Review Article

RESPIRATORY INFECTIONS AND THEIR EFFECT ON THE PAEDIATRIC LUNG MICROBIOME

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ABSTRACT
Respiratory tract infections, especially viral infections in children are a major cause of morbidity and mortality in many developing countries. The effect of infection and its potential sequelae are a consequence of the interaction of the lung microbiome, the pathogen and the immune system. The epidemiology of viral infections and *Bordetella pertussis* observed over a two-year period in a private-sector laboratory is presented. We then describe the paediatric respiratory microbiome on a basic level and briefly describe the effect of some of these pathogens on the developing paediatric microbiota.

INTRODUCTION
The human microbiome refers to the microbiota (all microorganisms including viruses, bacteria and fungi) existing in the human body and the habitat they colonise.[1] Microbiomes, be they in the lung or gut, are very complex with respect to the types and quantities of microbe present. Sequencing techniques have revealed that the lung microbiome is a diverse system that varies from the anterior nares to the distal airways with different combinations of diverse species. These differences may be due to genetics, environmental factors, anatomical factors, mucosal characteristics, immunity and microbe–microbe interaction. Emerging evidence suggests that the microbiome shapes the immune response therefore influencing the balance between health and disease.[2] Acute infections, especially viral, that disrupt the established microbiome may contribute to the development of bacterial pneumonia or asthma.[2, 3]

Viral infections account for 45–75% of childhood community-acquired pneumonias. These infections are responsible for significant morbidity and mortality in children under the age of five years and are a leading cause of paediatric hospital admissions, particularly in developing countries.[4] The number of recognised viruses causing clinically significant respiratory tract infections has increased due to improvements in molecular testing. Viruses associated with respiratory tract infections include influenza viruses A and B, parainfluenza viruses (PIV) 1 to 4, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), adenovirus, human coronaviruses (hCoV), rhinovirus, enteroviruses and human bocavirus (hBoV).[5] Testing a respiratory specimen with a multiplex **polymerase chain-reaction** (**PCR**) panel improves the likelihood of identifying the cause of a lower respiratory tract infection. Such testing:

- guides treatment decisions;
- prevents unnecessary investigations or procedures;
- minimises the unnecessary use of antibiotics;
- facilitates infection control, and
- provides knowledge of seasonality that influences healthcare planning.[6]

However, molecular testing has also brought about various dilemmas in the interpretation of results. These include ascertaining the dominant pathogen in mixed viral or viral–bacterial infections, the effect on the lung microbiome for future health and interactions with the host response.[3]

In the South African private healthcare sector, molecular diagnostics have been increasingly used for the investigation of community-acquired pneumonia. Respiratory specimens, usually nasopharyngeal swabs/aspirates, are submitted for testing on various panels that consist of common viruses and bacteria such as *Bordetella pertussis.*

STATISTICS
Data from 54 834 respiratory samples sent to a South African laboratory operating in the private healthcare sector were collated over a two-year period to ascertain the epidemiology of respiratory viruses and *Bordetella pertussis*. Specimen numbers indicate an increase in testing during March which correlated with the peak RSV season. Further peaks in specimen numbers occurred during the winter months correlating with the influenza season. Fifty-five per cent of specimens were from children under the age of five years. Multiple viruses were detected in 17% of specimens.

RESPIRATORY SYNCYTIAL VIRUS
RSV is associated with bronchiolitis, pneumonia and an increased risk of developing asthma.[7] RSV was detected

