**Title: The Nasopharyngeal Microbiome**

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**Abstract (250)**

Human microbiomes have received increasing attention over the last 10 years, leading to a pervasiveness of hypotheses relating dysbiosis to health and disease. The respiratory tract has received much less attention in this respect than that of, for example, the human gut. Nevertheless, progress has been made in elucidating the immunological, ecological and environmental drivers that govern these microbial consortia and the potential consequences of aberrant microbiomes. In this review, we consider the microbiome of the nasopharynx, a specific niche of the upper respiratory tract. The nasopharynx is an important site, both anatomically with respect to its gateway position between upper and lower airways, but also for pathogenic bacterial colonisation. The dynamics of the latter are important for long-term respiratory morbidity, acute infections of both invasive and non-invasive disease and associations with chronic airways disease exacerbations. Here we review the development of the nasopharyngeal microbiome over the life course, examining it from the early establishment of resilient profiles in neonates through to perturbations associated with pneumonia risk in the elderly. We focus specifically on the commensal, opportunistically pathogenic members of the nasopharyngeal microbiome that includes *Streptococcus* *pneumoniae*, *Staphylococcus* *aureus*, *Haemophilus* *influenzae* and *Moraxella* *catarrhalis*. In addition, we consider the role of relatively harmless genera such as *Dolosigranulum* and *Corynebacterium*. Understanding that the nasopharyngeal microbiome plays such a key, beneficial role in maintaining equilibrium of commensal species, prevention of pathogen outgrowth and host immunity enables future research to be directed appropriately.

**Summary**

* Composition of the nasopharyngeal microbiome is dynamic through the life course, with changes in part explaining resilience to respiratory disease.
* Cross-kingdom and inter-bacterial species interactions largely govern colonisation by pathogens and progression to disease.
* Future research should be directed at determining the combined roles of host immunity, mycobiome and virome in aberrant respiratory microbiomes.

**Abbreviations**

NP – nasopharyngeal; URT – upper respiratory tract; COPD – chronic obstructive pulmonary disease; MRSA – meticillin resistant *Staphylococcus aureus*; NTHi – non-typeable *Haemophilus influenzae*;RSV – respiratory syncytial virus; HRV –human rhinovirus; LRTI – lower respiratory tract infection; URTI – upper respiratory tract infection; AOM – acute otitis media; LPS – lipopolysaccharide; CF- cystic fibrosis

**Introduction**

Humans play host to both a staggering number and variety of microorganisms with whom we have co-evolved over the millennia. The study of these microbiomes, being the communities of bacteria, archaea, viruses and fungi found on or within our bodies, has expanded considerably over the last decade. This expansion has been driven mainly as a consequence of the developments in ‘omics technology, the growing availability of data and the computational approaches to analysis. The Human Microbiome Project revealed an extraordinary inter- and intra-individual diversity, linked to body site ([1](#_ENREF_1)). Crucially the functional capacity of these microbiomes was robust to differences found between individuals ([1](#_ENREF_1)). Since then countless studies have examined the role of specific microbiomes in health and disease. These include anatomically local associations, such as the role of gut microbiomes in inflammatory bowel disease ([2](#_ENREF_2)), obesity and diabetes ([3](#_ENREF_3)), or more distally through the gut-brain axis where co-morbidities of gut dysbiosis have been linked to cognitive decline and Alzheimer’s ([4](#_ENREF_4)), multiple sclerosis ([5](#_ENREF_5)) and depression ([6](#_ENREF_6)). The definition of a ‘healthy’ microbiome is one that still escapes definition but the supposition that dysbiosis has a profound impact on our health remains ([7](#_ENREF_7)). From an infectious disease perspective, it has also become clear that infection being a consequence of simply an interaction between susceptible host and pathogen is no longer the extant paradigm. Here the role of the larger, complex network of interactions that is greatly influenced by the host commensal microbiota has begun to be understood ([8](#_ENREF_8)). Whilst the gut microbiome has received the most substantive focus to date ([9](#_ENREF_9), [10](#_ENREF_10)), clear aetio-pathogenic interplay within other body sites has been documented ([11-13](#_ENREF_11)).

In this context the upper respiratory tract (URT) represents a multi-species and dynamic ecological environment that in contrast to the human gut has received only modest attention. Here we consider the microbiome of an important niche within the URT, the nasopharynx, focussing on the bacterial members of this consortium. First we examine the nasopharynx and place the dominant microbiota within the context of that present in other regions of the URT. Then we will review how the nasopharyngeal (NP) microbiome arises from early colonisation and the implications of this for long-term health and disease risk. We summarise some of the direct and indirect interactions that occur between members of the microbiome and/or external selective pressures such as vaccination. Finally the viromes and mycobiome are considered in the context of interactions with the bacterial consortia of the microbiome.

