].

Several respiratory viruses have been extensively studied related to AOM pathogenesis; among the more common and important are IAV, RSV, human rhinovirus (HRV), and adenovirus.

**IAV:** Influenza virus is the only respiratory virus for which effective vaccines and antiviral drugs are currently

available. This makes it possible for the use of influenza vaccines and/or antiviral drugs to prevent of AOM associated with influenza. Between one-third and two-thirds of young children with influenza-associated URI develop AOM [7, 30]. IAV replicates in respiratory epithelial cells and circulating leukocytes. It produces chemokines and cytokines which induce the extravasation of blood and mononuclear cells to the extra-vascular matrix and the development of an antiviral and a Th1-mediated immune response [31]. IAV secretion of neuraminidase has been shown to increase pneumococcal adhesion and invasion capabilities in the ET and middle ear [32]. IAV-mediated inflammation caused ET congestion and further reduced ciliary function, which resulted in bacterial clearance inhibition [33]. Early viral challenge studies in adult volunteers infected with IAV have shown the development of an ET obstruction, negative middle ear pressure, and the development of MEF to a lesser extent (3–19 %) [34]. In an experimental model utilizing human middle ear epithelial cells, IAV infection led significant changes in host interferon-inducible genes, chemokine and cytokine genes, pro- and anti-apoptotic genes, signal transduction and transcription factors, cellular immune response, cell cycle and metabolism genes [35]. In an animal model, in which mice were infected intranasally with IAV, middle ear inflammation and even hearing loss were documented [36]. IAV co-infection with *S. pneumoniae* resulted with a higher bacterial load when compared with bacterial infection alone.

**RSV** is a large RNA paramyxovirus which is most commonly associated with bronchiolitis and pneumonia in very young children; it may also cause acute respiratory disease, including URI in any age group. RSV was detected for the first time from the MEF in the 1960s [37], and to date RSV is considered to be one of the most ototropic viruses [9, 14]. Nearly half of young children with RSV-URI developed AOM within 4 weeks of URI onset, mostly within 1 week [7]. In children presenting with AOM, RSV has been found in specimens obtained from nasal wash and MEF samples [8]. RSV detection from the MEF ranged from 2 % to 22 % of AOM cases, with or without positive bacterial cultures [7, 38, 39]. It has also been demonstrated that RSV is an important contributing factor for the occurrence of AOM in young children hospitalized with respiratory distress [40]. In a recent study in children aged 3–18 months who were hospitalized with acute bronchiolitis, more than half had AOM at entry or developed AOM within 14 days, and 25 % more developed MEF throughout the 2-week observation period [41]. RSV was identified in more than half of the MEF aspirates obtained. RSV has also been demonstrated to significantly prime the expression of several pro-inflammatory interleukins (IL) such as IL-6 and IL-8, which are the most important mediators of fever and of the acute

phase response and required for resistance against *S. pneumoniae* [42].

**HRV** is one of the most common causes of URI in adults and children, and has also been strongly associated with AOM. The primary site of inoculation of HRV is the nasal mucosa; it attaches to respiratory epithelium and spreads locally. The major HRV receptor is the intercellular adhesion molecule-1 (ICAM-1). The natural response of the human defense system to injury involves ICAM-1, which aids the binding between endothelial cells and leukocytes [43]. Approximately 30 % of the children 6–48 months of age with HRV present in their nasopharyngeal secretions developed AOM within 4 weeks of URI onset [7]. Advances in molecular biology using nucleic acid detection methods such as PCR, real-time PCR, microarray and genomic sequencing have not only allowed the discovery of HRVs, and have also made possible studies of the serotype diversity of HRV infections [43]. HRV has also been detected in the MEF; one recent study reported a high HRV detection rate in 58 % from children with a history of recurrent AOM [44]. HRV has also been associated with poor bacteriologic outcome for AOM treated with antibiotics, compared to other respiratory viruses [45].

