Interactions between respiratory syncytial virus and *Streptococcus pneumoniae* in the pathogenesis of childhood respiratory infections: a systematic review

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Lower respiratory tract infections, commonly caused by respiratory syncytial virus (RSV) or *Streptococcus pneumoniae* (pneumococcus), pose a substantial global health burden, especially in children younger than 5 years of age. A deeper understanding of the relationship between RSV and pneumococcus would aid the development of health-care approaches to disease prevention and management. We completed a systematic review to identify and assess evidence pertaining to the relationship between RSV and pneumococcus in the pathogenesis of childhood respiratory infections. We found mechanistic evidence for direct pathogen–pathogen interactions and for indirect interactions involving host modulation. We found a strong seasonal epidemiological association between these two pathogens, which was recently confirmed by a parallel decrease and a subsequent resurgence of both RSV and pneumococcus-associated disease during the COVID-19 pandemic. Importantly, we found that pneumococcal vaccination was associated with reduced RSV hospitalisations in infants, further supporting the relevance of their interaction in modulating severe disease. Overall evidence supports a broad biological and clinical interaction between pneumococcus and RSV in the pathogenesis of childhood respiratory infections. We hypothesise that the implementation of next-generation pneumococcal and RSV vaccines and monoclonal antibodies targeting RSV will act synergistically to reduce global morbidity and mortality related to childhood respiratory infections.

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Introduction

Despite improvements in health care, immunisation programmes, and nutrition, lower respiratory tract infections (LRTIs) remain the leading cause of childhood morbidity and mortality worldwide. Pneumonia accounts for up to 1 million global deaths each year in children younger than 5 years of age, most of which occur in low-income and middle-income countries.[1] Respiratory syncytial virus (RSV) and *Streptococcus pneumoniae* (pneumococcus) are two leading causes of childhood pneumonia.[2] As part of the normal airway microbiome, pneumococcus is carried asymptomatically in many children. However, asymptomatic colonisation can become pathogenic during viral respiratory infection, with evidence suggesting that RSV is a common trigger of such a transition.[3,4]

Although epidemiological studies report an association between RSV seasonal activity and pneumococcal disease in infants and older children,[5–7] the clinical relevance of the co-occurrence of RSV and pneumococcus is controversial. The traditional view has been that, in contrast to influenza, RSV is rarely associated with bacterial co-infections or secondary infections with bacterial pathogens, such as pneumococcus,[8,9] although severe disease in children who are admitted to an intensive care unit (ICU) could be the exception.[10] Further understanding of the interrelationship between these two pathogens is warranted to inform and improve health-care practice, especially given recent advances in RSV prevention, including monoclonal antibodies for immunoprophylaxis in infants and maternal vaccines, which are in late-stage development or approved for use[11] and could potentially affect the burden of associated pneumococcal infections. For example, increased knowledge of RSV–pneumococcus interactions might inform the development of RSV vaccination strategies, including the selection of target populations and the calculation of potential societal and health-economic costs of these strategies, as well as the design of future approaches to prevent or treat childhood LRTIs. The recently introduced extended-spectrum pneumococcal vaccines could enhance the impact of RSV vaccination, resulting in further reductions in severe forms of both RSV and pneumococcal disease, which might support reduced antibiotic use and have implications for health-care planning.

We established the *S pneumoniae* Interaction with RSV Initiative (SPIRIT), an international, multidisciplinary collaboration, to complete a detailed systematic review of the interrelationship between RSV and pneumococcus in the pathogenesis of childhood LRTI and to critically assess the evidence. First, we considered the different biological and molecular mechanisms that provide the basis of RSV and pneumococcus interactions. We then reviewed global epidemiology and clinical data to understand the association, co-occurrence, and interaction of RSV and pneumococcus during childhood LRTI. Lastly, we assessed the effects of interventions, mainly the impact of pneumococcal vaccination on RSV burden. In this Health-care Development paper, we present the findings of this systematic review, discuss the implications of these findings for clinical practice and health policy, and identify priorities for future research.

Methods

The SPIRIT collaboration was led by RD and LB, and involved authors with the following areas of expertise: pneumococcal infection (RD), airway microbiome (DB), modelling of epidemiological patterns of respiratory

Summary of systematic review

Aim

RSV and *Streptococcus pneumoniae* are two leading causes of childhood respiratory infections worldwide. We aimed to systematically identify and critically assess evidence regarding the interrelationship between RSV and pneumococcus with a view to supporting the development of health-care approaches for the prevention and management of disease.

Methods

We searched MEDLINE (PubMed), EMBASE, and the Cochrane Library or SCOPUS online databases, from inception to March 4, 2024, for reports on each of four pre-defined research questions. We identified other relevant articles through the bibliography or reference lists of key articles, and via targeted web searches for non-indexed articles, theses, dissertations, and meeting abstracts. All citations that included at least an abstract in English were considered. Eligible studies covered the following: preclinical evidence for RSV–pneumococcus interaction; children with RSV–pneumococcus co-infection; or interventions targeting either pathogen. Titles, abstracts, and full-text reports were assessed for eligibility for inclusion. Quality was assessed using the CAMARADES checklist (preclinical studies), the RTI Item Bank (observational studies), and the Cochrane Collaboration RoB 2 tool (randomised controlled trials).

Findings

Titles and abstracts for 19 522 citations were screened and 1631 full-text reports were reviewed for eligibility. After removal of duplicates across research questions, 124 reports met the inclusion criteria. RSV infection affects susceptibility to pneumococcal infection and disease severity by multiple host-dependent and host-independent mechanisms. Epidemiological evidence indicates a synergistic association between yearly epidemics of RSV and pneumococcal pneumonia. Most studies support a link between RSV–pneumococcus co-detection and enhanced disease severity, which is mirrored by the reduction in infant RSV hospitalisations associated with pneumococcal vaccination.

Implications

Our systematic review highlights broad biological and clinical interactions between pneumococcus and RSV in the pathogenesis of childhood respiratory infections. Greater understanding of these interactions will help to facilitate a cross-pathogen approach to the prevention and management of RSV and pneumococcus disease, with the ultimate aim of improving health outcomes for millions of children worldwide.

CAMARADES=Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies. RoB 2=version 2 of the Cochrane Collaboration Risk of Bias tool. RSV=respiratory syncytial virus.

See Online for appendix

infections (DMW), immunopathogenesis of respiratory infections (AM), pathogenesis of RSV infection (OR and SSB), and RSV prevention (LB). The systematic review was not registered prospectively.

Research questions

Searches were conducted and data extraction and quality assessment completed for each of four research questions. First, what is the biological evidence for the different modes of direct and indirect interaction between RSV and pneumococcus? Second, what is the epidemiological or ecological evidence of RSV–pneumococcal interaction? Third, what is the clinical evidence of RSV–pneumococcal interaction? And fourth, what is the evidence from intervention studies for RSV–pneumococcal co-occurrence and interaction? The protocol for each question is described in the appendix (pp 2–17).

Search strategy and selection criteria

A comprehensive literature search for each research question was undertaken using MEDLINE (PubMed), EMBASE, and either the Cochrane Library or SCOPUS online databases, from database inception to March 4, 2024. Search terms relating to RSV, pneumococcus, and co-infection (with corresponding Medical Subject Headings [MeSH] in PubMed and Emtree Subject Headings in Embase) were combined with specific criteria related to the review objectives—ie, mechanisms, epidemiology, clinical data, and interventional data (see appendix pp 2–17 for study protocols and complete search strings). No language limits were set, with the caveat that English translations of at least the abstract were required. Other relevant articles were identified through the bibliography or reference lists of key articles, and via targeted web searches for non-indexed articles, theses, dissertations, and meeting abstracts (the grey literature).[12] Titles and abstracts were screened and full-text reports were sought for retrieval and assessed for eligibility by two independent reviewers using pre-defined inclusion and exclusion criteria based on the research questions and search terms developed by the authors (appendix pp 2–17). SBB, LB, and RD reviewed the results; studies that were not considered to be relevant to the objectives of SPIRIT were excluded after further review and the reason(s) for exclusion were recorded (appendix pp 18–21). Additional studies identified by the authors during the drafting of this paper that met the pre-defined inclusion criteria were also included. All authors agreed on the final list of included studies.

Quality assessment

Risk of bias was assessed by SBB, LB, and RD for all studies for which full publications were available (appendix pp 22–30). For in-vitro, in-vivo, and ex-vivo studies, a modified version of the Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist[13] was used to assess risk of bias. A modified version of the RTI Item Bank[14] was used for observational studies, and version 2 of the Cochrane Collaboration Risk of Bias tool (RoB 2)[15] was used for randomised controlled trials (RCTs). Studies were not excluded solely on the basis of the quality score, but we considered risk of bias for selected studies in our data synthesis.

Results

A total of 24 154 articles were identified. After removal of duplicates for each research question, 19 522 titles and abstracts were screened and 1631 full-text articles were sought for retrieval. 1466 articles were retrieved and assessed for eligibility, of which 124 (after removal of duplicates across research questions and additional exclusions by authors) met the inclusion criteria and

were included in the final review (figure 1; appendix pp 31–39).[3–10,16–131] Risk of bias was assessed for 112 of these studies; 12 records were not assessed for quality because they were review articles,[26,27,81,103,131] commentaries,[75,79] letters to the editor,[104] meeting reports,[50] or abstracts.[32,51,112] The overall quality of studies was high, with most demonstrating a low risk of bias (six low risk and 21 moderate risk for in-vitro, in-vivo, and ex-vivo studies; 59 low risk and 18 moderate risk for observational studies; 7 low risk and 2 with some concerns for RCTs; appendix 22–30). In the following sections, we present our synthesis and interpretation of the reported data.

Biological evidence

In this section, we review the biological evidence for interactions between RSV and pneumococcus, with consideration of host-dependent and host-independent interactions between RSV and pneumococcus, the dynamics of pneumococcus colonisation in RSV LRTI, and RSV–pneumococcus interaction in relation to the respiratory microbiome. Figure 2 provides a summary of the different modes of RSV–pneumococcal interaction.