**The Nasopharynx and Surrounding Anatomy**

The nasopharynx is located above the soft palate and oropharynx at the back of the nasal cavity, effectively between the nose and throat and acts as a conduit between the upper and lower airways (Figure 1). Important anatomical features within this region include the adenoids and the Eustachian tube connecting the middle ear to the pharynx. The environment of the nasopharynx is distinct from that of the nasal cavity that, from a respiratory perspective, is the area of first contact between our bodies and external environment. The nasal cavity consists of the vestibule and anterior nares (the opening of the cavity) and the respiratory and olfactory regions. Here a number of key physiological and respiratory effects occur including the humidification, warming or cooling of incoming air and the entrapment of particulates by short coarse hairs known as vibrissae ([14](#_ENREF_14)). In addition, the nasal mucosa within the respiratory region is lined with pseudostratified columnar epithelium overlaid with mucus generated by interspersed goblet cells. This is in contrast to the anterior nares that are lined with skin-like keratinised squamous epithelia, containing sebaceous and serous glands, and the oropharynx where non-keratinised stratified squamous epithelium is found ([14](#_ENREF_14)). The mucus of the respiratory epithelium, of the nasal cavity and nasopharynx, consists of an aqueous layer, within which sit the cilia protruding from the mucosa, and a superior, highly proteinaceous layer made up of secreted antibodies such as IgA and various antimicrobials e.g. lysozyme ([14](#_ENREF_14)). The role of the mucus and ciliated cells is to trap particulates, including incoming bacteria, and move this material to the pharynx where it is swallowed ([15](#_ENREF_15)). This environment, rich in oxygen and blood supply, and warm at 34oC relative to the nasal cavity, thus provides a fertile niche to a wide variety of microorganisms.

**Nasopharyngeal Microbiome through the Life Course**

Microbiomes are governed by the same ecological principles that organise, regulate and structure other environments - categorised as random or selection driven, these include dispersal, selection, speciation and drift ([16](#_ENREF_16)). In practise this means that we can examine the development of the NP microbiome through the lens of these processes and begin with early colonisation.

Whilst the evidence that any form of *in utero* colonisation occurs is controversial ([17](#_ENREF_17)), it is clear that neonates rapidly acquire a microbiome that is in part determined by mode of delivery i.e. caesarean versus vaginal ([18](#_ENREF_18)). Whether these differences persist long term is questionable ([19](#_ENREF_19)), however there is evidence that the mode of delivery is associated with long-term respiratory co-morbidities ([20](#_ENREF_20), [21](#_ENREF_21)), and that this is potentially a consequence of a failure to quickly acquire a resilient respiratory microbiota ([22](#_ENREF_22)). Selection occurs as a consequence of comparative fitness and interactions between early colonisers, particularly as the host’s immune system is too immature at this stage to mount a selective response ([23](#_ENREF_23)). Environmental exposure is also an important factor with siblings and, as the child develops, greater social contact through nursery environments, being predicators of carriage for specific bacterial species ([24](#_ENREF_24)). Perturbations as a consequence of antibiotic prescription and exposure to smoking also significantly impact the composition of early microbiomes ([25](#_ENREF_25), [26](#_ENREF_26)).

In infants, NP microbiomes are characterised by high diversity, a rank abundance of the phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria and Fusobacteria, and common genera being *Moraxella*, *Haemophilus*, *Streptococcus*, *Dolosigranulum*, *Corynebacterium* and *Neisseria* ([27](#_ENREF_27)) (Figure 2). In the first two years of life distinct microbiome profiles can be observed ([28](#_ENREF_28)). These are based on the presence, or combinations of, 10 bacterial species which can be used as taxonomic biomarkers for each profile ([28](#_ENREF_28)). Whilst this structuring appears robust, it should be remembered that early-life NP profiles are not fixed and change as an infant develops. For example, at 1.5 months old, children could be classified as belonging to *Streptococcus* dominated, *Moraxella*, *Staphylococcus*, and *Corynebacterium* or *Corynebacterium*-*Dolosigranulum* profiles. Interestingly the latter profile is associated with breastfeeding and lower reported incidences of URT infection (URTI) ([28](#_ENREF_28)). Progression through to two years of age is marked by the disappearance of the *Staphylococcus* profile, which is unsurprising given the previously noted decreases in carriage through infancy ([29](#_ENREF_29)), and may reflect the impact of increased *Streptococcus* and *Haemophilus* presence which are known to affect *Staphylococcus* colonisation of the NP, as will be discussed later. In addition to the reduction in *Staphylococcus* a transition of the *Corynebacterium*-*Dolosigranulum* to a *Moraxella* dominated-*Dolosigranulum* profile and the appearance of *Haemophilus* have also been described ([28](#_ENREF_28)). It is interesting that the species that dominates the profile can have a significant effect on the overall stability of the NP microbiome ([28](#_ENREF_28)). *Moraxella*-dominated profiles, whether they were the primary profile for an infant or were transitioned into, are more stable over the course of the first two years of life ([28](#_ENREF_28)). This is in spite of the fact that early-life NP microbiomes are also the most vulnerable to alteration ([28](#_ENREF_28)).

