

# Viral Bacterial Interactions in Children: Impact on Clinical Outcomes

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Respiratory viral infections are associated with significant morbidity and mortality in children < 5 years of age worldwide. Among all respiratory viruses, respiratory syncytial virus (RSV) is the world's leading cause of bronchiolitis and pneumonia in young children. There are known populations at risk for severe disease but the majority of children who require hospitalization for RSV infection are previously healthy. Viral and host factors have been associated with the pathogenesis of RSV disease; however, the mechanisms that explain the wide variability in the clinical presentation are not completely understood. Recent studies suggest that the complex interaction between the respiratory microbiome, the host's immune response and the virus may have an impact on the pathogenesis and severity of RSV infection. In this review, we summarize the current evidence regarding the epidemiologic link, the mechanisms of viral–bacterial interactions, and the associations between the upper respiratory tract microbiome and RSV disease severity.

**Key Words:** RSV; bacterial colonization, disease severity, infants

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## BACKGROUND

Among all respiratory viruses associated with respiratory morbidity in infants and young children, respiratory syncytial virus (RSV) represents the most common cause of bronchiolitis and pneumonia, and one of the world's leading causes of death during the first year of life.<sup>1</sup> The clinical spectrum of the disease, however, is broad ranging from mild upper respiratory symptoms, to severe lower respiratory tract infection (LRTI) requiring hospitalization. Among hospitalized infants with RSV LRTI ~15% will require intensive care management.<sup>2–4</sup> There are specific populations at high risk for severe RSV disease (ie, prematurity, chronic lung

disease or congenital heart disease) but the vast majority of infants hospitalized with RSV LRTI are previously healthy.<sup>5–7</sup> In addition to these predisposing conditions, other factors including those specific to the virus, a dysregulated host immune response, or genetic predisposition, have been associated with severe disease.<sup>2,8–10</sup> Nevertheless, these factors do not completely explain the variability of RSV disease severity in children (Fig. 1).

Studies have shown that severe bacterial infections, such as bacteremia and meningitis, are extremely rare in children with bronchiolitis<sup>7,11</sup> and current guidelines do not support the routine use of antibiotics in this children.<sup>12–14</sup> Despite these recommendations, antibiotics are commonly used among hospitalized infants because of the difficulty to exclude superimposed bacterial pneumonia, and the severity of the infant's clinical presentation at such young age. Recently, there has been an increasing interest in understanding the complex interplay between the host, the virus and the respiratory microbiome, and how this interaction may affect disease pathogenesis and severity. In this review, we summarize the current evidence regarding viral–bacterial interactions and their influence on clinical manifestations, with a special emphasis on RSV infection.

## VIRAL–BACTERIAL INTERACTIONS: EPIDEMIOLOGIC LINK

The respiratory tract is colonized early in life with an abundant number of bacterial communities including commensal and potentially pathogenic bacteria (PPB). This ecosystem known as the microbiome plays an important role in human health.<sup>15</sup> Studies suggest that there is an association between early nasopharyngeal colonization with PPB and the development of bronchiolitis and recurrent wheezing in childhood.<sup>16,17</sup> In addition, there are data showing how the early composition of the respiratory microbiome can be transiently affected during acute viral infections, and how the incursion of pathogenic bacteria during these episodes can potentially affect both the acute course of the infection as well as the long-term respiratory morbidity.<sup>15,18</sup>

The association between the peak activity of respiratory viral infections, mainly RSV and influenza, and the incidence of invasive pneumococcal disease (IPD) in children has been previously described.<sup>19,20</sup> A retrospective study, conducted in a large tertiary pediatric hospital in the United States showed that one-third of children with IPD had a concomitant respiratory viral infection, and that the peak activity of RSV and IPD overlapped.<sup>19,20</sup> The mechanisms and timing underneath this association are not well understood and the association is likely bidirectional.

## MECHANISMS OF VIRAL–BACTERIAL INTERACTIONS

Respiratory viruses are thought to promote bacterial infections by enhancing the outgrowth of pathogenic bacteria within the respiratory tract. Studies in vitro and in animal models have proposed different mechanisms to explain this phenomenon including decreased bacterial clearance, increased bacterial adherence to the airway epithelium and suppression of immunity during recovery