**Adenovirus** is also a common URI virus, and is one of the most ototropic viruses; nearly half of children 6–48 months old with adenovirus URI developed AOM within 4 weeks of URI onset [7]. Children infected with adenovirus were 3 times more likely to have AOM with or without perforation when compared to children who had other respiratory virus infections [46]. In a chinchilla model of AOM, adenovirus has been shown to have synergistic effect with non-typeable *H. influenzae* [47]. One of the debates with adenovirus URI and AOM development is the fact that adenovirus nucleic acids can be detected by PCR several months after the initial infection. Therefore, detection of adenovirus nucleic acids in nasopharyngeal secretions may not always indicate that the virus is the cause of the current URI or AOM [48]. Among children with frequent URIs, Kalu et al. have shown that repeated positive PCR results for adenovirus may represent a new serotype/strain or prolonged presence of the same strain with continuous or intermittent detection during symptomatic URI.

In addition to the above viruses, **parainfluenza viruses** (types 1, 2, and 3) and human **enteroviruses** have been found associated with AOM [7, 49]; approximately one-third of URI associated with these viruses developed AOM within 4 weeks of URI onset. Many other types of common URI viruses, including **coronaviruses 229E, OC43, and NL63** have also been reported associated with AOM development after URI [7, 50–52].

The use of sensitive PCR techniques has enabled the detection of a few relatively new viruses. **Human bocavirus** (hBoV) has been detected in the upper airway of young

children with respiratory tract infections [53, 54]. This small single-stranded DNA parvovirus can be found alone or, more often, in combination with other viruses known to cause respiratory symptoms. Because of frequent hBoV co-infection with other viruses and its presence in the respiratory tract of asymptomatic children [55–57], the pathogenic role of hBoV has been questioned. In a recent study, hBoV1 seroconversion and primary infection was associated with respiratory illness and with AOM in young children [58]; this study has helped to clarify the significant role of hBoV1. HBoV DNA has been detected in the nasopharynx, MEF, and in the serum of children with AOM in several studies [2, 59–61]. HBoV infection may worsen clinical symptoms and prolong the clinical outcome of AOM [62]. In addition, concurrent presence of hBoV and *H. influenzae* in the nasopharynx has been associated with higher risk of AOM in children [14]. First identified in 2001, the role of **human metapneumovirus** (hMPV) in AOM complicating URI has also been studied. Many studies have shown that hMPV is prevalent and infects almost all children by age 5 years causing respiratory infections [63]. During AOM, hMPV has been detected from up to 13 % of nasopharyngeal samples and in 2.3 % MEF samples [64, 65]. Approximately one-quarter of hMPV-associated URI episodes were complicated by AOM; this is the lowest rate compared with other respiratory viruses [66].

To date, data from animal models as well as clinical studies have clearly shown that any virus that cause URI and compromise the upper airway may lead to AOM, even in the absence of pathogenic bacteria. Earlier studies in chinchillas have shown IAV and adenovirus alone to cause AOM [67, 68]; a more recent study in mice has also shown the ability of IAV to induce non-bacterial AOM and hearing loss [36]. In a study using comprehensive microbiologic diagnostics to detect bacteria and a broad spectrum of viruses from the MEF from children with AOM draining through tympanostomy tubes, Ruohola et al. reported 4 % of cases with viruses alone; 4 % no pathogen; 27 % bacteria alone; and 66 % bacteria and viruses [2]. Another clinical study of AOM development after 709 URI episodes reported 51 % incidence of AOM after URI in cases with all 3 types of pathogenic bacteria colonizing the nasopharynx, and only 10 % incidence in cases with no pathogenic bacteria colonized [28]. These reports continue to indicate the pathogenic role of viruses in AOM, even in the absence of bacteria.

### Specific Viral–Bacterial Interactions

There have been numerous reports on viral–bacterial interactions in the context of AOM pathogenesis, many of which are reviewed above. In animal models of AOM, synergism

between bacteria and viruses was evident as higher proportion of animals infected with both bacteria and virus developed AOM than those infected with bacteria or virus alone. This has been shown in animals infected with influenza, adenovirus, and/or *S. pneumoniae* [67, 69], and with adenovirus and/or non-typeable *H. influenzae* [47]. RSV can also facilitate bacterial adhesion to epithelial cells. An animal study demonstrated that in the presence of RSV in the nasopharynx, certain strains of *M. catarrhalis* have an enhanced ability to adhere to epithelial cells [70]. In a recent report, intranasally challenged chinchillas with *M. catarrhalis* followed by RSV infection resulted in subtle signs of AOM, and multiple signs of inflammation were observed in the middle ear mucosa, such as vasodilatation, submucosal edema, erythema, and bullous myringitis following sacrifice [71].