In contrast, adult NP microbiomes have been shown to be of greater microbiota diversity, yet with an overall lower abundance ([30](#_ENREF_30)). Observed genera, whilst *Staphylococcus*, *Haemophilus* and *Streptococcus* are present*,* also include *Sphingobacterium*, *Prevotella*, *Bifidobacterium*, *Rothia* and *Propionibacterium* but not *Moraxella* nor *Corynebacterium* ([30](#_ENREF_30)). Community structuring in adults, at least for nasal microbiomes, is evident and distinct ‘types’ can be determined based on the dominance of particular species and/or genera ([31](#_ENREF_31)). For the nasopharynx, five ‘types’ have been noted previously with the presence of *Corynebacterium*, *Dolosigranulum* and *Staphylococcus* of note; the latter being an interesting contrast to the nasal communities ([32](#_ENREF_32)). In contrast to children the overall differences in NP microbiota can be distilled down to an increased abundance of Actinobacteria and a reduction of Proteobacteria ([32](#_ENREF_32)).

Of the many effects of ageing, perhaps two of the most important with respect to our understanding of microbiomes are waning immunity and increased inflammation ([33](#_ENREF_33)). Altered homeostasis with commensal microbiota impacts mucosal and systemic immunity through the generation of chronic pro-inflammatory responses ([34](#_ENREF_34), [35](#_ENREF_35)). In the respiratory tract this is manifest by a more disordered microbiome ([36](#_ENREF_36)), which has been correlated with lower respiratory tract infections (LRTI) ([37](#_ENREF_37)), and in combination with immunosenescence may explain why the elderly are so much more susceptible to disease ([33](#_ENREF_33), [38](#_ENREF_38)). The directionality of these interactions as yet is not fully understood however. Interestingly colonisation of the elderly URT with pathogens capable of causing pneumonia or more invasive disease, for example *Streptococcus pneumoniae*, is low ([39](#_ENREF_39)). Certainly in mouse models the composition of the NP microbiome in aged mice is more susceptible to colonisation with *S. pneumoniae* that in turn reduces the efficiency of clearance ([40](#_ENREF_40)). However this carriage dynamic in itself is not too dissimilar to that found for adults in general, being also less frequent than in children ([41](#_ENREF_41)). Nor does it suggest that NP microbiome is the key contributor as the most diverse adult profiles are the least likely in which to find *S. pneumoniae* ([42](#_ENREF_42)). Whilst NP carriage is low in the elderly, contrasting evidence for higher carriage levels when saliva was examined does exist ([43](#_ENREF_43)) – perhaps this shift in carriage accounts for the higher disease in this population. Regardless, disease in the elderly is likely multi-factorial being a combination of a microbiome less resistant to incoming pathogens and impaired immunity ([44](#_ENREF_44)).

**Bacterial Pathogens that Colonise the Nasopharynx**

Exhaustive introductions to each of the human pathogens that can be found resident in the NP microbiome are beyond the scope of this review and have been covered elsewhere ([45-48](#_ENREF_45)). Nevertheless it is useful to identify those which are of most concern from the perspective of infectious disease surveillance and control.

*S. pneumoniae*, the pneumococcus, is a Gram-positive diplococci and a leading cause of childhood mortality, particularly in the developing world ([49](#_ENREF_49)). It is characterised by the heterogeneity of its polysaccharide capsule, of which there are in excess of 97 types as defined by antisera cross-reactivity ([50](#_ENREF_50)). Carriage, which can be anything from 10 to 90%, peaks in young children <5 years of age ([51-58](#_ENREF_51)) where disease burden, in addition to that in the very old ([33](#_ENREF_33)), is highest. Although colonisation is a prerequisite for disease, it is not predictive ([59](#_ENREF_59)). The introduction of polysaccharide conjugate vaccines (PCV), a seven valent (Prevenar7™) and, subsequently, 13-valent (Prevenar13™) formulation, has substantially reduced invasive pneumococcal disease ([60](#_ENREF_60), [61](#_ENREF_61)). Carriage however has remained at pre-vaccine levels with serotype replacement dictating pneumococcal epidemiology and disease ([58](#_ENREF_58), [62-65](#_ENREF_62)).

The Gram-negative nasopharyngeal commensal *H. influenzae* is one of twelve recognized species of the genus *Haemophilus* ([66](#_ENREF_66)). Serotype b, one of six capsulated varieties and once a substantial burden in causing severe invasive infections such as meningitis and septicemia, was largely ameliorated by the introduction of *H. influenzae* type b (Hib) conjugate vaccines in the 1980s/1990s ([67](#_ENREF_67)). Carriage, and as a consequence disease, although to a much reduced extent, is now dominated by the Non-typeable form – NTHi. In the European Union/European Economic Area, NTHi accounted for 78% of the 8,781 cases of invasive disease between 2007 and 2014, with the burden highest in infants and those ≥60 years of age ([68](#_ENREF_68)).In addition to invasive disease, NTHi is also responsible for some of the most common causes of acute, mucosal or chronic infection. Acute otitis media (AOM) for example affects over 10% of the global population, mostly in the under 5s, with NTHi accounting for over 60% of cases ([69](#_ENREF_69)). It is also implicated in exacerbations of chronic lung disease in between 13 and 50% of cases ([70](#_ENREF_70)).