Host-dependent RSV–pneumococcus interactions

Damage to the respiratory tract caused by RSV increases host susceptibility to bacterial infections (including pneumococcus) in a non-specific manner. However, specific RSV–pneumococcus mechanisms have also been described. RSV infection has been shown to enhance adherence of pneumococcus to human airway epithelial cells via two main mechanisms: (1) by increasing bacterial binding to airway epithelial cells; and (2) via interference with the host immune response.[16–27] First, pharyngeal cells co-infected with RSV and pneumococcus show increased transcription of genes involved in adhesive functions (*psaA*, pilus islet), as well as transport and binding.[16] Next, RSV upregulates the expression of mediators that facilitate pneumococcal adhesion such as platelet-activating factor (PAF) receptor and intercellular adhesion molecule 1 (ICAM-1).[21–23] Accordingly, RSV-induced adhesion of pneumococcus to a human pulmonary epithelial cell line, A549, is suppressed when the PAF receptor is blocked by monoclonal antibodies or when the expression of PAF receptor is suppressed by clarithromycin.[21–23] Fluorescence and scanning electron microscopy showed redistribution of adherent pneumococci over the surface of RSV-infected epithelial cells, with dense bacterial accumulations near the syncytia, suggesting co-localisation of virus and bacterium.[17] Importantly, adherence did not depend on the specific pneumococcal strain or the sequence of the RSV attachment (G) glycoprotein.[19] These in-vitro studies show that multiple mechanisms contribute to RSV-induced adhesion of pneumococcus to host epithelial cells.

Further biological evidence comes from the interaction between RSV and the host immune system, resulting in an altered innate immune response with diminished pneumococcal clearance and increased local airway inflammation. RSV infection resulted in diminished in-vitro uptake of pneumococcus by monocyte-derived human macrophages, and RSV infection of a human monocytic leukaemia cell line, THP-1, contributed to diminished binding and killing of pneumococcus.[28,29] In vivo, RSV-mediated upregulation of growth arrest-specific protein 6 (Gas6) and subsequent binding to receptor tyrosine kinase Axl attenuated macrophage-mediated protection against pneumococcal infections in mice.[3]

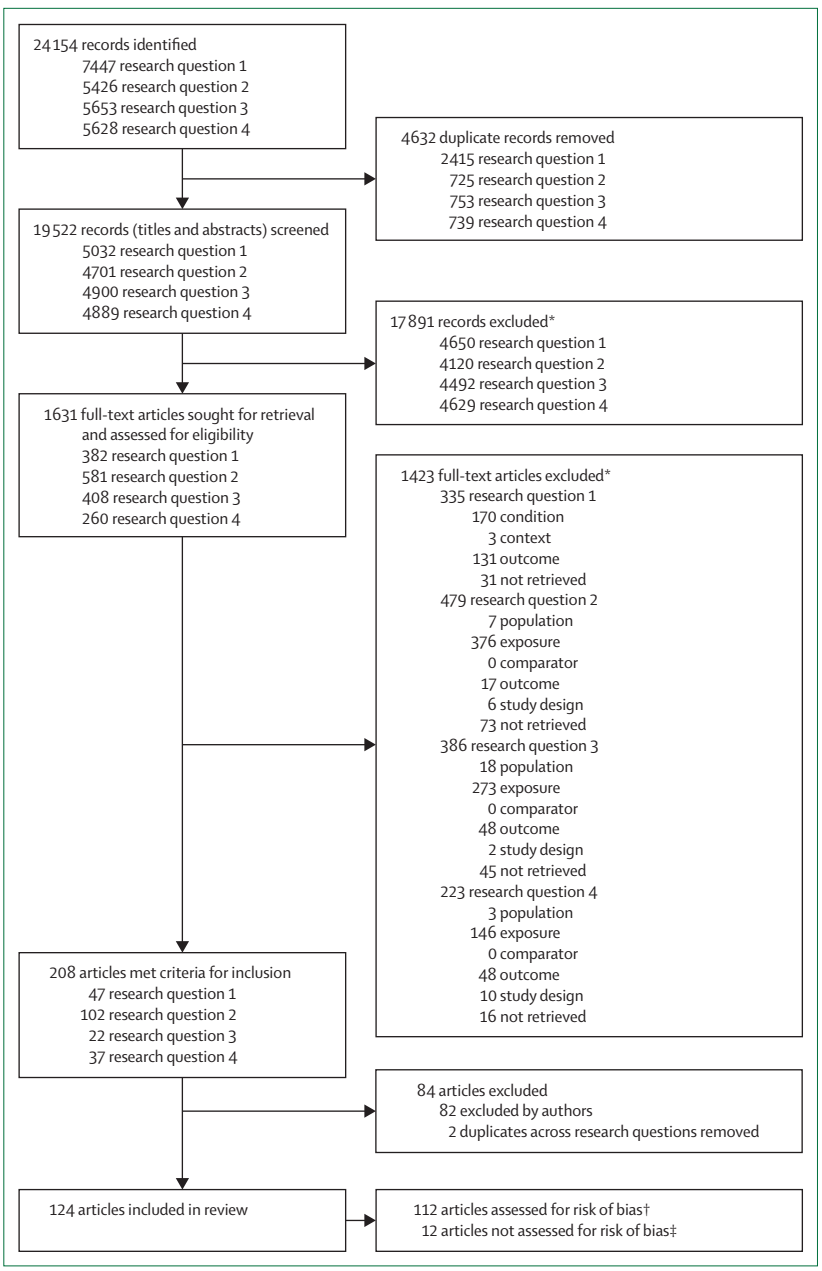

Figure 1: **Selection of studies**

*See appendix pp 2–17 for details of exclusion criteria. †Studies were not excluded solely on the basis of the quality score, but we considered risk of bias for selected studies (appendix pp 22–30) in our data synthesis. ‡Records not assessed for quality because article types did not allow extraction of the necessary data.