Although viruses are intracellular microorganisms and thus ‘infect’ the cells, as opposed to ‘colonize’ on the cell surface, studies using PCR have detected more and more respiratory viruses from the nasopharynx of asymptomatic children [72, 73]. In a study of the association between respiratory viruses and pathogenic bacteria in asymptomatic Aboriginal and non-Aboriginal children, viruses were detected in 42 % and 32 %, respectively [73]. HRV was the most frequent virus detected and associated with the presence of *H. influenzae* and *M. catarrhalis* in Aboriginal children. Adenovirus was positively correlated with *H. influenzae* in Aboriginal children and *M. catarrhalis* in non-Aboriginal children, but negatively associated with *S. pneumoniae* in Aboriginal children. The associations between viruses and AOM bacterial pathogens may have implications in future AOM preventive strategies.

A recent study examined risks of AOM development after URI in association with specific combinations of respiratory viruses and AOM bacterial pathogens detected during URI [14•]. Data were from a prospective, longitudinal study that included 194 children (6–36 months of age) with URI; microbiologic studies included nasopharyngeal bacterial and viral cultures and quantitative PCR for RSV, HBoV, and hMPV. The presence of adenovirus, bocavirus, *S. pneumoniae*, non-typeable *H. influenzae*, and *M. catarrhalis* was significantly associated with AOM occurrence. High RSV viral load ( $\geq 3.16 \times 10^7$  copies/ml) was associated with AOM risk, while high viral load of HBoV and hMPV was not. Adjusted for the presence of key viruses, bacteria, and AOM risk factors, AOM risk was associated with high RSV viral load with *S. pneumoniae* (OR 4.40; CI 1.9–10.19) and non-typeable *H. Influenzae* (OR 2.04; CI 1.38–3.02). The risk was higher for the presence of HBoV and *H. influenzae* together (OR 3.61, CI 1.90–6.86). The authors suggested that AOM prevention methods should consider methods for reducing infections caused by RSV, HBoV, and adenovirus, in addition to AOM bacterial pathogens.

## Effects of Viral–Bacterial Interactions on Clinical Course and Recovery of AOM

AOM commonly occurs as a bacterial complication of viral URI, and by the time AOM is established viral URI may have been aborted or the virus may never enter the middle ear. If the virus infects the middle ear along with bacteria or viral URI is still ongoing at the time of AOM diagnosis, many earlier studies have shown that viral–bacterial interaction can lead to adverse AOM outcome. Prolonged AOM symptoms and bacteriologic failure result from concurrent viral infections [6, 74, 75], through mechanisms such as virus-induced inflammation [76, 77] and interference of antibiotic penetration into the middle ear [23, 24]. These earlier studies detected viruses by the conventional diagnostics (viral culture and antigen detection), which are less sensitive than molecular diagnostics such as PCR and, therefore, detected viruses only in cases with larger inoculums. Due to widespread use of PCR for respiratory virus detection from the nasopharynx and MEF, virus yield has been achieved in a higher proportion of cases, with a broader spectrum of viruses. However, debate is still ongoing whether detection of viral nucleic acids, as opposed to live viruses in the MEF, indicates a significant role of the detected virus [78•]. Future studies are still required to confirm the effects of low quantity of viruses in the MEF, as detected by PCR only, on the clinical course and recovery of AOM.