*Moraxella catarrhalis* is a Gram-negative, exclusively human commensal and pathogen of the URT ([45](#_ENREF_45)). The role of this bacterium in disease went unnoticed until the last forty years and was generally thought of as harmless. Now however the important role of *M. catarrhalis* in acute mucosal infections, as well as exacerbations in chronic obstructive pulmonary disease (COPD) is becoming more evident ([45](#_ENREF_45)). More serious infections are also documented and include bacteraemia, sepsis, meningitis, mastoiditis, septic arthritis and endocarditis ([71-77](#_ENREF_71)).

Unlike those above, the principle site of colonisation in the URT for *Staphylococcus aureus* is the anterior nares and around 25-30% of the population are consistently colonised, although others may be so transiently ([78](#_ENREF_78)). Carriage in the nasopharynx in infants has also been found to be between 20 to 30%, which is followed by an increase in early adolescence to 40–50% ([79](#_ENREF_79)). By adulthood however (> 18 years of age) this has reduced to ~25% and substantial heterogeneity in the level of carriage can be found ([79](#_ENREF_79)). *S. aureus* can cause bacteraemia, infective endocarditis, skin and soft tissue infections as well as both hospital- and community-acquired pneumonias ([47](#_ENREF_47)). Although the evolution of meticillin resistant *S. aureus* (MRSA), and the associated significant clinical consequences ([80](#_ENREF_80)), is now thought to have occurred prior to the widespread use of this antibiotic ([81](#_ENREF_81)), it remains nevertheless the most pressing challenge with respect to this pathogen.

*Neisseria meningitidis*, the meningococcus, is a Gram-negative that can cause a number of diseases - notably meningitis and sepsis. Carriage can occur asymptomatically and disease progression is a rare event ([82](#_ENREF_82)). The prevalence of carriage is often reported at ~10% but this can vary with age ([83](#_ENREF_83)) and is impacted upon by increasing social contact i.e. transmission rates tend to be highest in those living in close confines such as universities, and military barracks ([84](#_ENREF_84)). Vaccination, for example with MenB and Hib/MenC, has been effective at reducing invasive disease levels however as with the pneumococcus increased disease by capsular serogroups not covered by the vaccine does occur ([85-87](#_ENREF_85)).

**Interactions between Nasopharyngeal Bacterial Species**

Competition, synergism and antagonism (including ammensalism) are all potential microbial interactions in the NP microbiome and as a consequence there exist a plethora of inter-species relationships (Figure 2). For example, it is well known that *S. aureus* and *S. pneumoniae* exhibit an inverse correlation in carriage ([88](#_ENREF_88), [89](#_ENREF_89)). A well-studied mechanism for this is the production of H2O2 by SpxB, pyruvate oxidase in *S. pnuemoniae* ([90](#_ENREF_90), [91](#_ENREF_91)). Inhibition of other bacterial species, including *N. meningitidis*, has also been shown to occur through this mechanism ([92](#_ENREF_92)). The *in vivo* relevance of this is still very much in discussion ([93](#_ENREF_93)) and clearance of *S. aureus* may rather be CD4+ T-cell dependent as hydrogen peroxide mediated clearance was not observed in an HIV +ve cohort ([94](#_ENREF_94)). Moreover contact-dependent clearance of *S. aureus* biofilms has also been noted ([95](#_ENREF_95)).

Similar correlations with *S. aureus* have also been observed with both *H. influenzae* and *M. catarrhalis* ([96](#_ENREF_96)), with the later exhibiting an even stronger negative impact on *S. aureus* colonisation than *S. pneumoniae* ([24](#_ENREF_24)). Mechanistics are scant on this association and it is not clear if this is an inter-bacterial dynamic or one mediated indirectly through other commensal interactions or via immune system modulation in the host epithelium.

Strong positive associations in colonisation between *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* have been extremely well established ([24](#_ENREF_24), [97](#_ENREF_97), [98](#_ENREF_98)). There did exist a prior *in vitro* paradox where both latter species were susceptible to hydrogen peroxide-mediated killing by *S. pneumoniae* ([92](#_ENREF_92)). However this has been resolved with studies showing beneficial interactions within polymicrobial biofilms ([99](#_ENREF_99)) and potential protective mechanisms involving phase-variable modulation of global regulators that prevent oxidative killing of NTHi ([100](#_ENREF_100)). The positive association is such that colonisation density of both *S. pneumoniae* and *H. influenzae* increases when both species are present ([101](#_ENREF_101)). For *M. catarrhalis*, it has a synergistic relationship with *H. influenzae*, preventing complement mediated killing via complement C3 inhibitory binding, accomplished with secreted proteins in outer membrane vesicles (OMVs) ([102](#_ENREF_102)).

