**Title: The Nasopharyngeal Microbiome**

David W. Cleary1,2 and Stuart C. Clarke1,3\*

1 Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK, SO17 1BJ

2 NIHR Southampton Biomedical Research Centre, University Hospital Southampton Foundation NHS Trust, Southampton, UK, SO16 6YD

3 Global Health Research Institute, University of Southampton, Southampton, UK, SO17 1BJ

\*Corresponding author email: S.C.Clarke@soton.ac.uk

**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.

**Acknowledgements**

None

**Declarations of Interest**

SCC has been a principal investigator for clinical trials and other studies conducted on behalf of University Hospital Southampton NHS Foundation Trust/University of Southampton that are sponsored by vaccine manufacturers but receives no personal payments from them. In addition SCC has participated in advisory boards for vaccine manufacturers but received no personal payments for this work. DWC has been employed on grants to the University of Southampton from GSK and Pfizer.

**Funding Information**

None

**Author Contribution Statement**

The manuscript was written by DWC and reviewed by SCC.

**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*

**References**

1. HMPConsortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486.

2. Halfvarson J, Brislawn CJ, Lamendella R, Vázquez-Baeza Y, Walters WA, Bramer LM, et al. Dynamics of the human gut microbiome in inflammatory bowel disease. Nature Microbiology. 2017;2:17004.

3. Yassour M, Lim MY, Yun HS, Tickle TL, Sung J, Song Y-M, et al. Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. Genome Medicine. 2016;8(1):17.

4. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, Festari C, et al. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. Neurobiology of Aging. 2017;49:60-8.

5. Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, et al. Alterations of the human gut microbiome in multiple sclerosis. Nature Communications. 2016;7:12015.

6. Kelly J, Kennedy P, Cryan J, Dinan T, Clarke G, Hyland N. Breaking Down the Barriers: The Gut Microbiome, Intestinal Permeability and Stress-related Psychiatric Disorders. Frontiers in Cellular Neuroscience. 2015;9(392).

7. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Medicine. 2016;8:51.

8. Cho I, Blaser MJ. The Human Microbiome: at the interface of health and disease. Nature reviews Genetics. 2012;13(4):260-70.

9. Kamada N, Seo S-U, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. Nature Reviews Immunology. 2013;13(5):321-35.

10. Stecher B, Maier L, Hardt W-D. 'Blooming'in the gut: how dysbiosis might contribute to pathogen evolution. Nature Reviews Microbiology. 2013;11(4):277-84.

11. Whiteside SA, Razvi H, Dave S, Reid G, Burton JP. The microbiome of the urinary tract - a role beyond infection. Nat Rev Urol. 2015;12(2):81-90.

12. Fujimura KE, Demoor T, Rauch M, Faruqi AA, Jang S, Johnson CC, et al. House dust exposure mediates gut microbiome Lactobacillus enrichment and airway immune defense against allergens and virus infection. Proceedings of the National Academy of Sciences. 2014;111(2):805-10.

13. Theriot CM, Koenigsknecht MJ, Carlson PE, Hatton GE, Nelson AM, Li B, et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. Nature communications. 2014;5:3114-.

14. Fokkens WJ, Scheeren RA. Upper airway defence mechanisms. Paediatric Respiratory Reviews. 2000;1(4):336-41.

15. Voynow JA, Rubin BK. Mucins, Mucus, and Sputum. Chest. 2009;135(2):505-12.

16. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. The application of ecological theory towards an understanding of the human microbiome. Science 2012;336(6086):1255-62.

17. Perez-Muñoz ME, Arrieta M-C, Ramer-Tait AE, Walter J. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. Microbiome. 2017;5(1):48.

18. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences. 2010;107(26):11971-5.

19. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med. 2017;23(3):314-26.

20. Kristensen K, Fisker N, Haerskjold A, Ravn H, Simões EAF, Stensballe L. Caesarean Section and Hospitalization for Respiratory Syncytial Virus Infection: A Population-based Study. The Pediatric Infectious Disease Journal. 2015;34(2):145-8.

21. Rusconi F, Zugna D, Annesi-Maesano I, Baïz N, Barros H, Correia S, et al. Mode of Delivery and Asthma at School Age in 9 European Birth Cohorts. American Journal of Epidemiology. 2017;185(6):465-73.

22. Bosch AATM, Levin E, van Houten MA, Hasrat R, Kalkman G, Biesbroek G, et al. Development of Upper Respiratory Tract Microbiota in Infancy is Affected by Mode of Delivery. EBioMedicine. 2016;9:336-45.