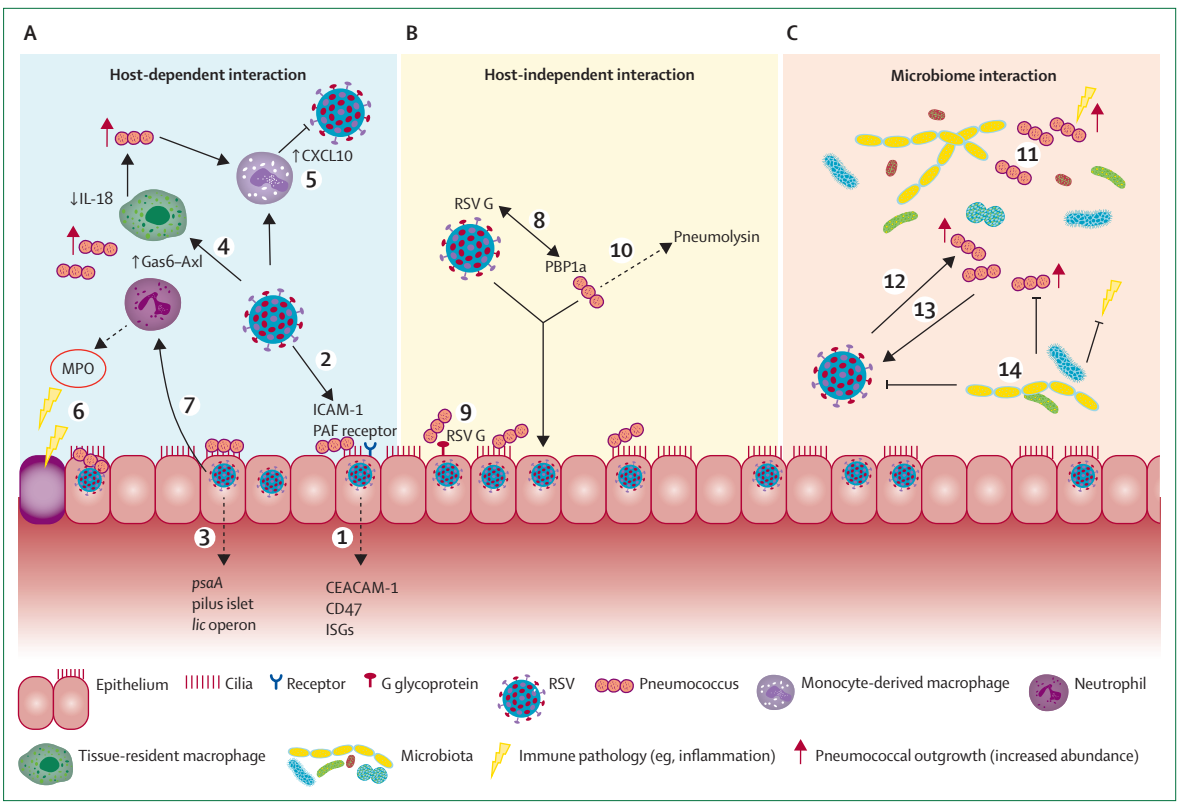

Figure 2: **Modes of interaction of RSV, pneumococcus, and the host respiratory epithelium**
RSV increases pneumococcal outgrowth in vitro and in vivo by increasing pneumococcal adhesion and by altering the antibacterial immune response by macrophages and neutrophils. (A) Host-dependent RSV–pneumococcus interactions include RSV-induced increases in epithelial gene expression of adhesion molecules such as CEACAM-1 and CD47, as well as ISGs (1).[22] Pneumococcal adhesion is mediated through the epithelial expression of PAF receptor and ICAM-1, which are upregulated in response to RSV infection (2).[21–23] Following RSV–pneumococcus co-infection, pharyngeal cells show an increase in transcription of genes involved in adhesion (*psaA*, pilus islet), choline uptake, and incorporation (*lic* operon), as well as transport and binding (3).[16] RSV also alters monocyte-derived macrophage, tissue-resident macrophage, and neutrophil function, which leads to reduced bacterial clearance (4–7). RSV induces upregulation of Gas6, which binds Axl to induce conversion of resident macrophages to an M2-like phenotype with reduced antibacterial activity against pneumococcus, characterised by diminished IL-18 production (4).[3] RSV–pneumococcus co-infection of monocyte-derived macrophages results in diminished viral load of RSV, possibly as a result of enhanced CXCL10 release by monocyte-derived macrophages (5).[38] Co-infection with RSV and pneumococcus increases pulmonary inflammation, alters lung neutrophil function (characterised by higher levels of MPO), and reduces ciliary function, thus contributing to immune-induced pathology (6, 7).[30] (B) Host-independent interaction between RSV G glycoprotein and PBP1a of pneumococcus is associated with increased bacterial adherence to human ciliated epithelial cell cultures (8).[20] RSV-infected cells expressing RSV G glycoprotein serve as a receptor for pneumococci, thereby increasing pneumococcal adherence (9).[25] RSV G and pneumococcus together induce changes in the pneumococcal transcriptome, with upregulation of key pneumococcal virulence genes, including the gene encoding pneumolysin, a pneumococcal toxin (10).[20] (C) Finally, there is a multidirectional interplay between the immune system, microbiome, and viral infections. For example, commensal bacteria *Corynebacterium pseudodiphtheriticum* and *Dolosigranulum pigrum* in the upper respiratory tract modulate respiratory immunity and increase resistance to RSV and pneumococcal replication, while pneumococcus abundance during RSV infection is associated with enhanced innate inflammation (11).[56–60] RSV induces pneumococcal outgrowth from the resident microbial community (12).[61–65] Pneumococcus load increases prior to RSV infection (13).[61–65] Beneficial commensals from the respiratory microbiome inhibit replication of and inflammation induced by (combined) RSV and pneumococcus infection (14).[56–58] CEACAM-1=carcinoembryonic antigen-related cell adhesion molecule 1. CXCL10=chemokine (C-X-C motif) ligand 10. Gas6=growth arrest-specific protein 6. ICAM-1=intercellular adhesion molecule 1. IL-18=interleukin-18. ISG=interferon-stimulated gene. MPO=myeloperoxidase. PAF=platelet-activating factor. PBP1a=pneumococcal penicillin-binding protein 1a. RSV=respiratory syncytial virus.

RSV infection, followed by exposure to pneumococcus 7 days later, not only decreased pneumococcal clearance, but also increased pulmonary inflammation and altered lung neutrophil function, characterised by higher levels of myeloperoxidase.[30] A study of neonatal lambs co-infected with RSV and pneumococcus found, despite having lower bacterial loads in the lungs, an increased pulmonary influx of neutrophils, higher myeloperoxidase levels, and alveolar thickening suggestive of enhanced inflammation.[31] Additionally, immune modulatory molecules, such as CD200R on myeloid cells, are involved in the pathophysiology of mortality related to RSV–pneumococcus co-infection by regulation of innate immunity and subsequent pathological inflammation in murine models.[32] Increased pulmonary inflammation enhances susceptibility to secondary infections through modification of airway epithelial cell function and composition. In addition, RSV–pneumococcus co-infection of ciliated airway epithelial cells has been shown to enhance the mucosal inflammatory response and reduce ciliary beat frequency, possibly contributing further to disease severity.[20]

Importantly, inactivated pneumococcus did not exacerbate pneumonia in RSV-infected mice,[33] suggesting

that live pneumococcus is responsible for disease enhancement. Exposure of bronchial epithelial cells to pneumococcus before and during RSV infection did not increase RSV-induced interleukin-6 (IL-6) or IL-8 release,[34] suggesting that other cytokines and chemokines are involved in the inflammatory response during RSV and pneumococcal co-infection. Intriguingly, in adult mice, RSV infection did not lead to enhanced susceptibility to secondary pneumococcal infection, although this effect was observed for influenza viruses.[35] The results of this study in adult mice,[35] however, are in contrast to those of most studies in infant mice.[3,24,36] This discrepancy could reflect differences related to age-dependent RSV–pneumococcus interactions. Age-dependent interactions have also been suggested by human studies. In infants, RSV significantly contributed to invasive pneumococcal disease (IPD), but influenza viruses did not; in adults, by contrast, influenza viruses significantly contributed to IPD burden, whereas RSV did not.[37] The age-specific differential between RSV and influenza with regard to pneumococcal co-infection requires further study. Altogether, in-vitro and animal data suggest that RSV infection affects pneumococcal clearance by monocyte-derived cells, and that RSV–pneumococcus co-infection increases pulmonary inflammation, contributing to disease severity.

In addition to the effect of RSV on pneumococcal binding, clearance, and inflammation, there is evidence that colonisation or co-infection with pneumococcus influences RSV replication. An in-vitro study reported that RSV–pneumococcus co-infection of human monocyte-derived macrophages resulted in increased levels of interferon-γ-induced protein 10 (IP-10), in turn actively suppressing viral replication.[38] Additionally, in-vivo co-colonisation of pneumococcus and pneumonia virus of mice (PVM), a mouse homologue of human RSV, increased bacterial loads and nasal cytokine secretion, and enhanced PVM clearance.[36] These observations could be due to strain-specific characteristics, as certain pneumococcus serotypes (8, 15A, and 19F), but not others (19A and 23F), enhanced RSV replication in vitro.[39] In cotton rats, intranasal RSV infection 3 days after pneumococcal colonisation resulted in pneumococcal strain-specific enhancements of RSV replication; this phenomenon was observed with serotypes 19F and 23F, but not with serotypes 8 and 15A.[39] Previous studies[132,133] have suggested that neuraminidase, a virulence factor for many bacteria, enhances RSV infection in vitro; however, increased viral replication in these cotton rats did not correlate with pneumococcal neuraminidase activity.[39]

Host-independent RSV–pneumococcus interactions

There is direct interaction between the surface G glycoprotein of RSV and pneumococcus, without involvement of the host.[24] In the murine pneumonia model, following pre-incubation with RSV or purified G glycoprotein, cultured pneumococci were able to induce significant increases in the inflammatory response and bacterial adherence to human ciliated epithelial cells, and markedly increased virulence.[24] RSV G glycoprotein and pneumococcus interaction has also been associated with extensive changes in the pneumococcal transcriptome and significant upregulation of the expression of key pneumococcal virulence genes, including pneumolysin, a major pneumococcal toxin.[20] Finally, RSV-infected epithelial cells express RSV G glycoprotein, which has been shown to serve as a receptor for pneumococci, thereby increasing pneumococcal adherence.[25] Mechanistically, the RSV G glycoprotein binds to the pneumococcal penicillin-binding protein 1a (PBP1a), resulting in increased bacterial virulence and worse disease outcomes.[20]

In summary, most preclinical studies show that RSV infection affects susceptibility to pneumococcal infection and disease severity by multiple host-dependent and host-independent mechanisms, such as increasing bacterial adherence and virulence, reducing macrophage-mediated and neutrophil-mediated bacterial clearance, and enhancing pulmonary inflammation. Moreover, pneumococcal colonisation affects RSV replication, an effect that could be specific to the pneumococcal strain.

Pneumococcus colonisation dynamics during RSV LRTI

As discussed, evidence exists of direct binding of RSV to pneumococcus, resulting in increased pneumococcal binding to epithelial cells, and of indirect effects through the host that result in increased pneumococcal binding to RSV-infected epithelial cells. Here, we aim to assess the question of how these interactions relate to pneumococcus colonisation in RSV-infected children. The frequency, time course, and density of pneumococcal colonisation of the nasopharynx and pneumococcus serotypes correlate with RSV infection in children.[4,40–50] In a study of children with upper respiratory tract infection, pneumococcal density was higher in those with a concomitant respiratory virus, irrespective of symptoms.[49] Density of pneumococcus has been reported to increase during RSV infection and decrease in the 4 weeks after infection, although the pneumococcal serotype was not specified.[4] Another study found that when pneumococcus was co-detected in children with RSV, the same serotype was often also detected in both the preceding and the subsequent 4 weeks.[41] Although RSV is associated with pneumococcal infection, the presence of pneumococcus did not predict the co-presence of RSV in children with acute respiratory infections.[42] In a group of hospitalised infants (median age 14 weeks) with bronchiolitis (85% positive for RSV), pneumococcal carriage density decreased with convalescence, which was not due to antibiotic treatment, suggesting a cause–effect relationship between high pneumococcal density and the acute phase of the disease.[40] However, in another study, influenza and parainfluenza viruses were significantly associated with acquisition of a new serotype of pneumococcus, whereas the association with RSV, although in the same direction, did not reach statistical significance.[44] Several studies suggest that RSV

facilitates pneumococcal outgrowth.[4,41,49] However, there are insufficient data to draw any firm conclusions on whether this outgrowth derives from new acquisitions or emerges from within the resident microbial community.

RSV–pneumococcus interaction in relation to the respiratory microbiome

The composition of commensal flora of the upper and lower respiratory tract has been shown to modulate the host immune response, possibly influencing susceptibility to pneumococcal respiratory infections.[51–55] For instance, in the upper airway, the beneficial commensal bacteria *Corynebacterium pseudodiphtheriticum* and *Dolosigranulum pigrum* have been shown to modulate respiratory immunity, reduce inflammation, increase resistance to RSV infection, and inhibit pneumococcal replication in vitro and in vivo.[56–58] By contrast, nasopharyngeal abundance of *Streptococcus*, *Haemophilus*, and *Moraxella* spp are associated with both the development of virus-associated LRTIs and bronchiolitis severity, regardless of the presence of RSV.[59,60] In line with animal studies, clinical evidence suggests that the microbiota can modulate the host response to RSV.[61–65] One study in children younger than 2 years with mild, moderate, or severe RSV infection, and healthy age-matched controls, identified five nasopharyngeal microbiota clusters, each dominated by different bacteria, including a *Streptococcus*-dominated profile.[61] RSV infection and hospitalisation were, independent of age, positively associated with *S pneumoniae* and *Haemophilus influenzae* abundance, and with increased expression of proinflammatory and neutrophil activation-related genes in the blood.[61] Two other clinical studies in infants with RSV infection confirmed that *Streptococcus*-dominated microbiota profiles were associated with risk of hospitalisation and, like abundance of *Haemophilus* spp, with a higher risk of ICU admission.[59,66] One smaller study, however, showed that abundance of *Haemophilus* spp in particular was associated with RSV disease and increased RSV viral load.[67] In several of these studies, the microbiota signatures were typified by the absence or low abundance of the protective commensal bacteria *Corynebacterium* and *Dolosigranulum* spp.[59–61]

These findings suggest that RSV-related airway microbiome dysbiosis, typified by a predominance of *Streptococcus* and *Haemophilus* spp and the absence of normal commensals, affects local innate immune responses and thereby contributes to disease severity. Further evidence is provided by studies that have used bioactive compounds with immunomodulatory properties (post-immunobiotics), which have shown protection against secondary infections with pneumococcus during RSV infection.[68–70] Together, these studies suggest an important role for the microbiome in modulating RSV–pneumococcal interactions.[61,68–70] The importance of ecological interactions in disease susceptibility and severity is further emphasised by a study showing that classification of infection was possible only by combining information on viral infection, microbial composition, and host variables.[53] In summary, the interaction between RSV, the microbiome, and the host immune response illustrates the multifactorial pathophysiology of the development of LRTI in children. Future studies need to address the question of whether the implementation of novel interventions aimed at restoring the local microbiome, with an abundance of protective commensal genera, could help to limit the development of severe viral or bacterial LRTIs. Furthermore, modulation of the direct interaction between RSV G glycoprotein and PBP1a offers a potential molecular target for the design of novel intervention strategies aimed at reducing disease severity.

