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Role of Upper Respiratory Microbiota and Virome in Childhood Rhinitis and Wheeze: Collegium Internationale Allergologicum Update 2021

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Abstract
There is emerging evidence that the respiratory microbiota influences airway health, and there has been intense research interest in its role in respiratory infections and allergic airway disorders. This review aims to summarize current knowledge of nasal microbiome and virome and their associations with childhood rhinitis and wheeze. The healthy infant nasal microbiome is dominated by *Corynebacteriaceae* and *Staphylococcaceae*. In contrast, infants who subsequently develop respiratory disorders are depleted of these microbes and are instead enriched with *Proteobacteria spp.* Although human rhinovirus and human respiratory syncytial virus are well-documented major viral pathogens that trigger rhinitis and wheezing disorders in infants, recent limited data indicate that bacteriophages may have a role in respiratory health. Future work investigating the interplay between commensal microbiota, virome, and host immunological responses is an important step toward understanding the dynamics of the nasal community in order to develop a strategical approach to combat these common childhood respiratory disorders.

Introduction

In recent years, the influence of commensal microbiota on the development of host immunity has been the focus of intense research. The commensal microbiota also maintains the integrity of mucosal and epithelial barriers and modulates an equilibrium between commensal and pathogen colonization, which in turn impacts upon health and disease states [1]. This, therefore, gives rise to the notion that establishing a symbiotic relationship between host and microbiota in early life is important and could influence health in later life [2]. In this regard, the influence of the gut microbiome on immunity has been the main focus of attention. However, emerging data have also provided evidence that the respiratory microbiota is associated with airway health and may affect the development of respiratory infections and disorders [3].

This review aims to present the current knowledge on the upper airway microbiota and its development through infancy and its influence on childhood respiratory infections and disorders such as rhinitis and wheeze. In view of the limited published work, we also intend to identify

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knowledge gaps and directions for future research. We also recognized that data comparisons between studies may be challenging as nasal sampling techniques (anterior nares vs. nasopharyngeal swabs) and molecular detection techniques varied considerably between studies. These factors have to be considered when interpreting data between studies.

Methodology

The literature search for this review, which included relevant cohort and case-control studies, was conducted between August and October 2020 using the Google Scholar and PubMed database. The search terms used were (1) ([nasal] OR [nose] OR [nasopharyngeal]) AND ([infant] OR [neonate]) AND ([rhinitis] OR [wheeze]) and (2) (nasal) OR (microbiome) OR (virome) OR (bacteriophage). The search was limited to English publications in the last 2 decades and children from birth to 18 years.

Early Establishment and Succession of Nasal Microbiota in Infants

The upper respiratory tract has been recognized as the main entry point [4] and reservoir [5] for respiratory commensal bacteria and pathogens. In adults, the bacterial composition of the nasal microbiome is stable and diverse as a result of maturation due to age progression and interaction with the environmental microbiome [6, 7]. In comparison, the nasal microbiome of children, especially under 3 years of age, demonstrates significant variability in composition, both between individuals and across age groups [8, 9]. The data from these studies are presented as relative abundance, and data on the bacteria density are not available with the 16S ribosomal RNA (rRNA) sequencing methods. Table 1 summarizes 5 studies performed on birth cohorts with repeated nasal sampling over first 1–2 years of life. Although general comparisons in nasal microbiome profiles can be made, it is recognized that there are some methodological differences between these studies. One such difference is the nasal sampling site. Two studies [10, 11] of the 5 studies sampled the anterior nares, whilst the other 3 utilized nasopharyngeal swabs [9, 12, 13]. It has been shown that the nasopharynx is more likely to be colonized by nonlipophilic skin colonizers such as *Haemophilus spp.* and *Streptococcus spp.*; thus, these species might be more likely to be enriched in studies using nasopharyngeal samples [11, 12, 14, 15]. In terms of DNA sequencing techniques, 4 studies [9–12] utilized the 16S rRNA 454 pyrosequencing technique, except for one [13] which applied the more advanced DNA shotgun sequencing techniques of the 16S rRNA gene. These techniques support comparisons for larger-order bacterial taxa. However, the low sequencing depth precludes species-specific comparisons [16].