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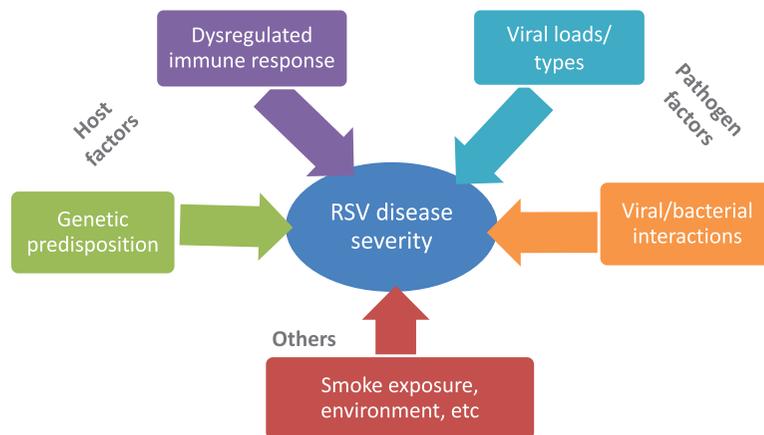
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**FIGURE 1.** Diagram depicting different factors that may influence RSV disease severity.



from viral infections.<sup>21–23</sup> Traditionally, it has been hypothesized that a previous respiratory viral infection predisposes to a more severe bacterial disease. This association has been frequently described in patients with influenza infection and subsequent development of severe pneumococcal and staphylococcal pneumonia, or between chickenpox and severe group A streptococcal infection. Evidence of bacterial superinfections in infants with RSV infection is limited.<sup>24</sup> We used a mouse model to define in a controlled setting, if a prior RSV infection was associated with more severe pneumococcal pneumonia. To this end, mice were inoculated with RSV and 5 days later with *Streptococcus pneumoniae* serotype 3. Compared with mice inoculated with *S. pneumoniae* alone, those coinfecting with RSV plus pneumococcus had significantly greater morbidity and mortality. The rate of bacteremia in the coinfecting group was 80% compared with 0%–30% in mice inoculated with pneumococcus only ( $P < 0.01$ ). In addition the coinfecting group demonstrated significantly worse clinical disease severity as defined by greater weight loss, airway obstruction, lung inflammation, and 80% mortality, suggesting that a prior RSV infection increased bacterial replication, predisposing to more severe bacterial pneumonia.<sup>25</sup>

On the other hand, emerging evidence suggest that prior colonization with PPB may enhance the severity of respiratory viral infections. Data from a large randomized placebo controlled trial using a 9-valent pneumococcal conjugate vaccine (PCV) in children, showed that vaccination with PCV-9 was associated with a 31% reduction of pneumonia of any viral cause, including RSV.<sup>26</sup> In another large retrospective time series study conducted in the United States investigators found that RSV was associated with a 20% increase in the

incidence of pneumococcal pneumonia in infants. Interestingly, following the introduction of PCV-7 there was a significant decline not only in the rates of pneumococcal pneumonia but also in the number of hospitalizations for RSV infection.<sup>27</sup> In addition, in a prospective longitudinal cohort study conducted in young children in Peru, investigators showed that nasopharyngeal pneumococcal density increased before the onset of an acute respiratory viral infection, peaked during the acute infection and decreased afterward.<sup>28</sup>

Altogether, these data support bidirectional interactions between respiratory viruses and bacteria. Furthermore, cotransmission of both group of pathogens simultaneously and acquisition by a new host is a possibility, given the common transmission of these pathogens through large droplet aerosols or direct contact with secretions.

### PPB AND RSV DISEASE SEVERITY

To understand the role of PPB on RSV disease severity, most clinical studies have been conducted in children with RSV and suspected bacterial pneumonia requiring pediatric intensive care unit (PICU) care. In those studies of different designs and various sample sizes, the frequency of PPB detected by culture varied from 20% to 50% in lower respiratory samples, and codection of both RSV and PPB was associated with longer duration of mechanical ventilation (Table 1).<sup>29–36</sup> Nevertheless, differentiation between colonization and true lower respiratory infection remains challenging.

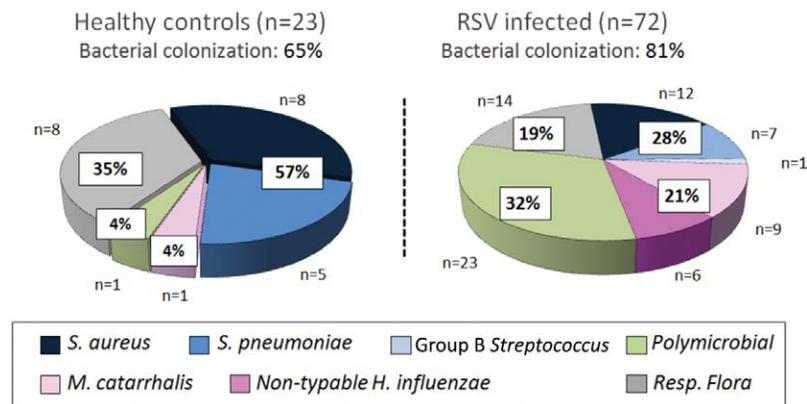
Emerging but limited evidence suggests that the nasopharyngeal microbiome may play a role in the pathogenesis and