These interactions however are seldom simple and between *S. pneumoniae* and *H. influenzae* there are both additional exclusionary, in the form of competition for epithelial platelet activating factor receptor (PAFr) binding ([103](#_ENREF_103)), and antagonistic interactions also. Here pneumococcal neuraminidase cleaves sialic acid residues on lipooligosaccharide making *H. influenzae* more susceptible to complement ([104](#_ENREF_104)). In retaliation *H. influenzae* enhances neutrophil recruitment and opsonophagocytic killing of *S. pneumoniae* ([105](#_ENREF_105)). A consequence of the latter interaction is that it selects for co-colonised pneumococci with thicker capsules ([105](#_ENREF_105)). At least in the case of *H. influenzae* then, interactions with *S. pneumoniae* may be serotype dependent. Given the noted increases in carriage following PCV introduction ([62](#_ENREF_62), [106](#_ENREF_106)) there is a cause to consider that post-intervention evolutionary dynamics on pneumococcal epidemiology ([107](#_ENREF_107), [108](#_ENREF_108)) may indirectly shift population structuring of *H. influenzae,* not solely as a consequence of niche availability but due to fitness alterations in circulating pneumococcal strains.

To date, perhaps understandably, the focus has been on the interaction between those species most often implicated in disease. A fledgling field of study concerns the interactions of other commensal species of limited pathogenic potential. For example, *Corynebacterium* and *Dolosigranulum* have previously been found to be predictors for the absence of *S. aureus* carriage mediated by both exclusionary and competitive interactions ([31](#_ENREF_31), [109](#_ENREF_109)). Although importantly in the case of *S. aureus* not all species of *Corynebacterium* have been found to be antagonistic ([109](#_ENREF_109)). In AOM, a correlation between the presence of *Corynebacterium* and *Dolosigranulum* with a reduced incidence of disease and a decrease in carriage of both *S. pneumoniae* and *H. influenzae* has also been observed ([110](#_ENREF_110)). Higher prevalence of both species were also seen in the nasopharynx of pneumococcal non-colonised children ([111](#_ENREF_111)). The mechanistics of this antagonism was linked to the production of free fatty acids by *Corynebacterium accolens* that were generated via a lipase mediated hydrolysis of triacylglycerols – a metabolic requirement for *C. accolens* as it lacks fatty acid synthase ([111](#_ENREF_111)). In a mouse model of RSV and secondary pneumococcal pneumonia, *C. pseudodiptheriticum* has also recently been shown to prime the innate immune system via Toll-like receptor(TLR)-3 activation and induce protective T-cell and alveolar macrophage responses ([112](#_ENREF_112)).

All of the above interactions must also be considered in the context of biofilms that have been shown to occur in the nasopharynx with pneumococcus ([113](#_ENREF_113)), *S. aureus* ([114](#_ENREF_114)), *N. meningitidis* ([115](#_ENREF_115)) and *H. influenzae* ([116](#_ENREF_116)). For the pneumococcus, multi-species biofilms with *H. influenzae* have been shown to increase biofilm density ([116](#_ENREF_116)) and are more resistant to antibiotics when *M. catarrhalis* is present; a consequence of the near ubiquity of beta-lactamases ([99](#_ENREF_99)). Thus these interactions may underpin important synergisms *in vivo*.

**The Nasopharyngeal Microbiome and Disease**

Respiratory tract infections remain a significant contributor to global morbidity and mortality, particularly in the very young and old ([117](#_ENREF_117)). As already stated, the URT serves as a reservoir for many of the responsible opportunistic pathogens; infection with which cause an estimated 4 million premature deaths globally with 3 million in those <5 years and pneumonia the biggest killer ([117](#_ENREF_117)).

Chronic conditions such as asthma, COPD and cystic fibrosis are all impacted to some degree by the respiratory microbiome ([118-121](#_ENREF_118)) through, for example, ecological dysbiosis that enables outgrowth of a particular pathogen or alteration in host responses to microbial encounters in the lower airways. We have seen already that nascent NP microbiota in neonates imparts a susceptible/non-susceptible phenotype for the development of asthma ([25](#_ENREF_25)), and dependent on the age of exposure and bacterial species involved, profoundly effects later risk for respiratory infection and bronchiolitis ([25](#_ENREF_25), [122](#_ENREF_122)). Cystic fibrosis (CF) also impacts the early life transitions between microbiome profiles in nasopharynx detailed earlier ([123](#_ENREF_123)). The switch to stable, resilient *Moraxella* dominated profiles does not occur and *S. aureus*, *Corynebacterium* and *Streptococcal* sp. are more prevalent. Antibiotic treatment, common in CF, exacerbates this situation, leading to an increase of Gram-negatives such as *Burkholderia* sp. with a commensurate reduction in beneficial commensals ([123](#_ENREF_123)).

The possibility that certain species generate lipopolysaccharide (LPS)-driven, low-grade, chronic mucosal inflammatory responses that alter colonisation dynamics has also been proposed ([124](#_ENREF_124)). *H. influenzae*, for example, generates a strong TLR4-mediated inflammatory response in models of chronic airways disease ([125](#_ENREF_125)). Equally, this is also of benefit to the host, exemplified by the observation that influenza virus infection is less efficient following LPS-priming of the innate immune system ([126](#_ENREF_126)).