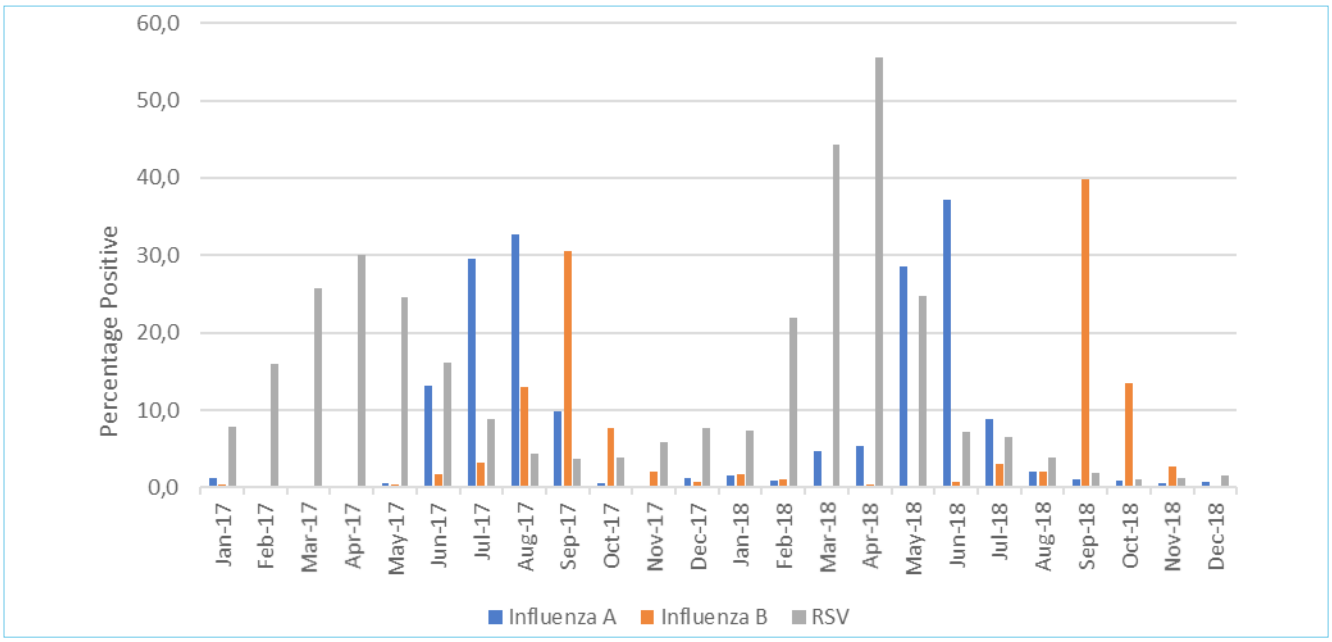

Figure 1: Influenza A, influenza B and RSV cases for 2017–2018

throughout the reported period, but the peak season was from February to May, preceding the influenza season (see Figure 1). During these peak months, RSV was detected in 16–55% of samples submitted for testing.

INFLUENZA A AND B

Influenza A and B cause seasonal epidemics that occur during the winter months (see Figure 1). Influenza A typically precedes influenza B. The influenza B season often extends into springtime. During the influenza season, influenza was detected in 14–46% of samples. Sporadic cases occur throughout the rest of the year and may be associated with travel to the northern hemisphere.

RHINOVIRUS

Rhinovirus causes upper respiratory tract infections, otitis media, asthma exacerbations and pneumonia.[5,8] Rhinovirus was the most prevalent virus, detected throughout the year in 10–33% of specimens.

ADENOVIRUS

Adenovirus infections are associated with pharyngitis, otitis media, bronchiolitis and pneumonia.[8,9] Adenovirus was detected throughout the year in 10–16% of specimens, making it the third most commonly detected virus after rhinovirus and RSV.

ENTEROVIRUS

Enterovirus infections may result in upper respiratory tract infections, bronchiolitis and pneumonia.[10] Enterovirus infections were detected throughout the year in 2–14% of specimens. Detection was above 10% in February–March 2017, September 2017 and November–December 2018.

PARAINFLUENZA VIRUSES

PIV types 1–4 were detected throughout the year with intermittent peaks (see Figure 2). PIV-3 was responsible for 54.8% of PIV infections over the two-year period. PIV-3 is more commonly associated with pneumonia than the other types.[5] A majority of patients represented in the described data were hospitalised and more likely to have lower respiratory tract infections, which may account for the predominance of PIV-3.

HUMAN METAPNEUMOVIRUS

hMPV is responsible for upper respiratory tract infections, bronchiolitis, pneumonia and exacerbation of asthma.[5] hMPV was detected in 1–7% of specimens on a monthly basis. There was a marked peak in April 2018 when it was detected in 18% of specimens.

HUMAN CORONAVIRUSES

HCoV-229E, hCoV-HKU1, hCoV-NL63 and hCoV-OC43 are associated with upper respiratory tract infections, although they may cause pneumonia in children.[5] HCoV were detected in 1–13% of specimens monthly. Seasonal peaks occur during the winter months.

BORDETELLA PERTUSSIS

Pertussis (whooping cough) is a respiratory infection caused by the bacterium *Bordetella pertussis*. It is an endemic infection that occurs in periodic cycles of 3–5 years. Immunity follows either vaccination or infection, but is not lifelong. Patients at risk include the unimmunised or partially immunised comprising mostly infants, those with impaired immune systems and those with chronic lung diseases. The most severe infections occur in infants, which lead to multiple pathogenic changes in the lower respiratory tract.[11,12,13]

In 2017, 62% of positive results were from children <5 years old with 53% <1 year old. Adults accounted for 9% of cases. In 2018, 67% of positive results were from children <5 years old with 69% <1 year old. Adults accounted for 23% of cases.