The overall results of these studies show that nasal microbiome undergoes dynamic changes in microbiota composition throughout the first few years of life. There is low microbial diversity soon after birth, and this increases over the next few months of life. Five longitudinal studies from European and American birth cohorts involving nasal sampling over the first 2 years of life showed that the nasopharyngeal microbiome of healthy infants is dominated by a high abundance of *Corynebacteriaceae* (*Actinobacteria* phylum), *Moraxellaceae* (*Proteobacteria* phylum), and *Staphylococcaceae* (*Firmicutes* phylum), with some studies describing also the presence of *Pasteurellaceae* (*Proteobacteria* phylum), *Propionibacteriaceae* (*Actinobacteria* phylum), and *Lactobacillaceae* (*Firmicutes* phylum) in the first few months of life. These early colonizers are subsequently replaced by a dominance of *Streptococcaceae* (*Firmicutes* phylum) and/or either *Corynebacteriaceae* or *Moraxellaceae* and other minor families [9–13]. Based on these studies, the changing profile of the nasal microbiome in the first 2 years of life is depicted in Figure 1. The adolescent/adult nasal microbiome profile is also included as a comparison [17, 18]. The study from the Netherlands and Switzerland demonstrated strong seasonal variability of microbial profiles [9, 11]. The winter profile was dominated by *Pasteurellaceae* (*Proteobacteria* phylum), while the summer was dominated by *Corynebacteriaceae* (*Actinobacteria* phylum) and/or *Bacteroidetes* phylum. The differences were postulated to be a result of interactions between seasonal infection patterns and the host-environment equilibrium, where disturbances to the native nasal microbiota could be induced by infection by pathogenic bacteria in the environment.

The consistent presence and dominance of the commensals *Corynebacteriaceae* and *Staphylococcaceae* in the nasal microbial profiles in early life have been substantiated by the longitudinal nasal microbial profiles of healthy controls from our study [19]. These early microbial profiles have also been shown to be associated with protection against any respiratory infection in later childhood [9, 14, 20]. The exact role of *Moraxellaceae* is still controversial. The *Moraxellaceae* family has been suggested to be a symbiotic stabilizer of the nasal microbiome in early life and associated with a lower probability of developing upper respiratory tract infections [11, 12]. However, this observation has not been consistent between studies [14, 21, 22].

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Table 1. Summary table of studies of healthy infants

Study design	Cohort size	Age range	Study aim and setting	Sample type	Analysis methods	Clinical significance	Reference
Cohort (Netherlands)	330 infants, randomly selected 150 nasopharyngeal swab	18 months	Assess composition of and variability in nasopharyngeal microbiota in young children sampled during different seasons	Naso-pharyngeal swab	Culture for *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus*; 16S rRNA 454 pyrosequencing for bacteria	Nasopharyngeal microbiota of young children is highly diverse and varies between seasons. Microbiota profiles in fall/winter were predominantly *Proteobacteria* and *Fusobacteria*, and in spring, there was a predominance of *Bacteroidetes* and *Firmicutes*	Bogaert et al. [9]
Cohort (USA)	81 children	6 weeks	To assess composition and metabolic function of neonatal microbiome across multiple body sites and to determine the effect of cesarean delivery and its confounders on neonatal microbiome	Anterior nasal swab	16S rRNA 454 pyrosequencing	At delivery, neonatal microbiome was relatively homogeneous across all body sites. In the first 6 weeks of life, the infant microbiota undergoes substantial reorganization, primarily driven by body site, not the delivery mode	Chu et al. [10]
Cohort (Netherlands)	47 infants (Bern Infant Lung Development Cohort)	First year of life	Describe dynamics of upper respiratory tract microbiota in healthy infants	Anterior nasal swab	16S rRNA 454 pyrosequencing	3-month-old infants possess increased relative abundances of *Staphylococcaceae* and *Corynebacteriaceae*. Significant seasonal differences in microbiome profiles for the summer and winter months	Mika et al. [11]
Cohort (Switzerland)	60 children, with validation cohort of 140 children	First 24 months	Study bacterial succession of respiratory microbiota in first 2 years and relation to respiratory health	Naso-pharyngeal swab	16S rRNA 454 pyrosequencing	Microbiota profiles with early colonization of *Moraxella* and *Corynebacterium*/*Dolosigranulum* tend to be more stable, with lower rates of respiratory infections reported. Less stable profiles are marked by the *Haemophilus* or *Streptococcus*-dominated clusters	Biesbroek et al. [12]
Cohort (Netherlands)	102 children	Six months	To find out if delivery mode affects the respiratory microbiota development	Nasopharyngeal swab	16S rRNA illumina MiSeq next-generation shotgun sequencing	Respiratory microbiome develops toward a *Streptococcus viridans* dominant profile, regardless of delivery mode. Cesarean-delivered infants have an overall delay in development of respiratory microbiome profiles	Bosch et al. [13]