AOM is one of the most common infections of early childhood and is caused by colonisation of the middle ear by bacteria ascending the Eustachian tube from the nasopharynx ([69](#_ENREF_69), [110](#_ENREF_110), [127](#_ENREF_127), [128](#_ENREF_128)). Nasal microbiomes in children with AOM caused by S. pneumoniae, have been shown to be more disordered (lower diversity and evenness) with marked signs of pathogen outgrowth ([129](#_ENREF_129)). The presence of Corynebacterium and Dolosigranulum again were highlighted as potentially protective species against AOM ([129](#_ENREF_129)). Given the interactions between species of the URT it is unsurprising that the role of polymicrobial infection has also been highlighted in AOM ([129](#_ENREF_129)).

The link between the microbiota of the URT and LRTI, in terms of risk and/or progression to disease, have not been firmly established. Examples of correlates between increased density of pneumococci, *H. influenzae* and *M. catarrhalis* in the nasopharynx of paediatric pneumonia cases, in addition to a more diverse microbiota, have been detailed ([130](#_ENREF_130), [131](#_ENREF_131)). Similarly, associations of severity of RSV disease, a common cause of LRTI ([132](#_ENREF_132)), have been linked to carriage of *H. influenzae* and *S. pneumoniae*, but not *S. aureus* ([133](#_ENREF_133)).

In addition to the role of the microbiome in disease, the converse situation whereby the microbiome may be impacted upon by medical intervention should also be considered. In this respect, and understanding the intricate balance of inter-species relationships, the roles of vaccination and antibiotic therapy in shaping the NP microbiome can be considered. For the former in particular, concerns regarding the introduction of pneumococcal conjugate vaccines (PCVs) into paediatric vaccine schedules stemmed from a worry that carriage of *S. aureus*, and of greater concern MRSA, would increase as carriage of the pneumococcus was altered ([134](#_ENREF_134), [135](#_ENREF_135)). Indeed whilst carriage of pneumococci has remained at pre-PCV levels, although serotype prevalence has altered, evidence for the increased carriage of both *S. aureus* and *H. influenzae* has been noted ([62](#_ENREF_62), [106](#_ENREF_106), [136](#_ENREF_136)) and shown to persist at least 4.5 years after PCV7 introduction ([137](#_ENREF_137)), although these are not ubiquitous findings ([138](#_ENREF_138), [139](#_ENREF_139)). Moreover, there is an absence of a concomitant rise in MRSA following PCV introductions, suggesting concerns over this indirect effect are, for now, unfounded. As for antibiotics, as is common for much microbiome research, the focus has been on how therapy impacts the gut ([140-142](#_ENREF_140)). Here varied, unwanted outcomes have been highlighted that include altered metabolic function linked to obesity risk ([143](#_ENREF_143)), diabetes ([144](#_ENREF_144)) and increased susceptibility to infection ([145](#_ENREF_145)), reflecting the importance of gut microbiota to general health and well-being. It has been demonstrated however that susceptibility to antibiotic-induced perturbation is not universal across microbiomes with, for example, oral microbiota being more resistant than faecal in a direct comparison of therapies ([146](#_ENREF_146)). Nevertheless, antibiotic usage in infants has been associated with higher abundances of *Haemophilus*, *Streptococcus*, and *Moraxella*, which was linked to an increased risk of RTI and development of asthma later in life ([25](#_ENREF_25)). In addition, the antibiotic therapy was also shown to impact negatively on the more protective taxa of *Alloiococcus* and *Corynebacterium* ([25](#_ENREF_25)). In contrast, for children with CF prophylactic antibiotic therapy reduced microbiome diversity and beneficially reduced airway inflammation ([147](#_ENREF_147)). There remains much to be investigated therefore, and the interplay of the gut and airways, particularly in the context of antibiotic use, will be a fruitful area of research in the future ([148](#_ENREF_148)).

The role of NP microbiota and microbiomes in disease is thus complex, with myriad roles of intra- and inter-species interactions that modulate respiratory health over the life course. Note however that the above examples are largely conducted from the perspective of the bacterial responses, species specific or within the microbiome context. How these change then when one considers the role of host-response and, as discussed below, the inclusion of other biological effectors remains to be seen.

