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# Viral-Bacterial Interactions in Childhood Respiratory Tract Infections

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## Abstract

Acute respiratory infection (ARI) is an important cause of childhood morbidity and mortality worldwide. ARIs are caused primarily by viruses and bacteria that are often co-detected in respiratory specimens. Although viral-bacterial co-infections are frequently reported in children with ARI, their clinical significance and the mechanisms leading to ARI are not well understood. The respiratory tract is a reservoir of a diverse community of microorganisms, including both commensals and potential pathogens and there is growing evidence that the interactions between viruses and bacteria play a key role in the development of ARI. A better understanding of the interactions between viruses and bacteria in the respiratory tract may enhance insight into the pathogenesis of ARI, and potentially reveal new prevention and treatment strategies. This chapter summarizes the current knowledge on viruses, bacteria and viral-bacterial interactions in childhood ARI and the possible mechanisms by which these interactions may lead to disease.

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## 8.1 Introduction

Acute respiratory infection (ARI), both upper and lower, is a significant cause of childhood morbidity and mortality worldwide. Lower respiratory infection (LRI), including pneumonia, is among the leading causes of childhood mortality worldwide, accounting for close to a million deaths in children under 5 years of age in 2013 [1]. Viruses and bacteria can be detected in most children with ARI. However, both are also frequently detected in asymptomatic children, and hence, the clinical

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significance of their detection has long been debated. Furthermore, a wide range of prevalence rates are reported for the detection of viruses and bacteria, most likely due to differences in case definitions, diagnostic tools and methodologies used between studies and hence, the epidemiology and etiology of ARI are still not clear. Viruses and bacteria are often co-detected in respiratory samples from children with ARI and their interaction is likely to play a key role in the development of disease. However, the clinical significance of viral-bacterial co-infections and the mechanisms leading to ARI are not well understood.

Viruses are identified more frequently than bacteria in children with ARI [2]. Respiratory syncytial virus (RSV) has long been believed to be the most important viral cause of childhood ARI, particularly bronchiolitis, accounting for at least three million hospitalizations and up to 200,000 deaths each year [3]. Human rhinovirus (RV) is the leading cause of upper respiratory infections (URI), but is also an important cause of LRI including pneumonia and bronchiolitis, and accounts for the majority of asthma attacks in children [4, 5]. Advances in molecular methods such as polymerase chain reaction (PCR) in recent years have led to the identification of new viruses and viral species, and the importance of other viruses is now widely acknowledged. Adenovirus, influenza virus, parainfluenza virus, human metapneumovirus, coronavirus and bocavirus are among other commonly identified respiratory viruses known to contribute to the burden of childhood ARI.

Although less frequently detected than viruses, bacteria were traditionally believed to be the cause of more severe ARI-associated morbidity and mortality, particularly in developing countries [6, 7]. However, traditional diagnostic methods such as blood culture, which are still considered the gold standard in most settings, lack sensitivity and hence, the disease burden attributable to specific bacteria is not well understood. The recent advent of sequencing technologies such as 16S rRNA gene sequencing have led to the discovery of far more diverse respiratory microbial communities than previously recognized [8]. However, advanced diagnostics are largely limited to countries with the most resources and studies detecting a comprehensive range of pathogens remain scarce or non-existent. *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* are acknowledged as the most common and important bacterial pathogens in childhood ARI, although more recent studies have reported substantial reductions of ARIs due to pneumococcus and *Haemophilus influenzae type B* (Hib), most likely owing to the widespread introduction of conjugate vaccines [9].

The respiratory tract is a reservoir of a diverse community of microorganisms, both commensals and potential pathogens [10]. There is growing evidence that synergistic and antagonistic interactions between viruses and bacteria play a key role in the development of ARI. A better understanding of the interactions between viruses and bacteria in the respiratory tract may provide improved insight into the pathogenesis of ARI, and potentially reveal new prevention and treatment strategies. This chapter summarizes the current knowledge on viruses, bacteria and viral-bacterial interactions in childhood ARI and the possible mechanisms by which these interactions may lead to disease.

## 8.2 Respiratory Viruses

Respiratory viruses are an important cause of both upper and lower ARI. The burden of viral respiratory infections is greatest in children, with infants and pre-school children experiencing an estimated 6–10 viral infections annually [11]. The role of viruses in URI is well established, with 90% of URI caused by viruses compared with only 10% caused by bacteria [12]. Respiratory viruses are also commonly identified in children with LRI, and although their role in LRI is acknowledged, evidence for the clinical significance of respiratory viruses in LRI is still debated. Lung aspiration is considered the gold-standard for sampling since it is obtained directly from the site of infection and would indicate etiological significance for LRI, but is limited due to its invasive nature and rate of complications [11]. Upper respiratory tract samples such as nasopharyngeal aspirates are easily obtained and hence, more frequently used in etiological studies. However, it is argued that viruses identified in the nasopharynx represent only colonization and are not indicative of LRI. Nonetheless, viruses of the upper respiratory tract are more commonly identified in children with LRI than in asymptomatic, healthy children. A 2015 systematic review and meta-analysis of case-control studies of children with and without LRI found evidence for causal attribution of RSV, influenza, parainfluenza virus, human metapneumovirus and RV in children presenting with LRI compared to asymptomatic or healthy children [13]. However, there was no significant difference in the detection of adenovirus, bocavirus or coronavirus between cases and controls.