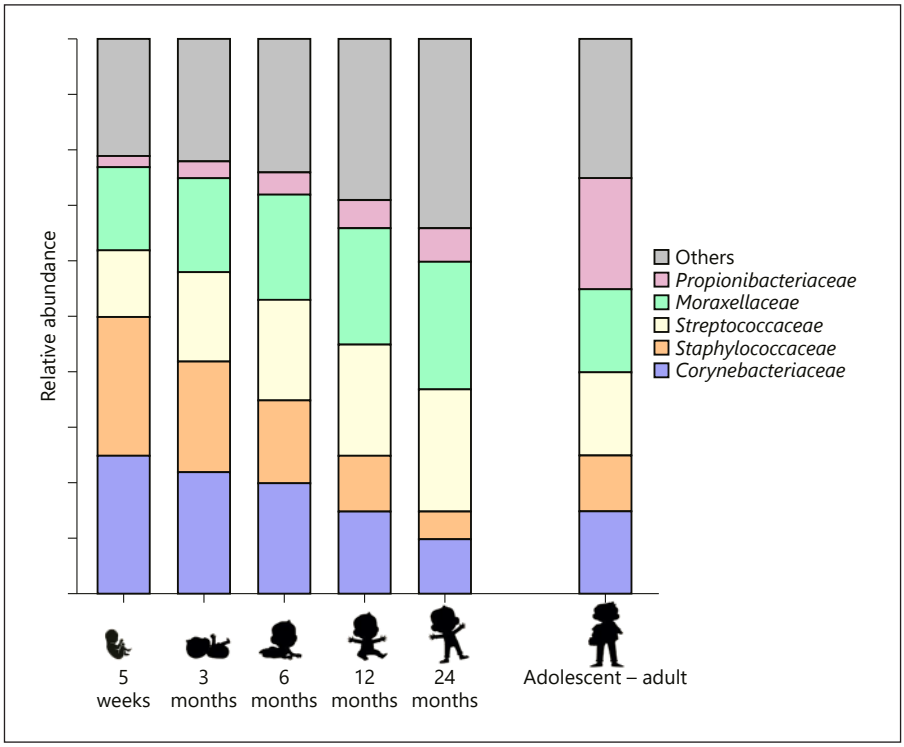

Fig. 1. Graphic representation of early establishment and succession of nasal microbiota (represented as relative abundance) in healthy infants in the first 2 years of life and adolescent/adult (not drawn to actual scale). Nasopharyngeal microbiome of healthy infants is dominated by a high abundance of *Corynebacteriaceae*, *Moraxellaceae*, and *Staphylococcaceae* in the first few months of life before subsequently replaced by a dominance of *Streptococcaceae* and/or either *Corynebacteriaceae* or *Moraxellaceae* and other minor families.

Bacteria with low abundance may also play a role in a healthy infant's respiratory tract. *Lactobacillus* spp. is a probiotic bacterium that is well known for its protective effects on the human gut microbiome and gut health. In recent years, longitudinal profiles of the healthy infants' nasal microbiome have shown that although *Lactobacillus* is a minor bacteria (<1% relative abundance), it could provide protection from infection and disorders, likely by inhibiting pathogenic bacteria growth through inhibitory substances such as bacteriocins [9, 10].

Infant Nasal Microbiota and the Development of Rhinitis and Wheeze Disorders in Childhood

Compared to healthy infants, a reduced diversity of nasal microbiota is associated with subsequent rhinitis and wheezing disorders, as well as allergen sensitization [19, 23, 24]. These studies suggest that colonization with a diverse array of microbes may be protective against inflammatory disorders of the upper and lower airways. The nasal microbiota promotes host resistance against infection with pathogens by (1) competing for sites of colonization and (2) direct production of inhibitory molecules and depletion of nutrients to prevent proliferation of pathogens. Table 2 summarizes 11 studies performed in both birth cohorts and cross-sectional studies which provide insights into the association between the infant nasal bacterial/viral microbiota succession/profiles. Their contribution to the subsequent development of respiratory disorders such as wheeze and rhinitis in childhood is also depicted in Figure 2.

There is evidence that *Corynebacterium spp.* is an important commensal for the development of healthy infant nasal microbiota. The Growing Up in Singapore toward Healthy Outcomes (GUSTO) birth cohort study showed that the overall abundance of *Corynebacterium spp.* found in the nasal microbiota was enriched in healthy infants compared to infants with rhinitis and/or wheeze in the first 18 months of life [19]. Moreover, *Corynebacterium spp.* in nasal epithelium of healthy infants has been shown to inhibit *S. aureus* colonization, which in turn reduced *S. aureus* infections [24]. Biesbroek and colleagues [12] also reported a lower incidence of upper respiratory tract infections in infants with a *Corynebacterium*-dominated nasal microbiome profile in early life.