**TABLE 1.** Studies evaluating the incidence of superimposed bacterial pneumonia in children with severe RSV infection admitted to the pediatric intensive care unit

Country/Year	Population	N	Microbiologic Diagnosis (culture)	Incidence/Suspected Bacterial Pneumonia	Outcomes
United States, <sup>29</sup> 2004; (R)	Children <1 yr (no high risk)	155	ETT	23%	N/A
Switzerland, <sup>30</sup> 2004; (R)	Children <1 yr	25	ETT	44% (Hi>Mc>Spn)	N/A
Netherlands, <sup>31</sup> 2005; (R)	Children <1 yr (+ high risk)	38	ETT/Blood	26%	Longer MV
United Kingdom, <sup>32</sup> 2006; (P)	Children <1 yr (+ high risk)	70	ETT	42% (Hi>Sa>Mc>Spn)	Longer MV
United States, <sup>33</sup> 2010; (P)	Children <1 yr (no high risk)	22	ETT	30%	Longer MV
South Africa, <sup>34</sup> 2012 (R)	Children <2 yr	54	ETT/blood blood Cx	12%	Longer MV, PICU and total LOS
Japan, <sup>35</sup> 2011 (P)	Children <5 yr	188	Sputum	44% (Hi>Spn>Mc)	N/A
China, <sup>36</sup> 2015; (P)	Children <2 yr (+ high risk)	250	Nasopharyngeal	30% Hi >Spn >Sa >Mc	increase fever, neutrophils % and LOS

Cx indicates culture; ETT, endotracheal tube; Hi, *Haemophilus influenzae*; LOS, length of stay; Mc, *Moraxella catarrhalis*; MV, mechanical ventilation; N/A, not evaluated; P, prospective; R, retrospective; Sa, *Staphylococcus aureus*; Spn, *Streptococcus pneumoniae*.

**FIGURE 2.** Percentage and type of PPB colonizing the nasopharynx in healthy controls and infants hospitalized with RSV LRTI. PPB identified by culture in RSV infected infants and age-matched healthy controls. Respiratory flora reflects the bacterial flora colonizing the upper respiratory tract. Pie charts represent the percentage of respiratory flora, Gram-positive, Gram-negative bacteria and >1 PPB present in nasopharyngeal samples from study subjects. Reproduced with permission from ref.<sup>6</sup>



severity of RSV infection. In a recent study conducted in low and high-risk children <2 years of age hospitalized with viral bronchiolitis, nasopharyngeal culture of PPB, specially *Haemophilus influenzae*, was associated with fever more frequently and longer duration of hospitalization.<sup>36</sup> In agreement with those findings, we found that previously healthy infants hospitalized with RSV LRTI had greater rates of nasopharyngeal PPB (*S. pneumoniae*, *H. influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus*) identified by culture versus age-matched healthy controls (81% vs. 65%;  $P < 0.02$ ). In addition, the distribution of the bacteria identified was different between groups. Infants with RSV infection were more frequently colonized with Gram-negative bacteria, as opposed to healthy controls in whom the proportion of Gram-positive bacteria was higher (Fig. 2). Moreover, infants with RSV LRTI and colonized with PPB had increased numbers of mucosal white blood cells and blood neutrophil counts, and specific colonization with Gram-negative bacteria was associated with higher plasma interleukin (IL)-6 and IL-8 concentrations and longer duration of supplemental oxygen.<sup>6</sup> These initial studies suggest that colonization with specific PPB may be more relevant than just a passive phenomenon.