While RSV and RV are widely acknowledged to be the most common and important viral causes of ARI in children, recent advances in molecular methods have highlighted the significance of other respiratory viruses.

### 8.2.1 Common Respiratory Viruses

#### 8.2.1.1 Respiratory Syncytial Virus

RSV has been considered the most important viral cause of ARI in children, although its contribution is variable [14, 15]. RSV is a seasonal virus that peaks in the cold season in temperate climates and in the rainy season in tropical climates [15]. It affects about 90% of infants and young children by the age of 2 years, with peak rates occurring in infants 6 weeks to 6 months of age [15]. There are two subtypes of RSV, A and B, which circulate concurrently, although some studies have suggested that RSV-A may be more virulent than RSV-B [16–18]. In 2005, there were an estimated 33.8 million new episodes of RSV-associated ARI worldwide in children less than 5 years of age, with at least 3.4 million RSV-associated ARI hospitalizations, and an estimated 66,000–199,000 deaths, 99% of which occurred in developing countries [3]. Hence, RSV is justifiably seen to be the most important cause of childhood ARI and a major cause of ARI-associated hospital admission [3]. Although RSV has been associated with higher rates of hospitalization than other respiratory viruses in several studies, estimates of RSV-associated ARI

incidence and hospitalization are highly variable between and within regions, likely due to methodological differences [15].

### **8.2.1.2 Human Rhinovirus**

RV is the most commonly identified respiratory virus in both adults and children and was found to be responsible for approximately two-thirds of cases of the common cold [19]. Hence, it is frequently referred to as the “common cold” virus. However, there is now abundant evidence from experimental and observational studies to support the role of RV as a lower respiratory tract pathogen. Early experiment studies with RV suggested that viral replication was optimal at 33 °C (91.4 °F) and was reduced at 37 °C (98.6 °F) and 39 °C (102.2 °F) [20, 21]. However, more recent studies have shown minimal differences in replication capacities at 33 °C (91.4 °F) and 37 °C (98.6 °F) for eight different RV strains, including when viruses were cultured and titrated at the same temperature [22]. Hence, RV is now recognised as an important cause of LRI including pneumonia and bronchiolitis and importantly, accounts for the majority of asthma attacks in children [4, 5]. Several studies worldwide have reported RV as the most common virus identified in children with LRI, with identification rates of up to 63% in some populations [23].

RV was first isolated and associated with respiratory clinical disease in human in 1956 [24] and by the 1980s, one hundred and one RV-A and RV-B serotypes, known as the reference or prototype had been preserved and distributed by the American Type Culture Collection (ATCC). The retrospective discovery of the third RV species, RV-C, reported in 2006 [25, 26], led to several new investigations of the prevalence of RV. The majority of these studies in children hospitalized with ARI found that RV-C was the most prevalent RV species and was often associated with more severe illness [5, 25–31].

### **8.2.1.3 Adenovirus**

Adenovirus is another common viral cause of ARI. Adenoviruses are classified into seven species, A to G, and different serotypes have been implicated in different clinical syndromes [32]. While the majority of adenovirus infections present as a mild URI, adenovirus is also known to cause LRI including pneumonia, bronchiolitis and bronchitis [32, 33]. Adenoviruses can also cause gastrointestinal, ophthalmologic, genitourinary and neurological infections. Adenovirus infections are most common during infancy and early childhood and is prevalent in up to 17% of children hospitalized with ARI [32]. Adenovirus is often associated episodes of recurrent wheezing, fever, hypoxia and lengthy hospitalizations [32].

### **8.2.1.4 Influenza Virus**

Influenza virus is an important cause of ARI morbidity and mortality, with the highest burden among children less than 5 years of age and adults over 65 years of age [34, 35]. The two main subtypes of influenza virus, A and B, routinely circulate and are responsible for seasonal flu epidemics each year. In the first study to estimate the global incidence of influenza-associated ALRI in children less than 5 years of age, there were an estimated 90 million new cases of influenza episodes, 20 million cases

of influenza-associated ARI and one million cases of influenza-associated severe ARI causing 28,000–111,500 deaths worldwide in 2008 [36]. In a more recent systematic analysis of the burden of influenza in paediatric respiratory hospitalizations between 1982 and 2012, influenza was associated with an estimated 870,000 hospitalizations in children less than 5 years of age annually [37].