**The Nasopharyngeal Virome**

The role of viral-bacterial interactions in the URT cannot be underestimated. It was the secondary infections with *S. pneumoniae* during the ‘Spanish Flu’ pandemic in the early 20th Century that led to devastating levels of mortality([149](#_ENREF_149), [150](#_ENREF_150)). A milieu of host-cell specific impacts of viral infection (none of which are limited to Influenza Virus) have been described that explain this association. Naturally these effects are both cell-type and virus specific ([151](#_ENREF_151)). For influenza these have been extensively reviewed recently elsewhere ([152](#_ENREF_152)). Viral infection for example causes significant epithelial damage that exposes basement membranes and other binding moieties such as fibrin, fibrinogen and collagen ([153](#_ENREF_153)). In combination with the up-regulation of bacterial receptor molecules such as ICAM-1, fibronectin and integrin ([154](#_ENREF_154), [155](#_ENREF_155)) this enables increased adherence, of *S. pneumoniae* for example, through surface proteins such as pneumococcal surface protein A (PsaP), choline-binding protein A (CbpA) and pneumococcal serine-rich repeat protein (PsrP) ([156](#_ENREF_156)). Viral neuraminidase facilitates this adherence by cleaving sialic acid residues that both unveils additional cellular receptors for bacterial binding but also removes mucin-related residues that otherwise would attract bacterial adhesion ([157](#_ENREF_157)). When coupled with impaired mucociliary bacterial clearance ([158](#_ENREF_158), [159](#_ENREF_159)) the opportunity for bacterial adherence and infection is clear. Influenzae virus infection also impairs the innate immune responses that otherwise would control and clear bacterial infections through the depletion of alveolar macrophages ([160](#_ENREF_160)). Additionally the production of type I interferon via viral nucleic acid-TLR interactions reduces natural killer cell responses by inhibition of pro-inflammatory cytokines and chemokine suppression ([161](#_ENREF_161), [162](#_ENREF_162)). Finally, preceding viral infection can increase inflammation responses by activation of TLRs that are also involved in innate sensing of Gram-positive bacterial infection ([163](#_ENREF_163), [164](#_ENREF_164)). This synergistic activation thereby leading to increased pathology in terms of lung damage ([165](#_ENREF_165)).

In terms of how these narratives endure in the context of the NP microbiome is arguably less clear. For example, does a particular consortium of virus and/or bacterial colonisers predispose to infection, either URTI or LRTI? Does that infection present to a greater or lesser severity dependent on the microbiota? For respiratory syncytial virus (RSV) and human rhinovirus (HRV), the second most common causes of bronchiolitis in infants ([166](#_ENREF_166)), there appears to be a correlation between etiological agent of infection and bacterial colonisation. In infants diagnosed with RSV, NP microbiomes have bacterial communities dominated by Streptococcus whereas for HRV it is Haemophilus and Moraxella that are more abundant ([167](#_ENREF_167)). For the former interaction, the interplay between RSV and *S. pneumoniae* is complex. Bacterial colonisation has been shown to alter the host response to RSV infection, including the over expression of Toll-like receptor signalling, neutrophil recruitment and activation, that leads to a more severe infection ([133](#_ENREF_133)). In fact associations between RSV and *S. pneumoniae* may be bidirectional given that RSV infection predisposes to invasive pneumococcal disease and that, conversely, pneumococcal vaccination has been shown to reduce RSV-related hospitalisations ([168](#_ENREF_168)). To add to this complex picture RSV can also bind to the *S. pneumoniae* penicillin binding protein, which promotes increased adherence to ciliated epithelium and increased virulence ([169](#_ENREF_169)). For *H. influenzae* and HRV, exposure of human bronchial epithelial cells to *H. influenzae* has been shown to induce a heightened sensitivity to some types of HRV, but not RSV, and increased IL-6 and IL-8 responses to subsequent RSV infection ([154](#_ENREF_154)). Modulations of innate responses to viruses by commensal bacterial species, specifically in the nasopharynx, have also been noted ([126](#_ENREF_126), [170](#_ENREF_170)). This highlights the vital role these bacteria have in mediating host responses to viral infection and colonisation. The clear point here however is that these interactions tend towards cell, virus and bacterial specificity ([171](#_ENREF_171)).

In attempts to examine viral respiratory carriage, it has been shown that viruses are near ubiquitous in the nasopharynx of children up to 2 years of age ([24](#_ENREF_24)), present in case control studies of respiratory disease ([172-174](#_ENREF_172)) and in asymptomatic carriers ([175](#_ENREF_175)). A defining feature of these studies it that most individuals presented with multiple viral species. Importantly this links to bacterial carriage, with *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* all more likely with increasing virome diversity ([24](#_ENREF_24)). Specific associations in asymptomatic carriage have also been noted that include coronavirus and adenovirus with *M. catarrhalis*, human rhinovirus and RSV with *H. influenzae* and human rhinovirus with enterovirus more common with *S. pneumoniae* ([24](#_ENREF_24)). These associations are somewhat mirrored in RTIs whereby the microbiota of infants with human rhinovirus and RSV are distinct from those healthy infants in that they are dominated by *Moraxella*, *Streptococcus*, *Haemophilus* as well as *Corynebacterium* ([176](#_ENREF_176)). This particular study focussed on infants less than 6 months old in whom *Haemophilus* is not particularly dominant and *Staphylococcus* is the more routinely observed genus ([176](#_ENREF_176)).

Whilst both *in vitro* and *in vivo* studies indicate that co-infection can drive pathogenesis of either bacterial or viral respiratory disease, the extent to which co-colonisation structures the NP microbiome during carriage is unclear and remains a field yet to be studied exhaustively.