Apart from *Corynebacterium spp.*, a high abundance of *S. epidermidis* is also associated with a reduced presence of potential pathogens such as *S. aureus* and *M. catarrhalis*. *S. epidermidis* was shown to exhibit high anti-

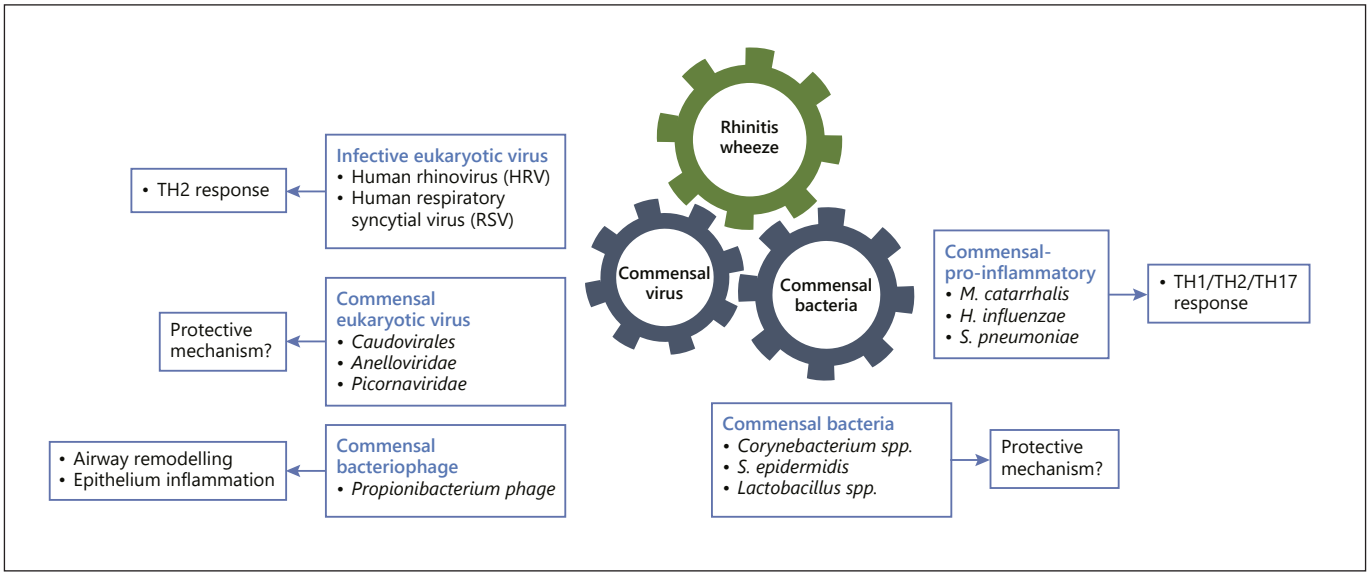

Fig. 2. Graphic representation of the potential role of upper airway bacteria, eukaryotic viruses, and bacteriophages in the pathogenesis of rhinitis and/or wheezing.

microbial peptide-inducing and biofilm-forming capacities which help to outcompete pathogenic bacteria during nasal colonization and has the ability to protect itself from epithelial antimicrobial peptides in vivo [25]. In addition, Rosas-Salazar et al. [26] found a lower abundance of *Lactobacillus* in the nasal microbiome of wheezing infants in the Infant Susceptibility to Pulmonary Infections and Asthma Following RSV Exposure (INSPIRE) longitudinal cohort. It could thus be postulated that the presence of *Lactobacillus* might be protective against development of viral-induced wheezing disorders.

On the other hand, early nasal establishment with pro-inflammatory bacteria species has been postulated to predispose infants to respiratory disorders such as rhinitis and wheezing. The *Proteobacteria* phylum, predominantly *Oxalobacteraceae* and *Moraxellaceae* genera, was found to be more abundant in infants with rhinitis and concomitant wheeze in the first 18 months of life (Singapore) and in dust mite-sensitized rhinitis and asthma infant at 3–5 years of age (Taiwan), respectively [19, 23].

In a culture-based study, Bisgaard and colleagues [27] observed an association between increased abundance of *M. catarrhalis* and *H. influenzae* (*Proteobacteria* phylum) and *S. pneumoniae* (*Firmicutes* phylum) in hypopharyngeal secretions of asymptomatic 1-month-old infants, and subsequent acute wheeze. Furthermore, Teo and colleagues [14] demonstrated with 16S rRNA sequencing that higher abundance of *Streptococcus spp*. in the nasal microbiome of 7–9-week-old infants was associated with an increased prevalence for chronic wheeze by 5 years of age. These earlier reports are consistent with findings of a Danish cohort of children born to mothers with asthma. The authors also found an association between colonization with *H. influenzae*, *S. pneumoniae*, *or M. catarrhalis* in the upper airways at 1 month and development of troublesome lung symptoms such as cough and wheezing within first year of life [28]. A more recent study involving the COAST birth cohort study reported that overrepresentation of these same bacteria, *H. influenzae*, *S. pneumoniae, or M. catarrhalis*, during wheezing illnesses in the first 2 years of life was associated with asthma in later childhood. Moreover, a *Staphylococcus*-dominant microbiome (predominance of *S. aureus*) in the first 6 months of life was associated with increased risk of recurrent wheezing by age 3 years [29]. In another study in Ecuadorian infants with noninfectious early-onset wheezing, there was a significantly higher abundance of *Neisseriaceae* and *Haemophilus* families in wheezing children at 10 months, compared to healthy subjects [30]. These longitudinal studies strongly support the notion that the early establishment of certain bacteria belonging to the *Proteobacteria* and *Firmicutes* phyla could predispose to subsequent development of rhinitis and wheeze disorders in early childhood.