### **8.2.1.5 Human Parainfluenza Virus**

Human parainfluenza virus (HPIV), first discovered in the late 1950s, is an important cause of ARI in children, accounting for 2–17% of hospitalized cases [38–40]. There are four types of HPIV, HPIV 1–4. HPIV-1 to HPIV-3 are common causes of ARI in infants and young children, while HPIV-4 is less commonly associated with respiratory illness [41]. Serologic studies have demonstrated that almost all children between 6 and 10 years of age have evidence of past infection, suggesting mild or asymptomatic primary infections [42].

### **8.2.1.6 Human Metapneumovirus**

Human metapneumovirus (hMPV) was first isolated from young children with respiratory tract disease in the Netherlands in 2001 [43]. Although hMPV was only recently discovered, phylogenetic analysis showed that hMPV has been circulating in humans for at least 50 years [44]. Clinical symptoms of hMPV are similar to those caused by RSV, ranging from URI to severe bronchiolitis and pneumonia [43, 45]. In a study evaluating the burden of hMPV infections in children in the USA, hMPV was detected in 6% of children hospitalized with ARI, 7% of children in outpatient clinics and 7% of children examined in emergency departments, with the greatest burden in children less than 1 year of age [46]. Other studies of hMPV in children hospitalized with ARI have reported prevalences as high as 11–25% [47–50].

### **8.2.1.7 Human Coronavirus**

Human coronavirus is often associated in respiratory illness. Four human coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1) are endemic in most populations and are associated with mild, self-limiting respiratory illnesses. Another two human coronaviruses, SARS-CoV and MERS-CoV cause severe respiratory syndromes and present a significant threat with their high fatality rates. The four non-severe human coronaviruses are implicated in both URI and LRI, with reported prevalence of approximately 10% in children hospitalized with ARI [51].

### **8.2.1.8 Human Bocavirus**

Human bocavirus 1 (hBoV1) was first discovered in 2005 from patients with LRI in Sweden and was the first virus to be discovered by molecular virus screening [52]. Since then, three additional species of HboV, hBoV2, hBoV3 and hBoV4 have been discovered, [53–55] although these species are found in the gastrointestinal tract and have been associated with gastroenteritis [53–55]. In contrast, HboV1 is associated with respiratory illness, and more specifically childhood ARI, with reported prevalence ranging from 1.5 to 19% [56]. As with other respiratory viruses, diagnosis of hBoV1 infection is not possible by clinical presentation and for URI common

symptoms include common cold-like symptoms and wheezing and for LRI clinical scenarios include pneumonia and bronchiolitis [57].

### 8.2.2 Viral Co-infections

The advent of improved molecular methods in recent years has increased the sensitivity in identifying viruses in children with ARI [58]. As a result, identification of multiple viruses in respiratory specimens from children with ARI is frequently reported [59, 60], with co-infection rates as high as 40–50% [23, 61]. However, the relationship between viral co-infections and severity of ARI in children is not conclusive. Some studies have reported increased risk of ARI hospitalization, increased length of hospital stay and worse clinical outcomes in children with viral co-infections [60, 62–65]. In contrast, recent systematic reviews and meta-analysis evaluating the relationships between respiratory viral co-infections in children have concluded that viral-coinfections were not associated with ARI severity [66–69].

To date, experimental studies of respiratory co-infections are scarce. An *in-vitro* study examining interactions between RSV and influenza virus demonstrated that growth of RSV was blocked by competitive infection with influenza A virus [70]. In the study by Shinjoh et al., RSV infection produced a higher peak viral load in single infections than in co-infections with influenza virus, if the infections were initiated at the same time [70]. However, if the influenza co-infection was initiated after the RSV infection, influenza growth was suppressed by RSV [70]. The study also demonstrated suppression of the growth of RSV by influenza A infection at the level of viral protein synthesis [70]. Indirect immunofluorescence revealed that a large proportion of infected cells synthesized both RSV and influenza A virus antigens, while scanning electron microscopy demonstrated that influenza A and RSV virions possessing surface antigens specific for each virus were selectively released from dually-infected cells [70].