**The Nasopharyngeal Mycobiome**

There is a notable absence of data concerning the role of fungi in the NP microbiome. *Aspergillus*, *Candida* and *Cryptococcus* have all been identified in respiratory samples from individuals with a variety of clinical confounders such as immunodeficiencies ([177](#_ENREF_177)) and there is evidence that fungal exposure can be associated with asthma and chronic respiratory disease ([178](#_ENREF_178), [179](#_ENREF_179)). In the former condition, clear differences in fungal species were found in induced sputum between healthy controls and asthmatics, suggestive of a role in disease ([180](#_ENREF_180)). In addition, *C. albicans* has been shown to impair macrophage-mediated clearance of *Pseudomonas aeruginosa* in a rat model of pneumonia ([181](#_ENREF_181)) and impact infection and mucosal inflammatory response in biofilm models of *Streptococcal* disease ([182](#_ENREF_182)). The most compelling argument that the mycobiome will ultimately prove a significant contributor to health and disease in the context of the NP microbiome is the, albeit limited, examples from LRTI and chronic lung conditions. For example, the presence of fungal species was found in CF patients with the lowest disease severity scores as well as other measures of increased morbidity ([183](#_ENREF_183)). Similarly, significant differences in the lung mycobiome of HIV patients compared to non-infected and HIV-COPD patients has also been described, with particular enrichment of *Pneumocystis* in the latter cohort ([184](#_ENREF_184)).

Despite these examples much remains to be learnt regarding this portion of the microbiome.

**Conclusions**

It is clear that the NP microbiome has an important role in infectious disease pathology and long-term respiratory health. An understanding as to the extent to which it underpins risk is critical to reduce the global burden of respiratory disease. The starting point here being that stability of a resilient NP microbiome provides protection from acquisition of new pathogens and/or the restriction of outgrowth from those already resident ([32](#_ENREF_32), [43](#_ENREF_43), [124](#_ENREF_124), [185](#_ENREF_185)).

Methodological improvements to current NP microbiome studies, and extrapolated to other sites in the URT, must be also be done to accumulate data that not only captures the residency of microbiota but also its activity; be that in terms of microbial or host gene expression. This would give insight into important interactions between host immunology and the various –omes present in the airways.

The presence of beneficial and protective species within the URT opens the door to the development of future therapeutic strategies. Early intervention in terms of microbiome augmentation is a possible avenue for future research ([186](#_ENREF_186)). This strategy should be combined with new understandings of the role of commensal species in preventing, and even perhaps replacing, colonising opportunistic pathogens ([111](#_ENREF_111), [112](#_ENREF_112)) as well as the impact of pre- and probiotics on microbiomes that are distal to the URT yet may have profound implications for respiratory disease ([187](#_ENREF_187)). This area has received only moderate attention to date and there are a plethora of examples that have been reviewed elsewhere ([188](#_ENREF_188), [189](#_ENREF_189)), having mainly focused on *Lactococcus sp*. with varying degrees of success in reducing risks of RTI. No example could be found that investigated the impact of *Dolosigranulum* sp., either alone or in combination with *Corynebacterium* sp.. This is a clear gap in knowledge, as too is the paucity of true microbiome studies that take into account the bacterial, fungal and viral communities in the context of microbiome perturbations during infection or treatment. Lastly, the microbiome should never be examined without due reference to the host. As such, the examination of host genetic variability and microbiome structure, as has been recently discussed in the context of the gut ([190](#_ENREF_190)), must also be done for the respiratory tract. It is beyond question that this is a vital piece to the jigsaw of microbiome structure and function, and future research should attempt to address this fully.

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**Figure Legends**

Figure 1: The microbiology of the nasopharynx and upper respiratory tract. The upper respiratory tract consists of the nasal cavity, nasopharynx and oropharynx. Anatomical structuring generates environmental gradients that, in combination differences in epithelium, result in contrasting microbiome compositions. Generally species diversity increases from the vestibule of the nose through to the oropharynx. Key bacterial taxa associated with each niche are listed. Anatomical illustration: © <https://www.123rf.com/profile_terriana>

Figure 2: Inter-bacterial associations within the nasopharyngeal microbiome through the life course. From infancy through adulthood the nasopharyngeal microbiome becomes more disordered with a greater diversity of bacterial species and a lower overall abundance of each coloniser. In the elderly this results in an increased susceptibility to colonisation with pathogens or a failure to restrict pathogen outgrowth. Positive associations between bacterial taxa are shown as blue lines, negative associations are red, dashed lines indicate where the mechanisms of the interaction are poorly described. The dashed green line represents the early disappearance of *Staphylococcus aureus* from the infant nasopharynx with increased colonisation in older children and young adults. C – *Corynebacterium*; D – *Dolosigranulum*; Spn – *Streptococcus pneumoniae*; Mc – *Moraxella catarrhalis*; Hi – *Haemophilus influenzae*; Sa - *Staphylococcus aureus*; N – *Neisseria*

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