Epidemiological evidence

Pneumococcus has rarely been isolated from blood or other sterile sites (ie, pleural effusion) in children with LRTI.[71] Isolation of pneumococcus from the lower airways is challenging. Therefore, we rely on indirect evidence of the relationship between pneumococcus and RSV from epidemiological studies. We begin with determination of the seasonal and spatiotemporal association between the two pathogens and then consider insights into their synergistic effects in LTRI from the COVID-19 pandemic. Clinical effects of the RSV–pneumococcus interaction are discussed in the following section.

Seasonality studies

Two large studies conducted in different parts of the world (the USA and Israel) found a strong association between the seasonality of respiratory viruses (dominated by RSV) and pneumococcal bacteraemic pneumonia, but not non-pneumonia IPD.[6,7] In the USA, non-bacteraemic alveolar pneumonia (considered mostly pneumococcal) coincided with the seasonality of viral and pneumococcal bacteraemic pneumonia.[7] In Israel, RSV was the most frequently detected virus in children with alveolar pneumonia, being 2·8 times more frequent than human metapneumovirus, which was second in rank.[72] An Ecuadorian study found that the yearly circulation pattern of RSV, and to a lesser extent human metapneumovirus, overlapped with that of pneumococcal pneumonia.[73] Further evidence comes from another US study, in which a high correlation between RSV activity and IPD, of which 51% were cases of empyema or bacteraemic pneumonia, was observed, mostly during the 4 weeks preceding hospital admission.[5] In addition to RSV and human metapneumovirus, the epidemiological interaction between respiratory viruses and pneumococcal pneumonia has been suggested for influenza in the 2 weeks preceding hospital admission, although to a lesser degree.[5]

Perhaps the most comprehensive study on the association between RSV activity and pneumococcal disease was conducted in the USA.[74] The study included more than 700 000 children aged 2 years or younger hospitalised with RSV and more than 16 000 hospitalised

with pneumococcal pneumonia across 36 states. RSV and pneumococcal pneumonia showed a distinctive spatiotemporal association. RSV was associated with a significant increase in incidence of pneumococcal pneumonia in infants younger than 12 months, and to a lesser extent in children aged 12–23 months. This interaction was specific to RSV, as influenza activity was not associated with pneumococcal pneumonia in these young children.[74] In summary, the available evidence shows an association between yearly epidemics of RSV and pneumococcal pneumonia.

Effect of the COVID-19 pandemic on seasonality

Suppression of the activity of four specific respiratory viruses (mainly RSV, but also human metapneumovirus, influenza, and parainfluenza viruses) during the pandemic coincided with remarkably reduced rates of pneumococcus-associated disease in children younger than 5 years, despite the persistence of pneumococcal carriage.[37,75–79] The off-season resurgence of these viruses coincided with a parallel off-season return of pneumococcus-associated diseases, strongly suggesting an important virus–pneumococcus synergistic interaction. Notably, rhinovirus activity and adenovirus activity were not suppressed, and their dynamics were not associated with those of pneumococcus-related disease.[76,77] The decline in IPD could have occurred because of the decline in pneumococcal exposure or because of a decline in susceptibility to severe infection.

Five studies have shown persistence of pneumococcal carriage in young children during the pandemic, with a prevalence and serotype distribution similar to those of the pre-pandemic years.[37,76,78–80] During the pandemic, one study showed the persistence of high-density pneumococcal carriage,[78] and one study showed a minimal reduction in density.[80] Together, the persistent carriage rates show that pneumococcal transmission was not affected by the pandemic. It is possible that the acquisition rate declined but prevalence remained high because of reduced competition between strains. Since no studies were truly longitudinal, this possibility could not be excluded, although it seems unlikely.

The dynamics of the four respiratory viruses observed in Israel (RSV, human metapneumovirus, influenza, and parainfluenza) were associated with pneumococcal bacteraemic pneumonia and non-bacteraemic alveolar pneumonia, but also with non-alveolar pneumonia LRTI.[76] No correlation was found with non-pneumonia IPD.[76] Studies in France[78] and Canada[37] found an association between RSV (or bronchiolitis as a surrogate for RSV) with IPD, but these studies did not distinguish between pneumonia IPD and non-pneumonia IPD.

In the Israeli study, the off-season return of respiratory viruses (with the predominance of RSV) and the sequential nature of the return enabled the construction of a model to quantify the attributable role of the viruses in pneumococcus-associated diseases.[76] The most important viral attribution in children younger than 5 years was RSV: 49% of alveolar pneumonia, 21% of non-alveolar pneumonia, and 18% of bacteraemic pneumococcal pneumonia. No attribution was found for RSV in non-pneumonia IPD.[76] These data provide epidemiological evidence of a synergistic relationship between RSV and pneumococcal invasive and non-invasive pneumonia.

Clinical effects of RSV–pneumococcus co-detection

To understand the link between population-level trends and individual-level interactions, we reviewed studies that identified pneumococcus in children with RSV LRTI and that identified RSV in children with pneumococcal infection. Rates of nasopharyngeal carriage of pneumococcus in healthy young children are high, at up to 90%.[81] In RSV-infected children, nasopharyngeal bacterial co-detection ranged from 6·1% to 97·0% (table 1).[10,41,43,82–102] Among those with bacterial co-detection, RSV–pneumococcus co-detection ranged from 0·0% to 74·7%, which was similar to the rates of co-detection of other common airway bacteria (*H influenzae* or *Moraxella catarrhalis*).[10,41,43,82–88,90,92–101] These wide ranges are probably related, among other factors, to differences in the study participants and design, different tools used for bacterial detection, previous antibiotic use, or epidemiological conditions. Here, we consider the evidence for co-detection in children who were or were not hospitalised with RSV and discuss the effects of pneumococcus, including serotype differences, on RSV disease severity.

RSV–pneumococcus co-detection in outpatients

In children with RSV infection not requiring hospitalisation, bacterial co-detection ranged from 73·1% to 80·4%.[41,82–84] In these patients, RSV–pneumococcus co-detection was in the range of 34·7–46·3% (median 40·5%), a higher incidence than for other bacteria, as shown in two different studies.[82,83] In most children with RSV infection, especially those with mild disease who do not receive medical attention, it is difficult to determine whether RSV is the only cause of disease because of a lack of testing, but evidence supports this theory, including the lack of response to antibiotics observed in treated episodes of mild disease.[103,104]

RSV–pneumococcus co-detection in hospitalised children

In hospitalised children with RSV infection, bacterial co-detection ranged from 6·1% to 97·0%.[43,85–94] Of the patients with positive bacterial co-detection, RSV–pneumococcus co-detection ranged from 36·6 to 74·7%, representing higher rates than for other bacteria.[85–87,92,94] However, in a South African case–control study of children younger than 5 years who were hospitalised for LRTI, prevalence rates of *S pneumoniae* in RSV-positive children were in the range of those for *H influenzae* and *M catarrhalis* (55·0%, 46·6%, and 66·4%, respectively).[89] In a case–control study from Tanzania, children with