In a mathematical model study investigating the dynamics of respiratory viral co-infections, Pinky et al. found that during co-infections, one virus could block another virus by being the first to infect the available host cells and that viral interference through immune response interaction was unlikely [71]. Interestingly, the study found that viral growth rate determines which virus will dominate a simultaneous infection [71]. For example, RV, the fastest-growing virus, reduced replication of the remaining viruses during a co-infection, while parainfluenza virus, the slowest-growing virus is suppressed in the presence of other viruses [71]. The authors of the study suggest that the blocking of one virus infection by the presence of another could be explained through resource competition and this finding has been supported by clinical studies of children with ARI [72, 73]. Canducci et al. found that co-infection of RSV and metapneumovirus in infants with ARI was a protective factor for length of hospital stay and hypoxia, when compared with RSV infection alone [72]. Marguet et al. also found shorter length of hospitalization in infants with RSV and RV co-infection comparing with single RSV infection [73].

Several other explanations for why identification of multiple viruses do not increase ARI severity have been suggested. One suggestion is that identification of a virus in a respiratory specimen using molecular methods may represent early detection of an infection, asymptomatic carriage, low-virulent infection or prolonged shedding [65]. Therefore, some cases of co-infection may simply be a subject of co-detection. It is also possible that the clinical outcome of viral co-infections is dependent on specific viral combinations, and this may explain the lack of consensus in the literature regarding whether viral co-infections is associated with an increased ARI severity. Many viral co-infection combinations have been reported, as reviewed by Scotta et al., with RSV and RV being the most frequently detected co-infection, although many studies do not even specify the viruses involved in viral co-infections [66].

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### 8.3 Respiratory Bacteria

Although less frequently detected than viruses, bacteria are also acknowledged to be an important cause of ARI-associated morbidity and mortality. Traditional diagnostic methods such as blood culture, which are still considered the gold standard in most settings, lack sensitivity, and hence the disease burden attributable to specific bacteria is not well understood. Given the absence of a reference standard for the detection of bacterial pathogens, the prevalence of bacteria in ARI is likely to be higher than often reported. *S. pneumoniae*, *H. influenzae*, *M. catarrhalis* and *S. aureus* are acknowledged as the most common and important bacterial pathogens in childhood ARI, although more recent studies have reported substantial reductions of pneumococcal and Hib disease, most likely owing to the widespread introduction of conjugate vaccines [9]. Culture-independent techniques have also demonstrated that the human microbiome is far greater in extent than previously recognised [74] and that only 1% of all bacteria can be cultured using standard diagnostic methods [8]. However, advanced diagnostics are still rare in most clinical settings and studies detecting a comprehensive range of pathogens remain scarce or non-existent.

The lower respiratory tract and lungs were traditionally believed to be sterile and free from bacteria. However, it is now widely accepted that the lungs are constantly exposed to diverse communities of bacteria from the upper respiratory tract and this has been confirmed with the use of culture-independent techniques such as the 16S rRNA gene sequencing. In one of the first studies in the field, Hilty et al. challenged the dogma that the lower respiratory tract is sterile by showing that bronchial tree contains a characteristic microbial flora that differs between health and disease [75].

The human respiratory tract is home to a diverse community of both commensal and potential pathogenic bacteria that cause respiratory disease. The term “microbiome” was first proposed in 2001 by Joshua Lederberg and is used to describe this “ecological community of commensal, symbiotic and pathogenic microorganisms that literally share our body space.” [76] In a balanced state, the respiratory microbiome is believed to play a beneficial role for the human host [77]. However, imbalances in the respiratory microbiome can contribute to the acquisition of new

pathogens, carriage of multiple pathogens or interactions among pathogens that lead to respiratory disease. In order to cause respiratory disease, bacterial pathogens must first colonize the nasopharynx. Since the nasopharynx lies between the nose, sinuses, ears, larynx, and the lower respiratory tract, pathogens of the nasopharynx can be the source for both upper and lower respiratory tract infections and hence, plays an important role in both the development of disease and the spread of pathogens [78]. Colonization is believed to be a dynamic and complex microbial process involving acquisition and elimination of species, interactions among microbes and between microbes and the host, and interference by environmental factors [10]. Given these bacteria often co-exist in the same ecological niche; it is likely that highly evolved relationships exist between these bacteria and their interactions with each other play a critical role in the pathogenesis of disease [79]. Furthermore, it is also likely that these species interact with one another even during healthy states [10].

### 8.3.1 Common Respiratory Bacteria

#### 8.3.1.1 *Streptococcus pneumoniae*

*Streptococcus pneumoniae*, or pneumococcus, is a Gram-positive, alpha-hemolytic (under aerobic conditions) or beta-hemolytic (under anaerobic conditions), facultative anaerobic member of the Streptococcaceae family [80]. It is responsible for a range of illnesses including pneumonia, meningitis, bacteremia, otitis media and sinusitis [81] and is the most commonly isolated organism in patients with community-acquired pneumonia [82]. *S. pneumoniae* colonizes the upper respiratory tract and is part of the normal flora of healthy individuals, particularly children. Although there are over 90 different serotypes, most cases of disease are caused by relatively few serotypes, with the ten most common serotypes accounting for 62% of invasive pneumococcal disease [83]. Since its isolation in 1881, there have been great efforts to treat and prevent *S. pneumoniae*. Antibiotic treatment for invasive pneumococcal infections typically includes broad-spectrum antibiotics until results of antibiotic sensitivity testing are available. However, emerging antibiotic resistance is a growing concern because of its potential negative impact on the outcome of patients who receive standard antibiotic therapy. Pneumococcal vaccines such as the pneumococcal conjugate vaccine or pneumococcal polysaccharide vaccine are now commonly administered to children globally.