Advances in molecular techniques have provided additional insights to understand the complex interactions between respiratory viruses, the airway microbiome and the host immune response in the pathogenesis of LRTI in children. In a small study conducted in Australia, in children <5 years of age, investigators found that nasopharyngeal bacterial detection by polymerase chain reaction (PCR) was overall higher in children with RSV compared with other respiratory viruses (73% vs. 56%, respectively;  $P 0.03$ ). Specifically, RSV infection was associated with a 3-fold increase in *S. pneumoniae* detection.<sup>37</sup> A subsequent study that included 29 previously healthy children <2 years of age with RSV infection suggested that nasopharyngeal codetection of RSV-*S. pneumoniae* by qPCR was associated with worst disease severity as demonstrated by higher disease severity scores.<sup>38</sup> A prospective multicenter study (MARC-35) in infants hospitalized with bronchiolitis showed high rates of colonization with nasopharyngeal PPB in these infants and predominance of certain bacterial species according to the respiratory virus causing the bronchiolitis.<sup>39</sup> Overall, the identification of a *Haemophilus* spp. enriched profile was associated with a dysfunctional local and systemic innate immune response, and with higher need for PICU admission and longer duration of hospitalization.<sup>40-42</sup> In agreement with those findings, we found that nasopharyngeal detection of *H. influenzae* and also *S. pneumoniae* by PCR in infants with RSV infection was associated with fever more frequently, higher clinical disease severity scores, and higher blood neutrophil counts. In addition, nasopharyngeal detection of *H. influenzae* in these infants was associated with worse radiologic

findings such as consolidation and atelectasis.<sup>43</sup> Altogether, these findings suggest a conceptual model in which both respiratory viruses and the airway microbiota contribute to the pathogenesis of airway disease. When the viral-bacterial balance is altered, there is an increase in the inflammatory response, greater damage of the airways that leads to greater disease severity.