	Age	Study design; diagnosis	Number enrolled, n (RSV positive, n)	Any positive bacterial culture, n/N (%)	*Streptococcus pneumoniae*, n/N (%)	*Haemophilus influenzae*, n/N (%)	*Moraxella catarrhalis*, n/N (%)	*Staphylococcus aureus*, n/N (%)
Community-based participants								
Brealey et al, 2020 (Australia)[41]	<2 years	Prospective longitudinal cohort study; URTI, LRTI	47 (47 with 54 episodes of RSV)	..	Nasopharyngeal samples: 33/54 (61·1%)	Nasopharyngeal samples: 8/54 (14·1%)	Nasopharyngeal samples: 26/54 (48·1%)	..
Chappell et al, 2013 (Australia)[82]	<5 years	Prospective cohort study; clinical RTI	67 (67)	Nasopharyngeal samples: 49/67 (73·1%)	Nasopharyngeal samples: 17/49 (34·7%)	Nasopharyngeal samples: 5/49 (10·2%)	Nasopharyngeal samples: 22/49 (44·9%)	Nasopharyngeal samples: 6/49 (12·2%)
Skevaki et al, 2015 (Greece)[83]	3 months to 6 years	Prospective cross-sectional cohort study; asymptomatic, URTI, LRTI	386 (51)	Nasopharyngeal samples: 41/51 (80·4%)	Nasopharyngeal samples: 19/41 (46·3%)	Nasopharyngeal samples: 25/41 (61·0%)	Nasopharyngeal samples: 15/41 (36·6%)	Nasopharyngeal samples: 15/41 (36·6%)
Esposito et al, 2013 (Italy)[84]	Mean 3·1 years (SD 2·9)	Prospective cohort study; CAAP	530 (126)	..	Nasopharyngeal samples: 65/126 (51·6%)	..	..	..
Patients admitted to hospital (non-ICU)								
Hishiki et al, 2011 (Japan)[85]	<5 years	Prospective cohort study; LRTI	188 (188)	Sputum samples: 82/188 (43·6%)	Sputum samples: 30/82 (36·6%)	Sputum samples: 36/82 (43·9%)	Sputum samples: 24/82 (29·3%)	..
Nguyen et al, 2017 (Laos)[86]	<5 years	Prospective cohort study; URTI, LRTI	383 (157)	Nasopharyngeal samples: 144/157 (91·7%)	Nasopharyngeal samples: 98/144 (68·1%)	Nasopharyngeal samples: 84/144 (58·3%)	..	..
Singh et al, 2016 (India)[87]	3–59 months	Prospective multicentre study; CAAP	377 (201)	Nasopharyngeal samples: 99/201 (49·3%)	Nasopharyngeal samples: 74/99 (74·7%)	Nasopharyngeal samples: 46/99 (46·5%)	..	..
Bénet et al, 2015 (Mali)[88]	<5 years	Prospective hospital-based case–control study; radiologically confirmed pneumonia	118 (30) cases with pneumonia; 98 (6) controls	..	Nasopharyngeal samples: cases 18/30 (60·0%); controls 2/6 (33·3%)	..	..	..
Morgan et al, 2023 (South Africa)[89]	<5 years	Cross-sectional case–control study; severe LRTI	454 (135); 319 controls without RSV	Nasopharyngeal and sputum samples: cases 131/135 (97·0%); controls 304/319 (95·3%)	Nasopharyngeal and sputum samples: cases 72/131 (55·0%); controls 168/304 (55·3%)	Nasopharyngeal and sputum samples: cases 61/131 (46·6%); controls 170/304 (55·9%)	Nasopharyngeal and sputum samples: cases 87/131 (66·4%); controls 208/304 (68·4%)	Nasopharyngeal and sputum samples: cases 46/131 (35·1%); controls 90/304 (29·6%)
Vissers et al, 2016 (Netherlands)[43]	<2 years	Prospective cohort study; mild, moderate, and severe RSV	105 (105)	..	Nasopharyngeal samples: 55/105 (52·4%)	Nasopharyngeal samples: 76/105 (72·4%)	..	..
Gan et al, 2022 (China)[90]	<12 years (86% <6 years)	Prospective cohort study; LRTI	254 (81)	..	Nasopharyngeal samples: 21/81 (25·9%)	..	..	..
Han et al, 2023 (China)[91]	<12 years (57% <3 years)	Prospective cohort study; RTI	1037 (188)	..	Nasopharyngeal samples: 22/188 (11·7%)	..	Nasopharyngeal samples: 6/188 (3·2%)	Nasopharyngeal samples: 25/188 (13·3%)
Lin et al, 2022 (Taiwan)[92]	Median 1·33 years (IQR 0·67–2)	Retrospective cohort study; RSV pneumonia	620 (620)	Nasopharyngeal samples: 201/620 (32·4%)	Nasopharyngeal samples: 82/201 (40·8%)	Nasopharyngeal samples: 51/201 (25·4%)	Nasopharyngeal samples: 32/201 (15·9%)	Nasopharyngeal samples: 82/201 (40·8%)
Valley-Omar et al, 2022 (South Africa)[93]	<5 years (data for ≥5 years not presented here)	Prospective hospital-based syndromic surveillance study; severe respiratory illness	2509 (601)	..	Nasopharyngeal samples: 35/404* (8·7%)	..	..	..
Otheo et al, 2022 (Spain)[94]	Median 37 months (IQR 18–66)	Prospective multicentre observational study; CAAP	495 (66)	Nasopharyngeal samples: 4/66 (6·1%)	Nasopharyngeal samples: 2/4 (50·0%)	Nasopharyngeal samples: 1/4 (25·0%)	..	..

(Table 1 continues on next page)

RSV were 8·4 times more likely to be diagnosed with community-acquired alveolar pneumonia (CAAP) than were otherwise healthy children.[105] Moreover, those with RSV were more likely to carry pneumococcus (adjusted odds ratio [OR] 1·6), although co-detection of pneumococcus and influenza A was more common (adjusted OR 4·2).[105] In a case–control study of children younger than 5 years from Ethiopia, the OR for detection

	Age	Study design; diagnosis	Number enrolled, n (RSV positive, n)	Any positive bacterial culture, n/N (%)	*Streptococcus pneumoniae*, n/N (%)	*Haemophilus influenzae*, n/N (%)	*Moraxella catarrhalis*, n/N (%)	*Staphylococcus aureus*, n/N (%)
(Continued from previous page)								
Patients admitted to ICU								
Duttweiler et al, 2004 (Switzerland)[95]	Median 1·7 months (range newborn to 5·8 years)	Prospective cohort study; severe RSV	127 (127)	Tracheal samples: 25/56 (44·6%)	Tracheal samples: 11/25 (44·0%)	Tracheal samples: 17/25 (68·0%)	Tracheal samples: 12/25 (48·0%)	Tracheal samples: 8/25 (32·0%)
Thorburn et al, 2006 (UK)[10]	Median 1·6 months (IQR 0·5–4·6)	Prospective cohort study; severe RSV	165 (165)	Tracheal samples: 70/165 (42·4%)	Tracheal samples: 12/70 (17·1%)	Tracheal samples: 28/70 (40·0%)	Tracheal samples: 18/70 (25·7%)	Tracheal samples: 22/70 (31·4%)
Levin et al, 2010 (USA)[96]	<1 year	Prospective descriptive study (and literature review); severe RSV	23 (23)	Tracheal samples: 17/22 (77·3%)	Tracheal samples: 1/17 (5·9%)	Tracheal samples: 11/17 (64·7%)	Tracheal samples: 3/17 (17·6%)	Tracheal samples: 3/17 (17·6%)
Kneyber et al, 2005 (Netherlands)[97]	<1 year	Retrospective observational study; moderate and severe RSV	82 (82)	Tracheal samples: 9/24 (37·5%)	Tracheal samples: 0/9 (0·0%)	Tracheal samples: 3/9 (33·3%)	..	Tracheal samples: 6/9 (66·7%)
Resch et al, 2007 (Austria)[98]	Median 2·75 months (range 0·25–96)	Retrospective cohort study; moderate and severe RSV	464 (464)	Tracheal samples: 9/36 (25·0%)	Tracheal samples: 4/9 (44·4%)	Tracheal samples: 2/9 (22·2%)	..	Tracheal samples: 1/9 (11·1%)
Randolph et al, 2004 (USA)[99]	<3 years	Retrospective cohort study; severe RSV	165 (165)	Tracheal samples: 24/47 (51·1%)†	Tracheal samples: 3/11 (27·3%)‡	Tracheal samples: 4/11 (36·4%)‡	Tracheal samples: 5/11 (45·5%)‡	..
Thorburn et al, 2011 (UK)[100]§	Median 2·8 months (IQR 1·3–11·5)	Retrospective review; severe RSV	352 (352)	Tracheal samples: 145/352 (41·2%)¶	Tracheal samples: 22/145 (15·2%)	Tracheal samples: 52/145 (35·9%)	Tracheal samples: 31/145 (21·4%)	Tracheal samples: 38/145 (26·2%)
Wiegers et al, 2019 (Netherlands)[101]§	<2 years	Retrospective review; severe RSV	167 (111)	Tracheal samples: 40/111 (36·0%)	Tracheal samples: 15/40 (37·5%)	..	..	..
Mixed population								
Lin et al, 2024 (Spain, UK, Netherlands)[102]	<1 year	Prospective cohort study and cross-sectional study; mild, moderate, and severe RSV	433 (433)	Nasopharyngeal samples: 406/433 (93·8%)	Nasopharyngeal samples: 294/406 (72·4%)	Nasopharyngeal samples: 195/406 (48·0%)	Nasopharyngeal samples: 329/406 (81·0%)	..

Studies are grouped by population: community-based participants, patients admitted to hospital (non-ICU), or patients admitted to the ICU, according to the setting in which all or most of the participants in each study were sampled. For some studies, the sum of the individual bacterial columns is more than 100% owing to bacterial co-infections or less than 100% owing to the presence of other bacteria not specified here. The data for individual bacteria are presented as a proportion of patients with RSV with a positive bacterial culture, with the exception of the studies by Brealey et al,[41] Esposito et al,[84] Bénet et al,[88] Vissers et al,[43] Gan et al,[90] Han et al,[91] and Valley-Omar et al,[93] for which individual bacteria data could be presented only as a proportion of all patients with RSV. CAAP=community-acquired alveolar pneumonia. ICU=intensive care unit. LRTI=lower respiratory tract infection. RSV=respiratory syncytial virus. RTI=respiratory tract infection. URTI=upper respiratory tract infection. *Only 404 of the 601 RSV-positive children underwent testing for *S pneumoniae*. †11/47 (23·4%) with probable pneumonia and 13/47 (27·7%) with possible pneumonia. ‡Individual bacteria specified only for patients with RSV with probable pneumonia. §Includes bronchoalveolar lavage samples. ¶72/352 (20·5%) had concomitant bacterial pneumonia and 73/352 (20·7%) had possible bacterial co-infection.

Table 1: **Proportion of positive bacterial cultures in studies of infants and children with RSV**

of RSV subtype B (RSV-B) was 2·53 (95% CI 1·01–6·75) in *S pneumoniae*-positive children compared with *S pneumoniae*-negative children.[106] The OR for RSV subtype A (RSV-A) was also higher, but did not reach statistical significance.[106] In a large study from China in children younger than 5 years with LRTI, co-detection of pneumococcus with RSV-A or RSV-B was common, but that of pneumococcus and rhinovirus was more common.[107] However, the study was uncontrolled and, in contrast to RSV, rhinovirus can often be detected in healthy children.[134,135]

Data regarding bacterial pneumonia in infants with RSV bronchiolitis who have been admitted to the paediatric ICU show more heterogeneity, with incidence of bacterial co-detection (cultured from tracheal aspirates) ranging from 25·0% to 77·3%.[10,95–101] Of these bacteria, *H influenzae*, *M catarrhalis*, and *Staphylococcus aureus* were isolated as often as pneumococcus.[10,95–101] The current data are not sufficient to enable comparisons with healthy children.