#### 8.3.1.2 *Haemophilus influenzae*

*Haemophilus Influenzae* is a Gram-negative, coccobacillary, facultative anaerobic pathogenic bacterium belonging to the Pasteurellaceae family [84]. It was first described in 1892 during an influenza pandemic and was mistakenly considered to be the cause of influenza until 1933 when the viral cause of influenza was known [85]. *H. influenzae* is commonly found in the upper and lower respiratory tract as a commensal but also causes a variety of both invasive infections, such as bacteremia, facial cellulitis, septic arthritis, and meningitis primarily in non-immune children

under age 4 years of age, and respiratory tract infections such as pneumonia, acute otitis media, bronchitis, epiglottitis and sinusitis in children and adults [86]. There are six different *H. influenzae* capsular serotypes, a through f, in addition to nonencapsulated or nontypeable strains. The two most important human pathogens are the capsular serotype b strains and the nontypeable strains which are nonencapsulated. Non-typeable *H. influenzae* (NTHi) live exclusively in the pharynges of humans and are increasingly recognized as pathogens that cause both localized infections of the respiratory tract (middle ear spaces, sinuses, and bronchi) and systemic infections such as bacteremia and pneumonia [86].

### 8.3.1.3 *Moraxella catarrhalis*

*Moraxella catarrhalis* is a Gram-negative, aerobic, oxidase-positive diplococcus belonging to the Moraxellaceae family that was first described in 1896 [87]. For most of the past century, *M. catarrhalis* was regarded as an upper respiratory tract commensal organism. However since the late 1970s, *M. catarrhalis* has been recognized as an important and common human respiratory tract pathogen [88]. Nasopharyngeal colonization is more prevalent among infants compared with adults, with colonization rates varying between 33 and 100% in infants from different parts of the world [89–91]. *M. catarrhalis* is also a common cause of otitis media in infants and children, causing 15–20% of acute otitis media episodes [88].

### 8.3.1.4 *Staphylococcus aureus*

*Staphylococcus aureus*, a Gram-positive coccal bacterium belonging to the Staphylococcaceae family, frequently colonizes the nasopharynx, respiratory tract and skin [92]. It is both a commensal bacterium and a human pathogen, with colonization rates of approximately 30% in the general population, although higher rates are observed in young children and the elderly [92, 93]. *S. aureus* is also a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections [93].

## 8.3.2 Bacterial Interactions

Several studies have investigated interactions between bacteria, although mostly in experimental and mathematical model studies. In an *in-vivo* study where *H. influenzae* was introduced into the nasopharynx of neonatal rats that had or had not been pre-colonized by *S. pneumoniae*, Margolis et al. reported that *H. influenzae* density increased when *S. pneumoniae* was present, suggesting synergism between these bacterial species [94]. However, when these two species were inoculated in the reverse order, inhibition was observed, indicating competition between both species [94]. Another *in-vivo* study by Lysenko et al. found that both *S. pneumoniae* and *H. influenzae* successfully colonized mice when each bacteria species was injected separately [95]. However, when *S. pneumoniae* was co-colonized with an *H. influenzae* strain, the density of *S. pneumoniae* was lower than when inoculated alone, and this was later proved to be fully dependent on complement- and

neutrophil-mediated killing of pneumococci [96]. These findings were supported by a large epidemiological study of the 9-valent pneumococcal conjugate vaccine and prevalence of bacterial colonization in HIV-uninfected and HIV-infected children in South Africa that reported inverse associations between *S. pneumoniae* and *S. aureus* and between *S. aureus* and *H. influenzae* in HIV-uninfected children but not HIV-infected children [97].

Mathematical models investigating bacterial interactions have produced conflicting results. Using a multivariate random effects model for longitudinal data, Jacoby et al. found a positive association between *S. pneumoniae* and *H. influenzae* colonization among Aboriginal and non-Aboriginal children in Australia [98]. In contrast, Pettigrew et al. modeled bacterial colonization in children and reported *S. pneumoniae* colonization to be negatively associated with colonization by *H. influenzae* [99]. The study also reported negative associations between *S. pneumoniae* and *S. aureus* and between *H. influenzae* and *S. aureus*, but interestingly, when *H. influenzae* was present with *M. catarrhalis*, the odds of *S. pneumoniae* colonization increased by more than two-fold [99]. These studies suggest that interactions between bacteria are complex and may shift from negative to positive when additional bacteria species are present.