23. Belkaid Y, Hand T. Role of the Microbiota in Immunity and inflammation. Cell. 2014;157(1):121-41.

24. van den Bergh MR, Biesbroek G, Rossen JWA, de Steenhuijsen Piters WAA, Bosch AATM, van Gils EJM, et al. Associations between Pathogens in the Upper Respiratory Tract of Young Children: Interplay between Viruses and Bacteria. PloS one. 2012;7(10):e47711.

25. Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe. 2015;17.

26. Greenberg D, Givon-Lavi N, Broides A, Blancovich I, Peled N, Dagan R. The Contribution of Smoking and Exposure to Tobacco Smoke to Streptococcus pneumoniae and Haemophilus influenzae Carriage in Children and Their Mothers. Clinical Infectious Diseases. 2006;42(7):897-903.

27. Bogaert D, Keijser B, Huse S, Rossen J, Veenhoven R, van Gils E, et al. Variability and Diversity of Nasopharyngeal Microbiota in Children: A Metagenomic Analysis. PloS one. 2011;6(2):e17035.

28. Biesbroek G, Tsivtsivadze E, Sanders EAM, Montijn R, Veenhoven RH, Keijser BJF, et al. Early Respiratory Microbiota Composition Determines Bacterial Succession Patterns and Respiratory Health in Children. American Journal of Respiratory and Critical Care Medicine. 2014;190(11):1283-92.

29. Harrison LM, Morris JA, Telford DR, Brown SM, Jones K. The nasopharyngeal bacterial flora in infancy: effects of age, gender, season, viral upper respiratory tract infection and sleeping position. FEMS Immunology and Medical Microbiology. 1999;25(1):19-28.

30. Stearns JC, Davidson CJ, McKeon S, Whelan FJ, Fontes ME, Schryvers AB, et al. Culture and molecular-based profiles show shifts in bacterial communities of the upper respiratory tract that occur with age. ISME J. 2015.

31. Liu CM, Price LB, Hungate BA, Abraham AG, Larsen LA, Christensen K, et al. Staphylococcus aureus and the ecology of the nasal microbiome. Science Advances. 2015;1(5).

32. Cremers AJH, Zomer AL, Gritzfeld JF, Ferwerda G, van Hijum SAFT, Ferreira DM, et al. The adult nasopharyngeal microbiome as a determinant of pneumococcal acquisition. Microbiome. 2014;2:44.

33. Krone CL, van de Groep K, Trzciński K, Sanders EAM, Bogaert D. Immunosenescence and pneumococcal disease: an imbalance in host-pathogen interactions. The Lancet Respiratory Medicine.2(2):141-53.

34. Zapata HJ, Quagliarello VJ. The Microbiota and Microbiomein Aging: Potential Implications in Health and Age-related Diseases. Journal of the American Geriatrics Society. 2015;63(4):776-81.

35. Buford TW. (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome. 2017;5(1):80.

36. Whelan FJ, Verschoor CP, Stearns JC, Rossi L, Luinstra K, Loeb M, et al. The Loss of Topography in the Microbial Communities of the Upper Respiratory Tract in the Elderly. Annals of the American Thoracic Society. 2014;11(4):513-21.

37. de Steenhuijsen Piters WAA, Huijskens EGW, Wyllie AL, Biesbroek G, van den Bergh MR, Veenhoven RH, et al. Dysbiosis of upper respiratory tract microbiota in elderly pneumonia patients. ISME J. 2016;10(1):97-108.

38. Kline KA, Bowdish DME. Infection in an aging population. Current Opinion in Microbiology. 2016;29:63-7.

39. Flamaing J, Peetermans WE, Vandeven J, Verhaegen J. PNEUMOCOCCAL COLONIZATION IN OLDER PERSONS IN A NONOUTBREAK SETTING. Journal of the American Geriatrics Society. 2010;58(2):396-8.

40. Thevaranjan N, Whelan FJ, Puchta A, Ashu E, Rossi L, Surette MG, et al. Streptococcus pneumoniae colonization disrupts the microbial community within the upper respiratory tract of aging mice. Infection and Immunity. 2016.

41. Le Polain de Waroux O, Flasche S, Prieto-Merino D, Edmunds WJ. Age-Dependent Prevalence of Nasopharyngeal Carriage of Streptococcus pneumoniae before Conjugate Vaccine Introduction: A Prediction Model Based on a Meta-Analysis. PloS one. 2014;9(1):e86136.

42. Cremers AJ, Zomer AL, Gritzfeld JF, Ferwerda G, van Hijum SA, Ferreira DM, et al. The adult nasopharyngeal microbiome as a determinant of pneumococcal acquisition. Microbiome. 2014;2(1):1-10.

43. Krone CL, Wyllie AL, van Beek J, Rots NY, Oja AE, Chu MLJN, et al. Carriage of Streptococcus pneumoniae in Aged Adults with Influenza-Like-Illness. PloS one. 2015;10(3):e0119875.