Association between pneumococcus co-detection and RSV disease severity

A positive correlation between pneumococcus and RSV disease severity has been widely reported.[43,46,48,84,108] Nasopharyngeal detection of pneumococcus or *H influenzae* in children younger than 2 years with RSV has been associated with fever, more frequent antibiotic treatment, worse radiological findings, and higher

neutrophil counts.[48,108] Similarly, in children younger than 3 years who were hospitalised with RSV, those in whom *S pneumoniae* or *H influenzae* was detected in the nasopharynx had higher rates of infiltrates on chest radiography than did those with no bacterial pathogens or with Group A *Streptococci* or *S aureus* (calculated from study data: 85·7% *vs* 58·7%, respectively; p=0·03).[109] In a large study conducted in three European countries, among RSV-infected infants younger than 12 months, the co-detection of *Haemophilus* spp and *Streptococcus* spp (mostly *S pneumoniae*) was generally positively associated with disease severity, and the co-detection of *M catarrhalis* was negatively associated with severity.[102] However, the increased severity with *Streptococcus* spp did not reach statistical significance.[102]

Pneumococcus and *H influenzae* co-detection in RSV-positive children has also been independently associated with greater odds of hospitalisation, higher disease severity scores, need for supplemental oxygen, and longer duration of hospitalisation.[48] In another study in which pneumococcus was more frequently detected during RSV infections compared with other viral infections (adjusted effect size 1·8), co-detection of both pathogens was associated with higher clinical disease severity scores.[46] Notably, in this study, severity was not associated with the presence of other pathogens or patient characteristics.[46] In a study of children younger than 5 years with RSV LRTI, those with RSV-associated alveolar pneumonia had significantly higher pneumococcus bacterial load, lower oxygen saturation, and higher hospital admission rates than did those without alveolar pneumonia.[84] It has also been reported that RSV-infected infants in need of oxygen supplementation had a significantly higher proportion of pneumococcal detection (62%) compared with infants who did not receive additional oxygen (15%).[108] In a further study of pneumococcus-positive children, pneumococcal density was correlated with higher RSV loads and higher levels of matrix metalloproteinase-9 (MMP-9), an inflammatory mediator that has been associated with severe RSV infections, suggesting that pneumococcal density influences both viral load and mucosal inflammatory response during RSV infection.[43]

Two studies did not demonstrate an effect of *S pneumoniae* co-detection on severity of viral LRTI disease (including RSV), as defined by increased length of stay, hospitalisation, or oxygen requirement.[110,111] Another study showed high rates of pneumococcal carriage in infants with RSV or human metapneumovirus, with no effect on illness severity.[112] Factors explaining these differences in study results might include the use of antibiotics prior to sampling or the presence of relevant comorbidities in the RSV group, the rates of which were 25·6% and 41·8%, respectively, in one of these studies.[110]

In conclusion, assessment of the effect of RSV–pneumococcus co-infection on clinical severity is difficult on the basis of available published data. Challenges include variability in the type of patients studied, the endpoints used, and the different entities studied. However, most studies support a link between RSV–pneumococcus co-detection and enhanced disease severity, particularly in relation to hospital admission and supplemental oxygen administration.

Serotype-specific pneumococcal effects on RSV disease severity

As discussed, pneumococcus serotypes 8, 15A, and 19F enhance RSV replication in vitro and serotypes 19F and 23F enhance replication in vivo (cotton rats).[39] In a clinical study from Italy, serotypes 5 and 19A were found almost exclusively among RSV-positive children with alveolar pneumonia compared with those with non-alveolar pneumonia.[84] Further evidence comes from an Israeli study that examined nasopharyngeal pneumococcal serotype distribution in children younger than 5 years with CAAP.[45] This study found that the prevalence of invasive pneumococcal serotypes (1, 4, 5, and 7F) was significantly lower in children with RSV-positive versus RSV-negative CAAP.[45] Conversely, nasopharyngeal detection of non-invasive serotypes (15B/C, 17F, 33F, and 35B) was higher in RSV-positive cases with CAAP.[45]

Effect of interventions

Quantification of the population-level consequences of the interaction between RSV and pneumococcus is challenging because these pathogens often share seasonality. Although statistical and mathematical models can be used to estimate the strength of the association between these pathogens, such approaches have limitations and cannot easily allow determination of causality. In this context, interventions that target RSV, pneumococcus, or both provide an opportunity to quantify the effect on each pathogen. The most notable example of this type of probe study involves the use of pneumococcal conjugate vaccines (PCVs). An inherent limitation in assessing the effect of PCVs on LRTI is the inability to directly attribute causality to pneumococcus for various clinical endpoints, which are often based on radiological examination.[113,136,137] In addition, the radiological diagnosis is not unequivocal, because the reported inter-reader agreement is poor, despite efforts to standardise radiological interpretation.[136] The best agreement scores are for alveolar consolidated LRTIs or pleural effusions, which are often attributed to pneumococcus. Nonetheless, even before PCV implementation, it was clear that pneumococcus contributed to non-consolidated pneumonia, including cases without the classic radiological findings, although to a lesser extent in such cases.[113] Thus, measurement of efficacy or effect of PCVs on different LRTI endpoints represents a useful method to estimate the attributable role of pneumococcus in LRTI.

A landmark RCT conducted in South Africa evaluated the effect of an experimental nine-valent PCV (PCV9).[114] In this study, infants who received PCV9 had a 22% reduction in hospitalisations due to RSV-positive pneumonia

(32% in HIV-uninfected children) compared with those who received placebo. Other virus-positive pneumonia cases, including influenza, parainfluenza, adenovirus, and human metapneumovirus,[115] also had lower incidences in the vaccinated group.[114] Another RCT reported one case of RSV pneumonia in the control group and no cases among the PCV recipients, which are insufficient numbers to draw conclusions.[116] In a double-blind study from southern Israel, toddlers aged 12–35 months attending day-care centres who received PCV9 had a 16% decline in outpatient visits for LRTI, including bronchiolitis, compared with recipients of a control vaccine.[117] However, no viral testing was done in this study.[117]

The introduction of PCVs for universal use in infants provided an opportunity to evaluate their real-life effect on both RSV and pneumococcus disease rates. In post-licensure studies, the effect of PCVs on RSV has been estimated using aggregate time-series data. These analyses typically evaluate changes in hospitalisation rates for both RSV and pneumonia over time, coinciding with the introduction of PCVs. Consistent with the aforementioned RCTs,[114–117] both alveolar and non-alveolar LRTI rates declined with the use of PCVs, confirming the important role of pneumococcus on these endpoints.[113,118,119] Furthermore, several studies showed a post-PCV implementation decline in RSV-related hospitalisations (table 2).[74,118,120–123] Among those still hospitalised, the proportion of RSV-positive cases was similar to that during the pre-PCV period,[118] suggesting that RSV-positive alveolar pneumonia cases were reduced in the same order of magnitude as all other alveolar pneumonia cases. A study from Australia found no change in rates of RSV LRTI among Indigenous infants during periods of PCV10 and PCV13 use compared with vaccination with PCV7,[123] suggesting no notable effect of increased serotype coverage by the extended-spectrum serotype vaccines; however, there was no comparison with the pre-vaccine period, limiting interpretation of the data. A recent detailed study, also from Australia, showed a clear effect of PCV administration on hospitalisation rates for RSV-positive children younger than 2 years.[124] In the post-PCV period (2005–12), fully vaccinated Aboriginal children (≥3 PCV doses) had a 30% reduction in hospitalisation rates, compared with the unvaccinated group. Among non-Aboriginal children, a 21% reduction was observed. The effect was dose-dependent, with a lower reduction in hospitalisation rates in partially vaccinated children.[124]

Aside from PCVs, interventions that specifically target RSV could enable analyses of the effect of RSV interventions on pneumococcal disease. To date, however, data from interventional studies targeting RSV are limited. A randomised trial of a monoclonal antibody targeting RSV did not directly evaluate potential effects on pneumococcal disease,[125] nor have other recent trials of RSV monoclonal antibodies (nirsevimab) or maternal vaccinations.[126–128]

Importantly, although not directly targeting pneumococcus, use of antibiotics provides an opportunity to evaluate the potential effects of bacterial pathogens on RSV disease. Indeed, some studies have shown a beneficial effect of antibiotics in children with RSV infection and respiratory failure, suggesting a role for bacteria in exacerbating the severity of the viral infection in that unique population with critical illness.[129,130] However, other studies have not shown a positive effect of antibiotic administration on RSV disease severity.[103,104] Thus, use of clinical judgement with regard to antibiotic use in such cases is warranted until further studies are conducted. In any case, antibiotic use would need to take into account potential unintended harm to the microbiome and contribution to antimicrobial resistance.[138]

Overall, interventional studies showing that PCV introduction was associated with reduced RSV-related hospitalisations indicate that RSV–pneumococcus co-infections are common, especially among young children requiring hospitalisation for LRTI. As new strategies for RSV prevention, including monoclonal antibodies for infants or maternal vaccination during pregnancy, are incorporated into clinical practice, there is a critical window of opportunity to study the effect of RSV prevention on the incidence of pneumococcal respiratory infections in more detail.[76,131]

Discussion

Key findings

Our extensive systematic search of the literature has allowed us to collate evidence related to RSV–pneumococcus synergistic interactions, including biological, experimental, epidemiological, clinical, and interventional data. The evidence implies that RSV and pneumococcus mutually contribute to the incidence and severity of LRTI, and that the elimination or reduction of the prevalence of one of these pathogens attenuates disease associated with the other. A striking example of these findings is shown in figure 3, which depicts the dynamics of hospitalisations for alveolar pneumonia in children younger than 5 years in southern Israel from the pre-PCV era through to 2022.[75,76,113] The introduction of PCVs in 2009–10 was associated with a substantial reduction in the incidence of pneumococcal pneumonia, but a further, deep reduction was seen when the activity of respiratory viruses (mainly RSV) was suppressed during the initial COVID-19 period, and incidence rebounded again with the re-emergence of these viruses. To further determine the steps preceding infection and the mechanisms of viral–bacterial interaction, a broader examination of the evidence base pertaining to multidirectional cause–effect relationships between microbiota, colonisation types, host immune response in early life, RSV-associated disease severity, and the ecological context is required. The relative importance of different factors, such as pneumococcal serotypes or changes in the respiratory microbiome