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## 8.4 Epidemiology of Viral-Bacterial Co-infections

### 8.4.1 Historical Context

Viral-bacterial co-infections are frequently detected in children with respiratory illness, and there is strong evidence for enhanced ARI severity in children during co-infections compared with single infections [100]. While most studies report viral-bacterial co-infection rates ranging from 20 to 50%, rates as high as 66–77% have been observed [70–72]. The clinical significance of viral-bacterial co-infections and mechanisms that drive these interactions are not well understood. It is difficult to determine the relative importance of individual viruses and bacteria involved in co-infections since both viruses and bacteria can be carried commensally in the respiratory tract and their detection may reflect colonization, rather than infection. Furthermore, it is not possible to distinguish between primary and secondary infections in clinical studies, making it difficult to elucidate the interactions between viruses and bacteria in co-infections.

The earliest suggestion that viral infections predispose to secondary bacterial infections has been attributed to French physician R. T. H. Laennec, who observed that the prevalence of pneumonia increased following an influenza epidemic in 1803 [69]. In 1947, British epidemiologist, William Farr, coined the term “excess mortality” to describe the increase in number of deaths during the influenza season that were not caused by influenza itself, and developed the methodology used today to quantitate mortality in influenza epidemics [70]. The association between influenza and secondary bacterial infections came into particular influenza pandemics during the twentieth century, as well as subsequent observational and experimental

studies, provided further evidence that viral infections predispose to secondary bacterial infections.

During the 1918 “Spanish flu” pandemic, over 50 million deaths occurred, most of which were not caused directly by influenza alone but rather as a result of secondary bacterial pneumonia [71]. The most frequently identified organisms in sputum, lung and blood samples of infected patients were *S. pneumoniae*, *H. influenzae*, *Streptococcus pyogenes* and *S. aureus*, and it was believed that the influenza virus acted synergistically with pathogenic bacteria resulting in increased incidence of disease and death [71]. These findings were supported by data from the 1957 “Asian flu” and 1968 “Hong Kong flu” pandemics showing that increased mortality was associated with increased incidence of bacterial pneumonia [72, 73]. The availability of antibiotics effective for secondary bacterial infections was believed to be a key factor for the lower number of deaths during the 1957 and 1968 pandemics compared with 1918. During the 2009 “swine flu” pandemic involving the H1N1 influenza virus, bacterial co-infection was frequently reported in fatal pneumonia cases, with *S. pneumoniae* being the most frequent bacteria identified [74, 75].

#### 8.4.2 Clinical Evidence of Viral-Bacterial Co-infections in Children

The best and most studied example of respiratory viral-bacterial co-infections involves influenza virus, and influenza virus-bacterial co-infections has been well described in both adults and children, with clear associations with increased disease severity [101–103]. However, with the exception of outbreaks, influenza virus is a relatively infrequent viral pathogen compared to other respiratory viruses including RV and RSV.

RSV is commonly implicated in viral-bacterial co-infections with reported co-infection rates of up to 17.5–44% in RSV-infected children [104–108]. In children with severe bronchiolitis studied by Thorburn et al., bacteria were isolated from 42% of lower airway secretions from infants with RSV using culture methods [107]. *H. influenzae* and *S. aureus* were the most common bacteria identified and furthermore, the study reported that children with bacterial co-infection were at increased risk for bacterial pneumonia [107]. In serological study of children with community-acquired pneumonia, 39% of children had viral-bacterial co-infections, of which RSV and *S. pneumoniae* was the most common combination, accounting for 33% of cases [109]. Like influenza, both RSV and *S. pneumoniae* infection peaks during the winter months [110] and RSV has also been linked to seasonal increases in *S. pneumoniae* [111]. In a case-control study by Benet et al., co-infection of RSV and *S. pneumoniae* was more common in cases than in controls but co-infection of RV and *S. pneumoniae* was not different between cases and controls [112]. RSV-bacterial co-infection has also been associated with increased disease severity compared with RSV alone including longer hospital stays and more frequent admission to pediatric intensive care unit [108] and longer ventilator support [105, 107]. However, associations identified in clinical studies are often weak and only

occasionally reach statistical significance. Additionally, some studies have reported bacterial co-infection rates below 2% in children with RSV [113–116]. In a study of infants hospitalized with RSV, bacterial co-infection was found in only 0.6% of children hospitalized for RSV-associated LRI [116].