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Table 2. Summary table of comparative longitudinal studies of infant nasal microbiome and the development of rhinitis and wheeze in childhood

Study design	Cohort size	Age range	Primary outcome	Study aim and setting	Sample type	Analysis methods	Clinical significance	Ref.
Cohort	234 infants	1st year	Chronic wheeze at 5–10 years of age	Elucidate the nasopharyngeal microbiome during respiratory health and illness, its longitudinal dynamics, susceptibility to exogenous factors, and association with future asthma	Naso-pharyngeal swabs	16S rRNA gene sequencing	Microbiome composition affects infection severity and pathogen spread to lower airways – *Haemophilus, Streptococcus,* and *Moraxella* in ARI infants and *Staphylococcus, Alloiococcus, and Corynebacterium* in healthy infants. Early asymptomatic colonization with *Streptococcus* increases risk of asthma	Teo et al. [14]
Case control	122 subjects	18 months	Prolonged rhinitis/wheeze at 18 months	To compare the development of nasal microbiota in infants with rhinitis and wheeze with healthy infants	Anterior nasal swabs	16S rRNA gene sequencing	Decrease in microbial diversity in children with rhinitis. Increased abundance of *Oxalobacteraceae* (*Proteobacteria*) and *Aerococcaceae* (*Firmicutes*) in rhinitis infants. Higher abundance of *Corynebacterium* in control subjects	Ta et al. [19]
Cohort	100 children hospitalized with diagnosed bronchiolitis	<2 years	Clinically diagnosed bronchiolitis at <2 years of age	Hypothesized that the *Proteobacteria* phylum would be associated with the viral etiology of the child's bronchiolitis (RSV, human rhinovirus, both) and with acute wheezing status (present/absent)	Naso-pharyngeal aspirate	RT-PCR for 16 viruses; then 16S rRNA gene pyrosequencing and whole genome shotgun sequencing	In children with severe bronchiolitis, *Proteobacteria* (*H. influenzae* and *M. catarrhalis*) was associated with viral bronchiolitis, and *Moraxella* species were more common among wheezing children. Increase in *H. influenzae* and *M. catarrhalis* discriminated between children with RSV/human rhinovirus coinfection and those children with only a single virus infection	Hyde et al. [22]
Case control	87 subjects (23 asthma, 23 rhinitis, and 32 control)	3–5 years	Allergic rhinitis/asthma	Evaluate relationship among airway microbiota, serum IgE, allergic sensitization, and relevance to rhinitis and asthma	Throat swab	16S rRNA gene sequencing	Healthy children have higher species diversity than rhinitis children. Higher abundance of *Haemophilus*, *Neisseria*, and *Moraxella* (*Proteobacteria*) are observed in rhinitis children	Chiu et al. [23]
Cohort	118 infants	First 2 years	At least 1 confirmed episode of RSV ARI in first 2 years	To examine if taxonomic composition, diversity, richness, and abundance of certain bacterial taxa of the upper airway microbiome during RSV ARI in infancy are associated with childhood wheezing illnesses	Nasopharyngeal aspirates	16S rRNA gene sequencing	Higher abundance of *Lactobacillus* during RSV ARI in nasopharyngeal region during infancy is associated with a reduced risk of childhood wheezing illnesses at 2 years	Rosas-Salazar et al. [26]
Cohort	361 children (bacteria) 299 children (viruses) 277 children (bacteria + viruses)	1–3 years	Wheezing episodes in the first 3 years	To study the association between wheezy symptoms in young children and the presence of bacteria in the airways	Hypopharyngeal aspirate for bacterial culture. Nasopharyngeal aspirate for virus identification	Bacterial culture and viral PCR	Acute wheezing was significantly associated with bacterial infections (*H. influenzae* and *M. catarrhalis*) but independent of virus infections	Bisgaard et al. [27]

Table 2 (continued)