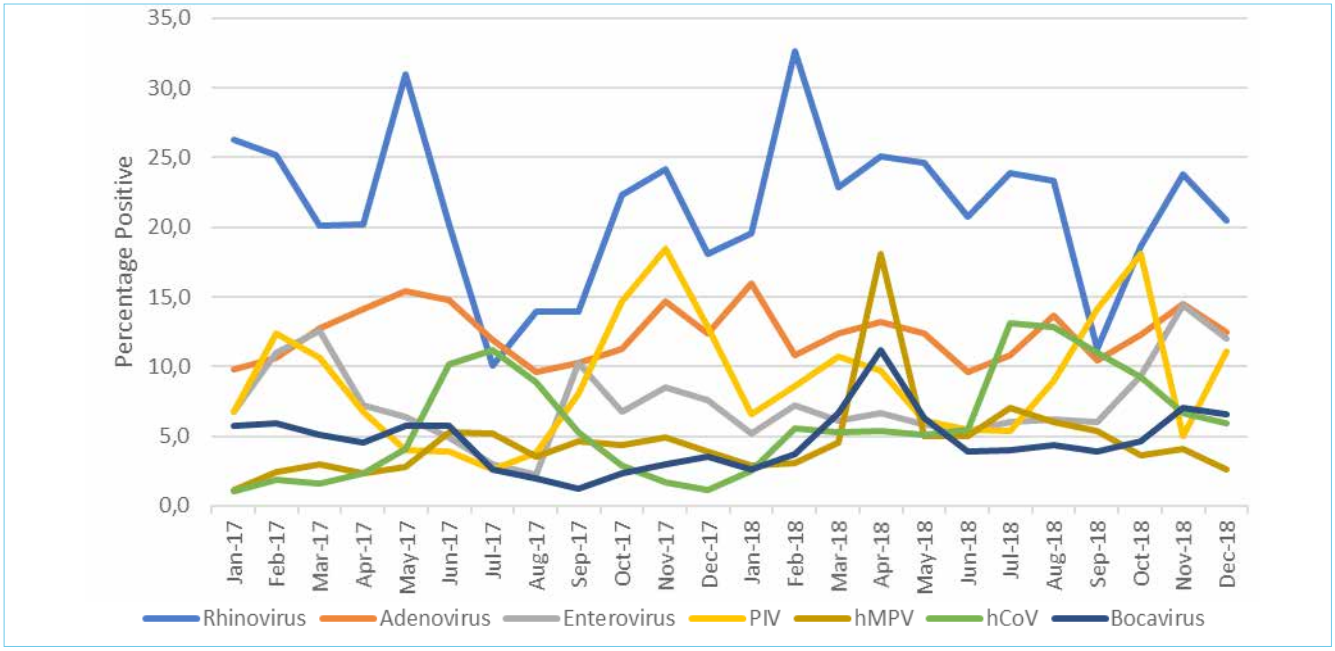

Figure 2: hCoV, PIV, rhinovirus, adenovirus, enterovirus, bocavirus and hMPV cases for 2017–2018

The number of requests for *Bordetella pertussis* testing between 2017 to 2018 increased markedly (1 631 specimens submitted in 2017 vs 8 853 specimens submitted in 2018). The percentage of specimens testing positive in 2017 and 2018 was 2.8% and 2.4%, respectively. This suggests that the increase in reported cases is due to an increase in testing rather than an increase in the number of infections as the detection rates were similar. However, in August of 2018, the National Institute of Communicable Diseases (NICD) reported a generalised increase in the number of diagnosed cases in South Africa from January 2018.[14] Our data suggest that the increase in diagnosed cases is due to an increased awareness with a subsequent increase in testing.

INFLUENCE OF INFECTIONS ON THE PAEDIATRIC LUNG MICROBIOME

The symbiotic relationship between the immune system and microbiome allows for appropriate and timely responses to infection. For an infection to occur in a site with an established microbiome, either displacement and/or elimination of the healthy state and changes in immunity are needed. Infection can occur either with a commensal (member of the microbiota) or an external pathogen. Any disturbance or stress on the system can cause an imbalance (dysbiosis) that impacts on overall health. Acute mucosal infections are characterised by dysbiosis associated with significant shifts in the microbiota leading to enhanced invasive properties and increased inflammation with tissue damage.