RV is also commonly implicated in viral-bacterial co-infections. In a study of children with invasive pneumococcal disease by Techasaensiri et al. 34% of children had a viral co-infection, of which 25% were influenza, and 21% were RV [117]. The study reported that children with viral-coinfections were admitted to the pediatric intensive care unit more frequently and had longer hospital stays than children without viral-coinfections [117]. In a study of children with community-acquired pneumonia by Honkinen et al., viral-bacterial coinfection was identified in 66% of children, of which RV and *S. pneumoniae* was the most common combination, accounting for approximately 7% of cases [118]. Furthermore, the study reported that all cases of treatment failure had a viral-bacterial co-infection. Lauinger et al. found that among RV-infected children, bacterial co-infections, identified in 8% of children, were associated with increased admission to ICU [119].

Other respiratory viruses have also been implicated in bacterial co-infections in children, but to a lesser degree. It has been suggested that the pathogenesis of hMPV infection is strongly affected by bacterial co-infection with *S. pneumoniae*. In a study of children hospitalized with LRI in South Africa, Madhi et al. found that administration of conjugate pneumococcal vaccine reduced the incidence of hMPV infection and the incidence of clinical pneumonia in both HIV positive and negative patients [120]. These findings suggest that a significant proportion of hMPV-associated hospitalizations may be prevented by vaccination with pneumococcal conjugate vaccine. Adenovirus co-infection was identified in 21% of children with invasive pneumococcal disease [117]. Some studies have found associations between overall respiratory virus incidence and bacterial incidence, without distinguishing the specific pathogens involved [110]. While clinical studies confirm that viral-bacterial co-infections are common in children with ALRI, the absence of a control population in most studies makes it difficult to elucidate the clinical significance of co-infections.

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## 8.5 Mechanisms for Viral-Bacterial Interactions

The historical context of viral-bacterial co-infections during influenza pandemics have led to a predominantly unidirectional view that primary viral infections increase the development of secondary bacterial infections leading to LRI. Viral-bacterial co-infections in children have been described in many clinical studies, with some associations with disease severity. However, in clinical studies, it is difficult to differentiate primary from secondary infections and to elucidate the clinical significance of co-infections. However, several *in-vivo* and *in-vitro* experimental model studies have proposed mechanisms to explain interactions between viruses and bacteria. Most mechanisms involve viral facilitation of secondary bacterial infections, for example through disruption of the respiratory epithelium or

modulation of innate and adaptive immune responses to decrease bacterial clearance or increase bacterial adherence. However, there is also growing evidence that bacterial infections may promote secondary viral infections, though direct interactions, bacterial interference with antiviral immunity or by synergism or complementation by virulence factors that have similar functions.

## 8.5.1 Viral Promotion of Secondary Bacterial Infections

### 8.5.1.1 Decreased Bacterial Clearance

The respiratory epithelium is the primary site of host-pathogen encounter in the respiratory tract and the first line of defence against infection [121, 122]. The respiratory epithelium restricts bacterial attachment through mucociliary clearance and maintenance of cell-cell junctions, which restricts access to bacterial receptors [123]. There is evidence that primary viral infections disrupt the respiratory epithelium leading to decreased bacterial clearance. *In-vitro* studies have shown that cells infected with RV, RSV, adenovirus and influenza led to impairment of mucociliary function and consequent decreased clearance of bacteria including *S. pneumoniae* and *H. influenzae* [124–127].

Modulation of innate immune cells following a viral-infection is also believed to decrease bacterial clearance in the respiratory tract. Among host innate immune responses, alveolar macrophages are the major cell population in the normal airway and form the first line of defence against respiratory pathogens. A deficiency in alveolar macrophage-mediated phagocytosis following influenza has been reported in several studies. Using a murine-model, Ghoneim et al. showed that influenza infection depleted and induced cell death of alveolar macrophages leading to impaired clearance of *S. pneumoniae* [128]. Influenza and *S. pneumoniae* co-infection in mice has been also shown to result in synergistic stimulation of type 1 interferons (IFNs) leading to impaired recruitment of macrophages and subsequently, increased bacterial colonization [129]. Another murine-model study by Jamieson et al. showed that influenza infection resulted in decreased production of inflammatory cytokines and chemokines through virus-induced glucocorticoid production, reduced recruitment of innate immune cells to the infection site and consequently, a dramatic increase in bacterial burden [130].