Study design	Cohort size	Age range	Primary outcome	Study aim and setting	Sample type	Analysis methods	Clinical significance	Ref.
Cohort	242 subjects	First 1 year	At least 1 respiratory episode during the first year of life	To investigate if bacterial colonization in healthy neonates is associated with respiratory symptoms during the first year of life	Nasal swab (n = 218)	RT-PCR	Colonization with *H. influenzae, S. pneumoniae,* or *M. catarrhalis* in the upper airways in neonates is positively associated with coughing and wheezing episodes in infants. Higher presence of pathogenic bacterial colonization was present in children with older siblings, compared with those without	von Linstow et al. [28]
Cohort	285 subjects	First 2 years	Presence of asthma at 6, 8, 11, 13, and 18 years of age	To investigate if nasopharyngeal microbiome composition and developmental trajectories are related to risk in developing childhood asthma	Nasopharyngeal mucus samples	16S rRNA gene sequencing	4 different developmental trajectories dominated the first 4–6 months of life of infants. *Staphylococcus*-dominant infants were associated with higher wheezing frequency, increased allergen sensitization, and asthma risk in subsequent years of childhood	Tang et al. [29]
Case control	48 subjects (24 wheezing and 24 healthy)	10 months	Early-onset wheezing	To compare the airway microbiome in infants with noninfective wheeze and healthy controls and to relate their findings to surveys of the airway microbiome in European children	Oropharyngeal swabs	16S rRNA gene sequencing	The airway microbiota contains higher abundance of *Streptococci* than in European survey studies. Higher prevalence of pathogens (*Haemophilus* and *Staphylococcus* spp.) may contribute to wheezing illnesses in this age group. Lower abundance of "protective" bacteria (*Veillonella, Pasteurellaceae, and Gemella*) found in noninfectious wheezing infants	Cardenas et al. [30]
Case control	1,237 subjects	First 18 months	Allergic/ nonallergic rhinitis at 18 months	Evaluate rhinitis (allergic and nonallergic) in the first 18 months of life, its link with other atopic manifestations, and the role of respiratory viruses	Nasopharyngeal swabs	Multiplex RT-PCR, human rhinovirus subtyping	Prolonged rhinitis was significantly associated with parental atopic history and atopic comorbidities of eczema and wheeze, but not allergen sensitization. HRV-positive infants (with prolonged rhinitis) had a higher rate of wheeze compared with HRV-negative infants, though insignificant	Hardjojo et al. [43]
Cohort	140 infants	First 1 year	Wheezing at 4 years	To study the role of HRV during infancy in the development of lower respiratory disease	Nose and throat swabs	RT-PCR	Viral presence (significantly HRV) is associated with lower respiratory symptoms during infancy and higher presymptomatic respiratory system resistance. HRV presence at infancy is not associated with childhood wheezing, but wheeze during a HRV episode could predispose the development of childhood wheeze, due to a reduced neonatal lung function	van der Gugten et al. [45]

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Table 2 (continued)

Study design	Cohort size	Age range	Primary outcome	Study aim and setting	Sample type	Analysis methods	Clinical significance	Ref.
Case control	584 children	4.2–73.6 months	Presence of rhinitis symptoms at point of collection	To evaluate if rhinitis is associated with bacterial colonization and whether rhinitis-inducing respiratory viruses interact with bacteria in ways which promote transmission	Nasal swabs	Bacterial culture and RT-PCR	Significant, age-independent associations between rhinitis symptoms and *H. influenzae* prevalence and abundance were observed. Close quantitative relationship between respiratory viral infection (including picornavirus infection) and *S. pneumoniae* colonization	Rodrigues et al. [54]
Case control	Children hospitalized for ARI	<15 years of age	ARI based on clinically diagnosed pneumonia or asthma	To describe virome and bacteriome in the upper respiratory tract of hospitalized children with asthma and pneumonia during acute episodes	134 nasopharyngeal swabs	Whole genome sequencing (bacteriome and virome)	Virome: the asthma group was dominated by RV-C, BoV-1, and RSV-B, while the pneumonia group was dominated by bacteriophage EJ-1 and TTMV. High presence of RV-C found in asthmatic children. Bacteriome: *Moraxella catarrhalis, Propionibacterium acnes*, and *Acinetobacter* were present in asthma and *Veillonella parvula* and *Mycoplasma pneumoniae* in pneumonia. *Streptococcus pneumoniae* and *Haemophilus influenzae* concurrently in both groups	Romero-Espinoza et al. [57]
Case control	109 children from a subcohort of 4,407 children with ARTIs + 20 healthy controls	4 months–11 years	Frequency of diagnosed ARTIs over a 6-year period	Comparing respiratory virome and serum cytokine profiles of children with multiple ARTIs versus single ARTI over 6-years	Nasopharyngeal swabs and serum samples	Metagenomic sequencing/ cytokine profile measurement	Multiple ARTIs: (1) higher rates of pathogenic respiratory viruses with higher diversity and richness; (2) positive association with *Propionibacterium* phages; (3) elevated serum levels of TIMP-1 and PDGF-BB	Li et al. [58]
Case control	Subcohort of 34 children	4-5 years old	Mild-to-severe asthma by 4-5 years	To identify DNA and RNA virus species in the upper respiratory system of healthy and asthmatic children	Nasopharyngeal samples	Metagenomic sequencing	During asymptomatic periods, asthmatic children demonstrate bacteriophage deficiency and increased eukaryotic viral richness and diversity (dominantly anellovirus/picornavirus)	Megremis et al. [59]