## Clinical Importance

### AOM Management

Knowledge and understanding on viral–bacterial in AOM has clinical implications on AOM management. It is known that many patients with mild to moderate AOM recover spontaneously without antibiotic therapy; viral AOM may explain some of the cases in this category. Initial observation or “watchful waiting” has become an acceptable option for primary AOM, according to the clinical practice guidelines around the world [79, 80]. On the other hand, when AOM is not a pure bacterial infection, results of antibiotic treatment may not be as expected. Response to antibiotic treatment in cases of viral co-infection may only be partial, although bacterial pathogens are sensitive to the antibiotic used [6, 74]. The clinician may then consider allowing more time for clinical response, without changing the antibiotic in cases with less severe persistent symptoms.

### Prevention of AOM

From the enormous data gathered on the role of respiratory viruses and pathogenic bacteria on AOM pathogenesis, it is

clear that prevention of AOM will be achieved through prevention of viral URI, prevention or elimination of nasopharyngeal colonization by pathogenic bacteria, and specific and early treatment of viral URI when possible. To date, effective prevention and treatment for respiratory viruses are only available for influenza. Both trivalent inactivated influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV) are now recommended for routine use for children of all ages. Both TIV and LAIV have been shown to be effective in preventing against influenza and influenza-associated AOM morbidity [30, 81–84]. A recent report analyzing data on the efficacy of LAIV against influenza-associated AOM concluded that children receiving LAIV had a high level of protection against influenza-associated AOM, when compared to placebo or TIV [85]. However, LAIV is currently licensed for only children older than 2 years of age. For antiviral drugs, a randomized, double-blind, placebo-controlled study of 695 children has shown that using oral oseltamivir within 48 h of influenza-like symptoms reduced new AOM diagnosis by 44 % [86]. Subsequent analyses from the same cohort showed that among laboratory-confirmed influenza cases, oseltamivir significantly reduced AOM occurrences (relative risk 0.57; CI 0.37–0.88) and the treatment effects were greatest in children aged 1–2 years old [87].

For reduction of bacterial colonization, currently available bacterial vaccines are for *S. pneumoniae*. Seven-valent pneumococcal conjugate vaccines (PCV-7) became available for routine administration in infants in the United States in 2000. The vaccine aimed against prevention of diseases caused by the most common 7 of more than 90 serotypes of *S. pneumoniae*. PCV-7 has dramatically reduced the incidence of pneumococcal invasive diseases [88]. In PCV-7-vaccinated children, *S. pneumoniae* strains expressing vaccine-type serotypes have virtually disappeared from the nasopharynx and MEF [89]. However, overall reduction of AOM cases was only 6–7 % following PCV-7 introduction [90]. The currently licensed vaccine has added benefit by aiming against 13 serotypes of *S. pneumoniae* (PCV-13) and has been approved in the United States since 2010. The impact of PCV-13 on AOM prevalence reduction has not been published, although there have been predictions of further reductions of AOM from this vaccine use [91].

One way to reduce colonization of pathogenic bacteria is by augmenting bacterial–bacterial interference. In infants, the negative association in nasopharyngeal colonization between *S. pneumoniae* or non-typeable *H. influenzae*, and *S. aureus* has been shown [92, 93]. In vitro studies have shown inhibition of AOM bacterial pathogens by bacterial flora such as alpha-hemolytic streptococci [94, 95]. Therefore, investigators have attempted to use probiotics, both by oral administration and nasal spray, to prevent recurrent AOM episodes and to reduce middle ear effusion [96–98]. To date,

studies have shown conflicting results; while some studies have shown positive effects of probiotics in reducing AOM incidence [97, 98], others have shown no positive outcomes [96]. The different types of probiotics, doses and route of administration may partly explain the differences in results. More studies are needed to identify the most promising probiotics that will be beneficial for AOM prevention.

## Conclusions

AOM is one of the most common infectious diseases in children, and the economical and individual burden of the disease is remarkable. To date, AOM is regarded as a multifactorial and polymicrobial disease which challenges researchers to further explore molecular, genetic, immunological, microbial, and metabolic pathways which participate in AOM pathogenesis. Viral–bacterial interactions play a significant role in AOM pathogenesis and have clinical implications. Further knowledge and understanding on viral–bacterial interactions will be a basis for successful management and preventive strategies for this common childhood disease.

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