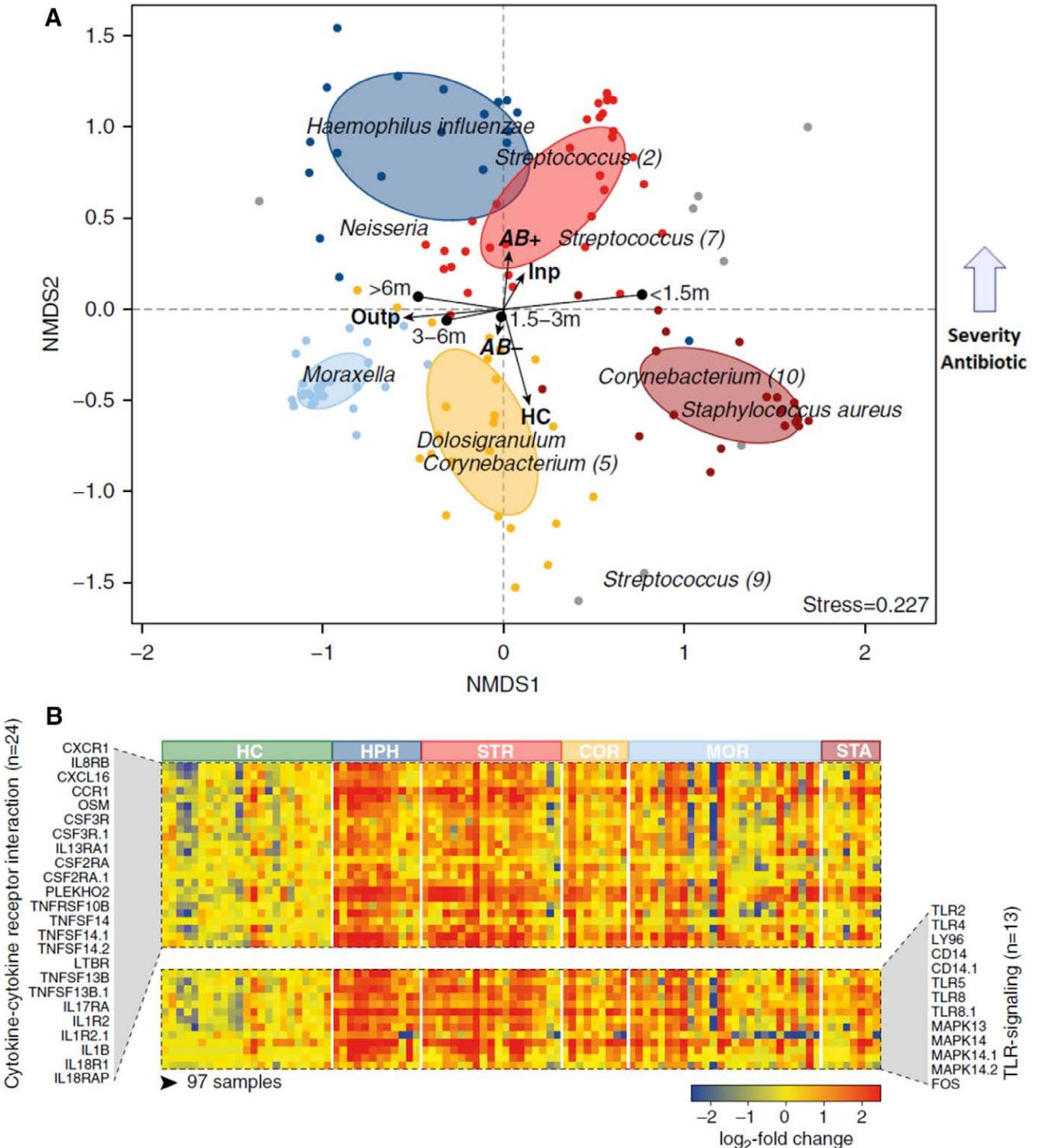
#### IS IT COLONIZATION OR INFECTION? THE ROLE OF TRANSCRIPTOMICS

The etiologic diagnosis of LRTI in children remains challenging. Despite the broad implementation of fast turn-around molecular tests that have facilitated the identification of a number of viruses in respiratory samples, assessing the contribution of these pathogens on disease severity is challenging.<sup>44</sup> These limitations have stimulated investigators to develop alternative diagnostic methods based on the global host immune response to the infection.

Blood leukocytes constitute an accessible source of clinically relevant information, and a comprehensive molecular phenotype of these cells can be obtained using transcriptional profiles.<sup>45</sup> These tools have demonstrated high sensitivity and specificity to discriminate between bacterial and viral pathogens and to assist with patient stratification based on disease severity.<sup>46-48</sup>

Using a combination of clinical data, blood RNA immune profiles and nasopharyngeal microbiome profiling by 16S-rRNA-based sequencing, we defined the specific nasopharyngeal microbiota profiles in infants with mild (outpatients) and severe (inpatients) RSV infection and their relationship with host immune responses and disease severity. We identified five different microbiota communities in infants with RSV infection characterized by enrichment of *H. influenzae*, *Streptococcus* spp., *Corynebacterium*, *Moraxella* and *Staphylococcus*. Using nonmetric multidimensional scaling analysis, we confirmed our previous observations using PCR assays and found that the abundance of *Haemophilus* and *Streptococcus* profiles were associated with a distinct systemic host immune response and greater RSV disease severity. While interferon related genes were overexpressed in infants with RSV infection, independent of their nasopharyngeal microbiota composition,<sup>47</sup> specific detection of *H. influenzae* and *Streptococcus* spp was associated with significantly greater overexpression toll-like receptor signaling and neutrophil pathways (Fig. 3).<sup>49</sup> These data confirm the importance of integrating the multifaceted components that may contribute to RSV disease severity in infants and open a complete new approach to analyzing the severity of RSV disease.

In summary, the development of the nasopharyngeal microbiome is complex and dynamic. The assessment of its contribution (specifically the predominance of pathogenic bacteria) on disease



**FIGURE 3.** Nasopharyngeal microbiome composition in infants with RSV infection. A: Nonmetric multidimensional scaling (NMDS) analyses were used to visualize the associations between nasopharyngeal microbiota clusters and host factors. More severe disease in infants with RSV infection was associated with younger age, lack of breast-feeding, greater antibiotic use and with *H. influenzae* and *Streptococcus spp.* profiles, whereas mild-moderate disease was related with *Moraxella* and *S. aureus* profiles. B: Heat map depicting the log<sub>2</sub>-fold change of blood expression of IL-8 and toll-like receptor signaling pathways in infants with RSV infection according to the nasopharyngeal microbiota profiles. Normalized expression is indicated as overexpressed (red) or underexpressed (blue) compared with the median expression of HC (yellow). HC indicates healthy controls; Outp, outpatients; inp, inpatients; AB, antibiotics HPH, *H. influenzae*; STR, *Streptococcus spp.*; COR, *Corynebacterium*; MOR, *M. catarrhalis*; STA, *S. aureus*. Infants with RSV infection and colonized with *H. influenzae* and *Streptococcus spp.* showed significantly higher overexpression of pathways associated with neutrophil activation and recruitment as well as Toll-like receptor signaling. Reproduced with permission from ref.<sup>48</sup>

severity during acute respiratory viral infections is challenging and still not well understood. The inclusion of modern molecular diagnostic tools with simultaneous detection of multiple pathogens in the clinical setting has become a frequent practice. Recent evidence suggests that codetection of respiratory viruses and pathogenic bacteria during the acute infection might be associated with greater clinical disease severity by triggering distinct host immune responses. Further research is needed to better understand the mechanisms and directionality of these interactions, both during respiratory health and during acute respiratory infections and their potential relationship with long-term lung morbidity (ie, asthma). Understanding these interactions and the contribution of the microbiome during respiratory infections may facilitate the development of therapeutic or preventive strategies, with the goal of improving patient outcomes.

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