ARI, acute respiratory infection; ARTI, acute respiratory tract infection; RSV, respiratory syncytial virus; HRV, human rhinovirus; PDGF-BB, platelet-derived growth factor subunit BB; TIMP-1, tissue inhibitor matrix metalloproteinase 1; RV-C, rhinovirus C; BoV-1, bocavirus 1.

Proinflammatory Commensals and the Development of Childhood Respiratory Disorders

Several mechanisms have been proposed to explain the links between early proinflammatory bacterial colonization, in particular *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, and the development of respiratory disorders. The survival of these commensals in the nasal mucosa has been attributed to their ability to form interspecies biofilms. Biofilm formation between *S. pneumoniae* and *H. influenzae* confers increased resistance to host antimicrobial agents (lysozyme, lactoferrin, and lipocalin), increased expression of cell surface appendages (type IV pili, lipopolysaccharide [LPS], or lipooligosaccharide) [31, 32], and the release of biomolecules such as extracellular DNA [33, 34], which further promotes the adhesion and cohesion properties of biofilm cells. Moreover, studies also showed that co-colonization with *M. catarrhalis* whose outer membrane vesicles contain phospholipids, adhesins, and immunomodulatory compounds, such as lipooligosaccharide, increases resistance of biofilms to antibiotics (amoxicillin) and host clearance [35]. Autoinducer-2 – an essential quorum signaling molecule for interbacterial communication – which is conserved among numerous bacterial species including *H. influenzae* enhances the establishment and maturation of polymicrobial biofilms containing these species [36].

Furthermore, colonization by these proinflammatory bacteria induces species-specific stimulation of the immune responses of the airway mucosa and increases inflammation accompanied by higher production and release of host antimicrobial peptides and proteins in the nasal epithelium via activation of Toll-like receptors (TLR). Lipoteichoic acids of *S. pneumoniae* are sensed by TLR-2 [37], whereas lipoproteins and LPS of *H. influenzae* are recognized by TLR-2 and TLR-4, respectively [38]; TLR-4 has also been reported to recognize the pore-forming toxin pneumolysin of *S. pneumoniae* [39]. To substantiate these in vitro findings, the Copenhagen Prospective Study of Asthma in Childhood 2010 birth cohort demonstrated that the upper airway lining fluid of neonates colonized with *M. catarrhalis*, *H. influenzae*, and *S. pneumoniae* had higher levels of innate/TH1/TH2/TH17-related cytokines and chemokines compared to noncolonized neonates [40]. Infants who had proinflammatory responses induced by these bacteria were subsequently shown to be at higher risk of asthma at the age of 7 years. The investigators also showed that peripheral blood mononuclear cells collected from these children at age 6 months induced aberrant production of TH2 and TH17 cytokines in response to *H influenza*, *M catarrhalis*, and *S pneumoniae* compared to nonasthma controls [41]. These studies suggest that early colonization with these proinflammatory bacteria could influence type 2 chronic inflammation and subsequent asthma.

Nasal Virome

The respiratory virome is also an integral component of the nasal microbiome, encompassing eukaryotic and prokaryotic (bacteriophages) viruses which are found in the upper respiratory tract and within the lung. However, little is known about the role of bacteriophages and their associations with eukaryotic viruses in respiratory health. Moreover, there is still a lack of mechanistic data to provide insights into the physiological role of commensal viruses in respiratory homeostasis.

The role of infectious eukaryotic viruses during episodes of acute respiratory tract infections (ARTIs) in young children and their impact on subsequent development of wheezing disorders are well documented. Human rhinovirus (HRV) and human respiratory syncytial virus (RSV) are major viral pathogens that trigger rhinitis and wheezing disorders in infants and are also risk factors for subsequent recurrent wheeze disorders in later childhood [27, 42–45]. These viruses incite lung damage through various immunological pathways such as the activation of proinflammatory mediators (such as TNF-α, interleukins, and chemokines) as well as activation of leukocytes at the infection site, resulting in lung function impairment, persistent bronchial hyperreactivity, airway inflammation, and promoting Th2 sensitization [46]. Moreover, children with concomitant allergen sensitization and skewed Th2-mediated responses were shown to have impaired type I and type III interferons leading to increased susceptibility to RSV and rhinovirus infections and recurrent wheezing [47, 48].