	Age	Number of cases	Type of PCV	Pre-PCV		Post-PCV		Change in rates after PCV introduction	
				RSV rate per 1000 children or 1000 child-years*	RSV hospitalisation rate per 1000 children or 1000 child-years*	RSV rate per 1000 children or 1000 child-years*	RSV hospitalisation rate per 1000 children or 1000 child-years*	RSV rate, %	RSV hospitalisation rate, IRR (95% CI) or %†
Weinberger et al, 2015 (USA)[74]	<2 years	>700 000 RSV hospitalisations and >16 000 pneumococcal pneumonia hospitalisations	PCV7	..	..	..	..	..	0–2 months: −6·3% (−14·7 to 2·2) 3–11 months: −18·4% (−25·4 to −10·6) 12–23 months: −9·2% (−15·1 to −2·9)
Fathima et al, 2018 (Australia)[120]	≤16 years	13 544 pneumonia hospitalisations	PCV7 and PCV13	..	..	..	..	..	..
Indigenous Australians	..	..	..	..	<6 months: 4·795 6–11 months: 4·803 12–23 months: 2·062 2–4 years: 0·814	..	<6 months: 4·883 6–11 months: 3·638 12–23 months: 2·167 2–4 years: 0·696	..	<6 months: 1·0 (0·6–1·7) 6–11 months: 0·8 (0·4–1·3) 12–23 months: 1·1 (0·6–1·9) 2–4 years: 0·9 (0·5–1·5)
Non-Indigenous Australians	..	..	..	..	<6 months: 1·214 6–11 months: 0·918 12–23 months: 0·794 2–4 years: 0·288	..	<6 months: 0·801 6–11 months: 0·607 12–23 months: 0·849 2–4 years: 0·309	..	<6 months: 0·7 (0·5–0·9) 6–11 months: 0·7 (0·5–0·9) 12–23 months: 1·1 (0·8–1·4) 2–4 years: 1·1 (0·8–1·4)
Foote et al, 2015 (USA)[121]‡	<5 years	..	PCV7 and PCV13	..	..	..	..	..	..
American Indian or Alaska Native	..	..	..	..	<1 year: 77·3 1–4 years: 4·6	..	<1 year: 44·6 1–4 years: 4·2	..	1 year: 0·58 (0·55–0·61) 1–4 years: 0·91 (0·83–0·99) <5 years: −36% (p<0·01)
General population	..	..	..	..	<1 year: 31·8 1–4 years: 1·9	..	<1 year: 26·1 1–4 years: 2·8	..	<1 year: 0·82 (0·72–0·93) 1–4 years: 1·43 (1·24–1·61) <5 years: −20% (p<0·01)
CDC, 2009 (USA)[122]§	<4 years	..	PCV7	..	<2 years: 28·1 2–4 years: 5·8	..	<2 years: 21·9 2–4 years: 5·6	..	<2 years: 0·8 (0·7–0·9) 2–4 years: 1·0 (0·9–1·0)
Triadou et al, 2020 (Israel)[118]	<5 years	12 271 pneumonia episodes	PCV7 and PCV13	..	..	..	..	..	..
PE-CAP	..	..	..	<5 years: 185	..	<5 years PCV7: 0 <5 years PCV13: 143	..	<5 years PCV7: −18·5% <5 years PCV13: −4·2%	..
NPE-CAP	..	..	..	<2 years: 370 2 to <5 years: 120 <5 years: 312	..	<2 years PCV7: 713 <2 years PCV13: 447 2 to <5 years PCV7: 528 2 to <5 years PCV13: 218 <5 years PCV7: 685 <5 years PCV13: 402	..	<2 years PCV7: 34·3% <2 years PCV13: 7·7% 2 to <5 years PCV7: 40·8% 2 to <5 years PCV13: 9·8% <5 years PCV7: 37·3% <5 years PCV13: 9·0%	..
Binks et al, 2020 (Australia)[123]	<1 year	4138 acute LRTI episodes	PCV7, PCV10, and PCV13	..	..	..	PCV7: 49 PCV10: 49 PCV13: 49	..	PCV10 *vs* PCV7: 0·99 (0·81–1·21) PCV13 *vs* PCV7: 0·98 (0·83–1·17) PCV13 *vs* PCV10: 0·99 (0·81–1·21)

CDC=Centers for Disease Control and Prevention. IRR=incidence rate ratio. LRTI=lower respiratory tract infection. NPE-CAP=community-acquired pneumonia without pleural effusion. PCV=pneumococcal conjugate vaccine. PE-CAP=community-acquired pneumonia with pleural effusion. RSV=respiratory syncytial virus. *Rate per 1000 children for Foote et al,[121] CDC,[122] and Triadou et al;[118] rate per 1000 child-years for Fathima et al[120] and Binks et al.[123] †IRR for baseline pre-PCV rates of RSV hospitalisation compared with post-PCV rates, except for Binks et al,[123] which compared rates between PCV eras as no pre-PCV rates were available, and for Weinberger et al[74] and Foote et al[121] (data for <5 years), which reported only percentage differences in rates. ‡Data for infants aged <1 year and children aged 1–4 years are for all bronchiolitis (ie, RSV and non-RSV); data for children <5 years are RSV-specific rates. §Data are non-pneumonia hospitalisation rates (not specifically RSV).

Table 2: **Rates of RSV and RSV hospitalisations for infants and children before and after PCV introduction**

during or preceding both symptomatic and asymptomatic respiratory infections, should be further investigated.

Although this systematic review provides compelling evidence for the additive or synergistic potential of RSV and pneumococcus in respiratory disease in young children, it is important to emphasise that the evidence does not suggest that most cases of RSV or pneumococcus represent co-infections. In fact, it is plausible that a substantial proportion of children with RSV-related disease do not have RSV–pneumococcus co-infections. Indeed, studies that used mostly macrolides did not show a positive effect of antibiotics on RSV disease.[103,104] On the basis of these findings, and the fact that in young children, radiological findings suggestive of pneumonia in classic bronchiolitis (mostly caused by RSV) are rare, the need for chest radiography in children with acute bronchiolitis was questioned.[139] However, biological, epidemiological, clinical, and interventional data suggest a role for pneumococcus (and potentially other bacteria) in the severity of RSV disease. This notion is supported by the beneficial effects of antibiotics in children with RSV and respiratory failure.[129,130] Moreover, the accumulated data show an unequivocally important role of RSV in increasing the risk and severity of disease caused by *S pneumoniae*. There is no evidence that the interaction of RSV with pneumococcus is modified by RSV subtype (A or B).

Limitations of the systematic review and data synthesis

We acknowledge the limitations of our systematic review. First, co-detection of nasopharyngeal pneumococcus and RSV during respiratory disease is often reported, since RSV is one of the most commonly detected viruses in young infants and children with LTRI and pneumococcus is one of the most commonly detected bacteria in the nasopharynx of both healthy and sick children. We aimed to focus on studies that were relevant to the discussion of a potential causative role of RSV–pneumococcus co-infection in respiratory disease. Second, the variability in conclusions between studies could be related to differences in study design or definitions of RSV-related disease, heterogeneous populations, or the inherent difficulty in estimating subtle effects over time from aggregate data. Regarding the epidemiological data on effects of PCVs, these studies generally do not attempt to disentangle changes due to PCVs from other changes in the population that might influence RSV rates, complicating the interpretation of the presence or absence of trends over time. Third, the role of RSV and bacterial co-detection has been studied in more depth in children admitted to the ICU. In many studies, only patients who are severely ill, which often means ICU admission, are tested for bacterial colonisation, resulting in selection bias; however, this challenge was addressed in our review by discussing patients inside and outside the ICU separately. Fourth, in many studies, objective parameters for disease severity in children with RSV are not included, which precludes a direct comparison of the influence of bacterial colonisation on clinical outcomes. Lastly, nasopharyngeal microbiota was often used as a proxy for lower respiratory tract microbiota in childhood LRTI, which, despite its limitations, is a previously validated approach.[53]

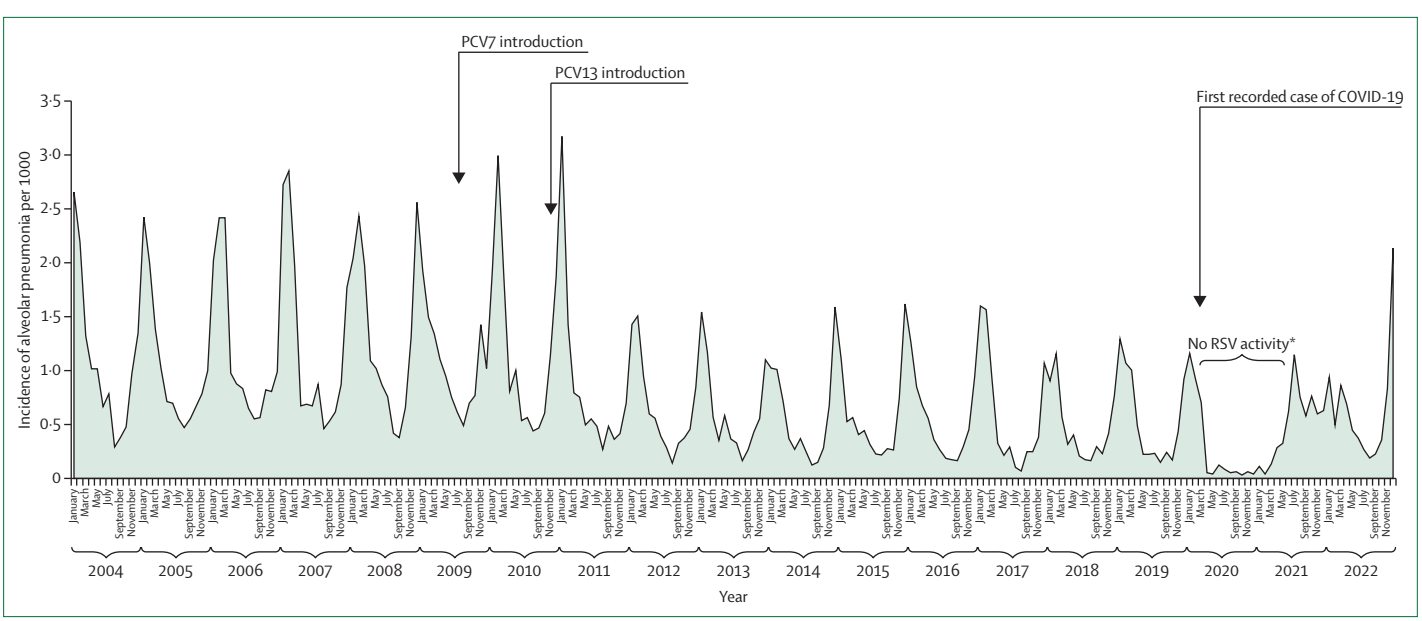

Figure 3: **Dynamics of hospitalisations for alveolar pneumonia in children younger than 5 years in southern Israel, 2004–22**