44. Adler H, Ferreira DM, Gordon SB, Rylance J. Pneumococcal Capsular Polysaccharide Immunity in the Elderly. Clinical and Vaccine Immunology. 2017;24(6).

45. Goldstein EJC, Murphy TF, Parameswaran GI. Moraxella catarrhalis, a Human Respiratory Tract Pathogen. Clinical Infectious Diseases. 2009;49(1):124-31.

46. Van Eldere J, Slack MPE, Ladhani S, Cripps AW. Non-typeable Haemophilus influenzae, an under-recognised pathogen. The Lancet Infectious Diseases. 2014;14(12):1281-92.

47. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clinical Microbiology Reviews. 2015;28(3):603-61.

48. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. Nat Rev Micro. 2008;6(4):288-301.

49. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. The Lancet.374(9693):893-902.

50. Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal Capsules and Their Types: Past, Present, and Future. Clinical Microbiology Reviews. 2015;28(3):871-99.

51. Huang SS, Hinrichsen VL, Stevenson AE, Rifas-Shiman SL, Kleinman K, Pelton SI, et al. Continued Impact of Pneumococcal Conjugate Vaccine on Carriage in Young Children. Pediatrics. 2009;124(1):e1-11.

52. Adetifa IMO, Antonio M, Okoromah CAN, Ebruke C, Inem V, Nsekpong D, et al. Pre-Vaccination Nasopharyngeal Pneumococcal Carriage in a Nigerian Population: Epidemiology and Population Biology. PloS one. 2012;7(1):e30548.

53. Bogaert D, van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rumke HC, et al. Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. Lancet. 2004;363.

54. Regev-Yochay G, Raz M, Dagan R, Porat N, Shainberg B, Pinco E, et al. Nasopharyngeal Carriage of Streptococcus pneumoniae by Adults and Children in Community and Family Settings. Clinical Infectious Diseases. 2004;38(5):632-9.

55. Turner P, Turner C, Jankhot A, Helen N, Lee SJ, Day NP, et al. A Longitudinal Study of Streptococcus pneumoniae Carriage in a Cohort of Infants and Their Mothers on the Thailand-Myanmar Border. PloS one. 2012;7(5):e38271.

56. Hill PC, Cheung YB, Akisanya A, Sankareh K, Lahai G, Greenwood BM, et al. Nasopharyngeal Carriage of Streptococcus pneumoniae in Gambian Infants: A Longitudinal Study. Clinical Infectious Diseases. 2008;46(6):807-14.

57. Kamng’ona AW, Hinds J, Bar-Zeev N, Gould KA, Chaguza C, Msefula C, et al. High multiple carriage and emergence of Streptococcus pneumoniae vaccine serotype variants in Malawian children. BMC Infectious Diseases. 2015;15(1):234.

58. Devine VT, Cleary DW, Jefferies JMC, Anderson R, Morris DE, Tuck AC, et al. The rise and fall of pneumococcal serotypes carried in the PCV era. Vaccine. 2017;35(9):1293-8.

59. Bogaert D, Groot R, Hermans PWM. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis. 2004;4.

60. Waight PA, Andrews NJ, Ladhani SN, Sheppard CL, Slack MPE, Miller E. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. The Lancet Infectious Diseases. 2015;15(5):535-43.

61. Moore MR, Link-Gelles R, Schaffner W, Lynfield R, Lexau C, Bennett NM, et al. Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: analysis of multisite, population-based surveillance. The Lancet Infectious Diseases. 2015;15(3):301-9.

62. Bosch AATM, van Houten MA, Bruin JP, Wijmenga-Monsuur AJ, Trzciński K, Bogaert D, et al. Nasopharyngeal carriage of Streptococcus pneumoniae and other bacteria in the 7th year after implementation of the pneumococcal conjugate vaccine in the Netherlands. Vaccine. 2016;34(4):531-9.

63. Gladstone RA, Devine V, Jones J, Cleary D, Jefferies JM, Bentley SD, et al. Pre-vaccine serotype composition within a lineage signposts its serotype replacement – a carriage study over 7 years following pneumococcal conjugate vaccine use in the UK. Microbial Genomics. 2017;3(6).

64. Croucher N, Finkelstein J, Pelton S, Mitchell P, Lee G, Parkhill J. Population genomics of post-vaccine changes in pneumococcal epidemiology. Nat Genet. 2013;45:656 - 63.

65. Huang SS, Platt R, Rifas-Shiman SL, Pelton SI, Goldmann D, Finkelstein JA. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. Pediatrics. 2005;116.

66. Nørskov-Lauritsen N. Classification, Identification, and Clinical Significance of Haemophilus and Aggregatibacter Species with Host Specificity for Humans. Clinical Microbiology Reviews. 2014;27(2):214-40.