Respiratory viruses such as HRV and RSV can trigger an outgrowth, rather than a new acquisition of *S. pneumoniae* and *M. catarrhalis* from the resident microbial community [49], as well as enhance their adherence to the respiratory epithelium by upregulating the expression of bacterial adhesion molecules such as fibronectin and platelet-activating factor receptor [50], which in turn facilitate secondary bacterial infection [51], and depress innate immune responses such as neutrophil and natural killer cell recruitment [52]. HRV was also shown to induce impairment on epithelial proliferation and promote airway remodeling [53].

Far less is known about the commensal virome, particularly its succession from birth, and its role in modulating respiratory health. Several technical challenges exist in studying the nasal virome. The low biomass and subopti-

mal viral nucleic acid extraction technology currently available limits viral detection capabilities. The odds of detecting viral agents in a study of infants and young children up to 5 years old decreased significantly (up to 70%) with increasing age [54], which might be due to fewer viral infections in older children. For these reasons, untargeted metagenomic sequencing for the identification of commensal viruses in nasal specimens has not been systematically evaluated. Furthermore, knowledge of bacteriophages has lagged substantially behind that of bacteria and other members of the microbiome due to technical difficulties in the isolation of bacteriophages and limited reference databases for phage genomes [55]. Nevertheless, the improvement of molecular detection techniques, including standardization, is already and will continue to facilitate the field [56].

A recent metagenomic sequencing study of the nasal virome in hospitalized asthmatic children below 15 years of age revealed that rhinovirus C, bocavirus 1, RSV-B, and parvovirus B19 were commonly detected, and bacteriophages such as *Propionibacterium* phage, *Staphylococcus* phage, and *Streptococcus* phage were also present, albeit with lower read counts [57]. Another study reported a significant elevation of relative abundance of *Propionibacterium* phages in children with multiple ARTIs compared to those with isolated ARTIs. This relative increase in abundance of these phages was associated with higher levels of serum tissue inhibitor matrix metalloproteinase 1 and platelet-derived growth factor subunit BB, which are involved in airway remodeling and epithelial inflammation conferring susceptibility to common respiratory viral infections or colonization by pathogenic bacteria [58]. These studies shed light on the possible role of bacteriophages on the upper respiratory tract and lung infections in children.

To the best of our knowledge, there is only 1 preprint study evaluating the nasal virome in asymptomatic children [59]. This study evaluated the nasal virome in European preschool children with stable asthma and who were symptom-free for at least 1 month. They showed a relative bacteriophage deficiency (richness and diversity) and increased eukaryotic viral presence, predominantly *Anelloviruses,* compared to healthy controls, indicating that children with asthma have a characteristically dysbiotic virome. The core eukaryotic virome in both groups consisted of *Caudovirales*, *Anelloviridae*, and *Picornaviridae* families. However, as this study was conducted in children with known asthma, it is not known if the differences observed in the respiratory virome during stable asthma episodes might be attributed to the disease itself or its treatment. Despite its small sample size, this study suggests that maintaining a stable respiratory core virome comprising eukaryote viruses and bacteriophages could be functionally important for the maintenance of a "healthy" equilibrium and could confer protection against asthma symptoms. Bacteriophages have been suggested to play an essential role in regulating bacterial populations within the respiratory tract that would in turn prime and modulate host immune responses in children [60–62].

Conclusion

This review provides a broad overview of the current body of evidence relating to the human upper respiratory microbiota and virome and its influence on rhinitis and wheezing disorders in children. There are substantial data showing that there are distinct differences in nasal microbiota composition in children with respiratory disorders and that colonization patterns in early life appear to impact development of disease in later life. There is also now emerging evidence that commensal respiratory virome, including bacteriophages, also play a role in childhood respiratory infections and asthma, but advances in viral detection technology are needed to improve our understanding of the host-virome interactions in disease pathogenesis. Understanding the influence of the commensal microbiota and virome on the integrity of the respiratory tract and mucosal immune responses is an important step toward the implementation of strategies to reduce susceptibility to these disorders.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this paper. C.J.X. Tay, L.D.H. Ta, Y.X.O. Yeong, and G.C. Yap prepared the manuscript. J.J.H. Chu, B.W. Lee, and E.H. Tham reviewed this paper. All authors reviewed and approved the final draft for publication.

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