Monthly incidence of hospital visits of children younger than 5 years for radiologically proven community-acquired alveolar pneumonia in southern Israel from the pre-PCV era through to 2022. PCV7 was implemented in Israel in July 2009 and PCV13 in November 2010. The first COVID-19 case was reported in February 2020. Data from Dagan et al[75,76] and Ben-Shimol et al,[113] updated to 2022. PCV=pneumococcal conjugate vaccine. RSV=respiratory syncytial virus. *Also suppression of human metapneumovirus, influenza, and parainfluenza viruses.

Implications for health-care development

This systematic review deepens our understanding of the interrelationship between RSV and pneumococcus, and summarises important evidence that should be used in developing global health-care approaches for children with co-infection or at risk of co-infection. A summary of recommendations for clinical practice, health policy, and research is provided in the panel. Further research will be essential to enhance understanding of RSV–pneumococcus interactions. It is imperative that future clinical studies, especially vaccine trials, include analysis of airway microbiome dynamics and local immune response in a bidirectional manner. Additionally, experimental studies, such as human challenge studies, offer the opportunity to examine RSV and pneumococcal interactions in a controlled in-vivo setting. With ample RSV immunisation trials on the horizon, we should include pneumococcal-related morbidity and mortality in the study outcomes. While maternal and infant RSV immunisation programmes are being rolled out globally, studies to accumulate real-world evidence are currently being undertaken, and many more will follow. These research efforts should include investigations of pneumococcal infections and antibiotic use, but there is also a unique opportunity to explore more broadly the effect of RSV prevention on the respiratory microbiome. This is a responsibility of manufacturers of RSV vaccines, but also a public health priority to understand how novel respiratory vaccines could shape the microbiological ecosystem, including the development of antimicrobial resistance, at the respiratory mucosal level. Pathogen-specific organisations, such as the International Society of Pneumonia and Pneumococcal Diseases (ISPPD) and the Respiratory Syncytial Virus Foundation (ReSViNET), will have an important role in future research and could increase their impact on global health by co-creating a collaborative research agenda.

In view of the evidence presented in this Health-care Development paper, we call for various stakeholders, including health economists, to increase understanding of the synergy between respiratory vaccine programmes. The expected synergistic effects of RSV and pneumococcal vaccines have the potential to substantially reduce the global burden of childhood pneumonia, leading to a reduction in antibiotic use. We also anticipate a reduction in the overall paediatric care capacity requirement, because respiratory infections are a major cause of health-care use, including hospitalisations, in the respiratory season. The greatest impact of the combined prevention of RSV and pneumococcal infection is expected in

For more on the **International Society of Pneumonia and Pneumococcal Diseases** see https://isppdsociety.com/

For more on the **Respiratory Syncytial Virus Foundation** see https://resvinet.org/

Panel: Recommendations for clinical practice, health policy, and research

Recommendations for clinical practice and health policy

- RSV and pneumococcus can mutually contribute to disease in young infants: on the one hand, RSV might facilitate the transition of pneumococcus from non-pathogenic to pathogenic, and on the other hand, the presence of pneumococcus probably increases the severity of RSV disease; instead of focusing on each of these pathogens separately, stakeholders should develop a cross-pathogen approach that focuses on the wider scope of respiratory infections in young children, including advocacy (eg, to increase awareness and education of the synergistic RSV–pneumococcus interaction for health-care professionals, policy makers and payers, and parents), management (eg, to promote the importance of diagnostic testing to support appropriate treatment), and prevention (eg, to support the introduction of a combined vaccination strategy)
- Indirect effects of RSV vaccination on pneumococcal disease and vice versa will have societal and health-economic consequences; these consequences should be incorporated into decision making by policy makers when considering the introduction of RSV prevention strategies and widening of the spectrum of pneumococcal vaccines
- Global efforts to increase the coverage of preventive measures against RSV and pneumococcus (such as widespread implementation and improved coverage of PCVs, infant monoclonal RSV antibodies, and RSV maternal vaccination) are needed

Research priorities

- Further mechanistic research on microbiological and host factors is required (at the mucosal and systemic levels) to better understand the synergistic relationship between pneumococcus and RSV that leads to increased disease rates and disease severity
- Respiratory microbiome studies are needed to further understand the complex interaction between RSV, pneumococcus, and the wider respiratory ecosystem that they are a part of, including bacteria, viruses, and fungi
- Implementation of newly licensed and future vaccines against RSV and pneumococcus in young children will provide a unique opportunity to understand their mutual effects on the burden of viral and bacterial lower respiratory disease; high-quality prospective surveillance and cohort studies will be required to assess these effects
- Antibiotic trials in all children with RSV bronchiolitis did not show any clear benefit, but benefit is expected for those with RSV–pneumococcus co-infections; to define the role of antibiotics in viral–bacterial co-infections, further research with a focus on diagnosis (eg, biomarkers to better define the target population), clinical evaluation (clinical judgement), and management is warranted
- Human challenge studies are needed to examine the dynamics of RSV and pneumococcus during the development of respiratory disease in controlled in-vivo and ex-vivo settings

PCV=pneumococcal conjugate vaccine. RSV=respiratory syncytial virus.

resource-poor settings, where mortality is highest. A better understanding of the correlation between pneumococcus and RSV-related pneumonia—and translation of such advances into policies for prevention and management—will help to improve respiratory health-care outcomes for millions of children worldwide.

Contributors

SBB, LB, and RD developed the concept for the literature review and synthesis of evidence pertaining to the relationship between RSV and pneumococcus in childhood respiratory infections; they designed the protocol for the systematic review together with Violicom Medical. The other authors (DB, AM, OR, and DMW) were consulted at an early stage and provided input into the study protocol. Violicom conducted systematic searches, screened the titles and abstracts of identified records, assessed full-text reports for eligibility, and collected relevant data from those eligible for inclusion. SBB, LB, and RD reviewed all full-text articles identified as eligible for inclusion, reviewed extracted data, interpreted the results, and wrote the first draft of the manuscript. Further input was provided for each section as follows: biological evidence by DB and OR; epidemiological evidence by DMW; clinical effects of RSV–pneumococcus co-detection by AM; and effects of interventions by DMW. All authors critically revised the manuscript for important intellectual content, including input into the panel, figures, and tables, and approved the final manuscript. All authors were responsible for the decision to submit the manuscript for publication.

Declaration of interests

LB and RD obtained a research grant from the Investigator-Initiated Studies Program of Merck Sharp & Dohme (MSD) to cover the costs of Violicom's searches and screening of the data. LB is the founding chairman of the Respiratory Syncytial Virus Foundation (ReSViNET Foundation); he has regular interactions with pharmaceutical and other industrial partners, but he has not received personal fees or other personal benefits. LB reports the following funding to his institution: funding for investigator-initiated studies from AbbVie, MedImmune, AstraZeneca, Sanofi, Janssen, Pfizer, MSD, and MeMed Diagnostics; funding for the RSV GOLD study from the Bill & Melinda Gates Foundation; funding as part of the Innovative Medicines Initiative-funded RESCEU and PROMISE projects with partners GSK, Novavax, Janssen, AstraZeneca, Pfizer, and Sanofi; funding for participation in clinical studies sponsored by MedImmune and Pfizer from Julius Clinical; and consulting fees and fees for invited lectures from AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, GSK, Novavax, Pfizer, Moderna, AstraZeneca, MSD, Sanofi, and Janssen. AM has received research grants to her institution from Janssen, Merck, and the US National Institutes of Health (NIH); fees for participation on advisory boards from Janssen, Sanofi Pasteur, Merck, Pfizer, and AstraZeneca; and fees for lectures from Sanofi Pasteur and AstraZeneca. OR has received research grants to his institution from Janssen, Merck, NIH, and the Bill & Melinda Gates Foundation; fees for participation on advisory boards from Sanofi Pasteur, Merck, and Pfizer; and fees for lectures from Pfizer, Sanofi Pasteur, and AstraZeneca. DMW is supported by a grant from the NIH National Institute of Allergy and Infectious Diseases (R01AI137093) and has received consulting fees from Pfizer, Merck, and GSK/Affinivax for work unrelated to this manuscript; he is the principal investigator on grants from Pfizer and Merck to his institution for work unrelated to this manuscript. RD has received grants to his institution from Pfizer, MSD, and MedImmune/AstraZeneca; consulting fees and fees for participation on advisory boards from Pfizer and MSD; payment for speakers bureaus from Pfizer, MSD, Sanofi Pasteur, and GSK; and payment for expert testimony from Pfizer. SBB and DB declare no competing interests.

Data sharing

The datasets generated and evaluated during the conduct of this Health-care Development paper are not publicly available but are available from the corresponding author on reasonable request.

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