The microbiome seen later in life is shaped primarily by its make-up and dynamics during infancy. Newborns usually acquire a microbiota resembling their mother's and this is dependent on the mode of delivery. Infants delivered by caesarean section are usually colonised with skin flora such as *Staphylococcus*, *Streptococcus* and *Corynebacterium* species, whereas those delivered vaginally acquire *Lactobacillus* or *Prevotella* species. Over the first six months of life this changes with a gradual decline of *Streptococcus, Corynebacterium* and *Staphylococcus* to the dominance of *Pneumococcus, Haemophilus* and *Moraxella* species.[15,16,17,20] The stages of development, for example, birth to infancy and factors such as choice of feeds, that is, breastmilk or formula, all influence the spectrum of microbiota that develop to maintain respiratory health. A longitudinal study of more than 200 infants showed that breastfed babies had an abundance of gram-positive bacteria such as *Corynebacterium* and lower numbers of *Staphylococcus* species in comparison to those who were formula-fed at six weeks. These changes in the microbial patterns were associated with lowered susceptibility to infections and development of wheeze in breastfed infants.[20]

Nasopharyngeal colonisation and its subsequent microbiome has been touted as one of the determinants of infection severity, spread to lower airways and chronic sequelae. Multiple studies have shown that microbial profiles in early airway colonisation with high abundance of species such as *Pneumococcus* and *Haemophilus* species predispose infants to acute severe respiratory tract infections such as bronchiolitis and pneumonia compared to those with more 'stable' profiles or other species.[16,17] The microbial dynamics associated with stable profiles have been described as the early presence in large numbers of *Corynebacterium*/*Dolosigranulum* followed by *Moraxella* species.

Viral infections may impair innate immune responses and mechanical mechanisms that clear bacteria in the lung through dysregulation of both alveolar macrophages and neutrophils. The degree of mucous production, reduction in mucociliary clearance and impairment of cellular repair impacts on the subsequent dysbiosis in the various microbial loads. This is a possible explanation as to why infection severity, co-infection with viruses and bacteria and development of chronic respiratory sequelae differ from individual to individual.[2,11,15]

PATHOGENS AND THEIR EFFECT ON THE LUNG MICROBIOME

In the respiratory tract, viruses can either cause primary infections, predispose to superinfection by bacteria or co-infect with bacteria.

RSV

Certain viruses such as RSV have been studied quite extensively to determine the factors determining acute infection, disease progression and long-term sequelae such as wheeze and asthma. Changes in the microbiome composition were studied in a cohort with no, mild, moderate and severe RSV infection, respectively. Hospitalisation and an exaggerated immune response were noted in the presence of higher predominance of *H influenzae* and *Streptococcus* species at the time of infection. The severity of infection is increased with bacterial co-infections such as *Moraxella* species. In addition, numerous studies have described the association between *Moraxella* respiratory tract colonisation and an increased severity of RSV infection, with RSV being reciprocally capable of altering the respiratory microbiome of the host.[7,15,16,18,20] Consequences include changes in the abundance of various species increasing predisposition to future infection and co-infections with other viruses such as hMPV and rhinovirus.[21]

BORDETELLA PERTUSSIS

B pertussis is a human-specific pathogen that utilises multiple virulence factors, for example, pertussis toxin, fimbriae and secretion systems, working in synergy to produce infection after adherence to the mucosal epithelium.

In vitro mouse studies have shown that nasal-cavity colonisation by this organism can be inhibited by certain murine microbiota, for example, *B bronchiseptica, Klebsiella* and *Staphylococcus* species. Conversely, the descent of *Bordetella pertussis* from the upper respiratory tract, where it colonises the nasopharynx, to the lower respiratory tract is potentially facilitated by microbiota such as *S mitis, S oralis, N subflava* or *Corynebacterium* species. The underlying mechanisms of these organism interactions, which include various virulence factors such as secretion systems, have not yet been well established. This study suggests that the organism has mechanisms to outcompete other species within its ecological niche, but not at the point of colonisation. Given that human upper respiratory tract microbiota is similar to that found in mice, manipulation of the nasal flora might prevent successful *B pertussis* colonisation and establishment of lower respiratory tract infection.[11,17,19]

Future research directed at interventions aimed at either mechanisms of maintenance or improvements to the 'healthy' respiratory microbiome are being studied. The ecological diversity of the microbiome based on age, nutrition and geographic location makes it difficult to establish a 'one size fits all' concept to prevent or treat infection. A necessary first step is the identification of the core processes necessary for host–microbe symbiosis. Delineating what the 'healthy' microbiota looks like and the impact of modifiable factors such as antibiotic use could provide a baseline. Current sequencing and non-culture-based microbiological techniques could further define which interactions or microbial relationships to target. These could possibly lead to new strategies, adjuvant therapies and vaccines to decrease morbidity and mortality with early intervention.

GLOSSARY

Microbiota: microorganisms inhabiting a specific niche or site
Microbiome: symbiosis between the human host, its resident microbes and its interactions

DECLARATION OF CONFLICT OF INTEREST

Dr Seetharam received a travel sponsorship to a congress (MSD). The authors declare no additional conflict of interests.

This article has been peer reviewed.

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