### 8.5.1.2 Increased Bacterial Adherence

Viral infections of respiratory epithelial cells can also promote bacterial adherence to host cells. In an experimental mouse model study, Hament et al. found that *S. pneumoniae* adherence to epithelial cells was enhanced by a preceding RSV infection [131] and it was later shown through *in-vitro* and *in-vivo* studies that RSV was capable of direct binding to *S. pneumoniae* [132]. Similarly, Avadhanula et al. showed that respiratory viruses including RSV, HPIV, and influenza virus enhanced adhesion of *H. influenzae* and *S. pneumoniae* to primary immortalized cell lines but only RSV and HPIV increased receptor expression for bacteria by primary bronchial epithelial cells and A549 cells [133]. Other studies have shown that RSV

virions can bind directly to *S. pneumoniae* and *H. influenzae* acting as a direct coupling particle between bacteria and epithelial cells and thereby increasing colonization by, and enhancing, invasiveness of bacteria [124, 134]. There is also evidence that during RSV infection, viral glycoproteins at the host cell surface, act as additional receptors for bacteria adherence [132, 134]. Respiratory viruses can also increase expression of host surface proteins to which bacteria can bind [133, 135, 136]. Host cell receptors for bacterial adherence have also been found to be exposed by viral neuraminidase activities in studies of influenza virus and *S. pneumoniae* [137, 138]. There is also evidence that viral-mediated epithelia damage can lead to exposure of the basement membrane and additional receptors for bacterial adherence [139, 140].

Although the majority of evidence for enhanced bacterial adherence during viral respiratory infection comes from studies of influenza virus or RSV, there is growing evidence for the role of RV in bacterial adhesion. In an *in-vitro* study by Wang et al., nasal epithelial cells were infected with RV, and then *S. aureus*, *S. pneumoniae*, or *H. influenzae* were added to the culture [140]. Compared with RV-uninfected control cells, the adhesion of *S. aureus*, *S. pneumoniae*, and *H. influenzae* increased significantly in RV-infected nasal epithelial cells. In another *in-vitro* study on the effects of RV infection on the adherence of *S. pneumoniae* to tracheal epithelial cells, the number of *S. pneumoniae* adhering to epithelial cells increased after RV infection [141].

### 8.5.2 Bacterial Promotion of Secondary Viral Infections

The historical emphasis on influenza—*S. pneumoniae* co-infections in adults and the unidirectional view that viral infections increase bacterial growth may be less relevant for children. While *S. pneumoniae* carriage rates are approximately 4% in adults, carriage rates are over 50% in children [142] and up to 80% in children under 5 in developing countries [143]. This supports growing evidence that bacterial infections may promote secondary viral infections, rather than vice versa. In a large, double-blind placebo-controlled trial in infants in South Africa, the 9-valent pneumococcal conjugate vaccine was shown to prevent 31% of pneumonias associated with respiratory viruses in children in hospital, leading to the suggestion that viruses contribute to the pathogenesis of bacterial pneumonia [144]. In another study of healthy children under 2 years of age, Verkaik et al. found that higher seroconversion rates to hMPV were associated with increased nasopharyngeal carriage of *S. pneumoniae* [145]. Furthermore, well-differentiated normal human bronchial epithelial cells pre-incubated *in-vitro* with *S. pneumoniae* resulted in increased susceptibility to infection with HMPV-enhanced green fluorescent protein, suggesting that *S. pneumoniae* can modulate HMPV infection [145].

Experimental models often show an increase in influenza virus titres following a bacterial challenge. In one study, influenza viral titres in mice were shown to increase when *S. pneumoniae* was present [146]. Subsequent mathematical modelling by Smith et al. established that the influenza infection reduced the bacterial clearance ability of alveolar macrophages and that the secondary *S. pneumoniae* infection enhanced viral release from infected cells [146]. In contrast, in another mouse model

study by McCullers et al., influenza infection preceding a pneumococcal challenge primed for pneumonia led to 100% mortality [147]. Interestingly, this effect was specific for viral infection preceding bacterial infection, and reversal of the order of administration led to protection from influenza and improved survival [147].

Further evidence of bacterial promotion of viral infections has been demonstrated by enhanced RSV and hMPV infection of primary epithelial cells with the addition of bacterial lipopeptides, suggesting that bacteria facilitated viral attachment to host cells [148]. *H. influenzae* has also been shown to increase airway epithelial cell ICAM-1 and TLR3 expression, leading to enhanced binding of RV and a potentiation of RV-induced chemokine release [149].

It has also been suggested that viruses might be capable of using their microbial environment to escape immune clearance, highlighting the importance of commensal microbiota in viral infections [150].

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## 8.6 Conclusion

Although viral-bacterial co-infections are common, the clinical significance of co-infections and the mechanisms leading to ARI are yet to be fully clarified. Complex synergistic and antagonistic interactions between viruses and bacteria most likely play a key role in the development of ARI. Viruses and bacteria of the respiratory microbiome may each influence the pathogenicity and consecutive development of infections of the other. Improving knowledge of the interactions between viruses and bacteria may lead to a better understanding of the pathogenesis of ARI and eventually to new prevention and treatment strategies.

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