67. Peltola H. Worldwide Haemophilus influenzae Type b Disease at the Beginning of the 21st Century: Global Analysis of the Disease Burden 25 Years after the Use of the Polysaccharide Vaccine and a Decade after the Advent of Conjugates. Clinical Microbiology Reviews. 2000;13(2):302-17.

68. Robert W, Assimoula E, Joana Gomes D, Elizabeth B, Miriam R, Lucia Pastore C. Epidemiology of Invasive <em>Haemophilus influenzae</em> Disease, Europe, 2007–2014. Emerging Infectious Disease journal. 2017;23(3):396.

69. Monasta L, Ronfani L, Marchetti F, Montico M, Vecchi Brumatti L, Bavcar A, et al. Burden of Disease Caused by Otitis Media: Systematic Review and Global Estimates. PloS one. 2012;7(4):e36226.

70. Rycroft CE, Heyes A, Lanza L, Becker K. Epidemiology of chronic obstructive pulmonary disease: a literature review. International Journal of Chronic Obstructive Pulmonary Disease. 2012;7:457-94.

71. Hays J. The genus Moraxella. In: Dworkin M FS, Rosenberg E et al, editor. The Prokaryotes. 6. 3 ed: Springer, New York.; 2006. p. 958-87.

72. Riley NMPTV. Molecular typing in bacterial infections Chapter 14 Moraxella. Vassil st G, editor. New York: Humana Press; 2013. xii, 482 p. p.

73. Shaikh SB, Ahmed Z, Arsalan SA, Shafiq S. Prevalence and resistance pattern of Moraxella catarrhalis in community-acquired lower respiratory tract infections. Infect Drug Resist. 2015;8:263-7.

74. Zemlickova H, Urbaskova P, Adamkova V, Motlova J, Lebedova V, Prochazka B. Characteristics of Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Staphylococcus aureus isolated from the nasopharynx of healthy children attending day-care centres in the Czech Republic. Epidemiology and infection. 2006;134(6):1179-87.

75. Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. Tonawanda/Williamsville Pediatrics. The Journal of infectious diseases. 1997;175(6):1440-5.

76. Ahmad S. Bronchopulmonary infection due to Moraxella (Branhamella) catarrhalis at a specialist hospital in Saudi Arabia. J Commun Dis. 1998;30(4):233-6.

77. Kobayashi Y. Bacteremic Moraxella catarrhalis pneumonia. J Infect Chemother. 2000;6(1):68.

78. Wertheim HFL, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. The Lancet Infectious Diseases. 2005;5(12):751-62.

79. Sollid JUE, Furberg AS, Hanssen AM, Johannessen M. Staphylococcus aureus: Determinants of human carriage. Infection, Genetics and Evolution. 2014;21:531-41.

80. Klein E, Smith DL, Laxminarayan R. Hospitalizations and Deaths Caused by Methicillin-Resistant Staphylococcus aureus, United States, 1999–2005. Emerging Infectious Diseases. 2007;13(12):1840-6.

81. Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, et al. Methicillin-resistant Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. Genome Biology. 2017;18(1):130.

82. Halperin SA, Bettinger JA, Greenwood B, Harrison LH, Jelfs J, Ladhani SN, et al. The changing and dynamic epidemiology of meningococcal disease. Vaccine. 2012;30:B26-B36.

83. Cartwright KA, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and Neisseria lactamica. Epidemiology and Infection. 1987;99(3):591-601.

84. Stephens DS. Uncloaking the meningococcus: dynamics of carriage and disease. The Lancet. 1999;353(9157):941-2.

85. Ladhani SN, Beebeejaun K, Lucidarme J, Campbell H, Gray S, Kaczmarski E, et al. Increase in Endemic Neisseria meningitidis Capsular Group W Sequence Type 11 Complex Associated With Severe Invasive Disease in England and Wales. Clinical Infectious Diseases. 2015;60(4):578-85.

86. Oldfield NJ, Cayrou C, AlJannat MAK, Al-Rubaiawi AAA, Green LR, Dada S, et al. Rise in Group W Meningococcal Carriage in University Students, United Kingdom. Emerging Infectious Diseases. 2017;23(6):1009-11.

87. Carville KS, Stevens K, Sohail A, Franklin LJ, Bond KA, Brahmi A, et al. Increase in Meningococcal Serogroup W Disease, Victoria, Australia, 2013–2015. Emerging Infectious Diseases. 2016;22(10):1785-7.

88. David MZ, Daum RS. Community-Associated Methicillin-Resistant Staphylococcus aureus: Epidemiology and Clinical Consequences of an Emerging Epidemic. Clinical Microbiology Reviews. 2010;23(3):616-87.

89. Lee GM, Huang SS, Rifas-Shiman SL, Hinrichsen VL, Pelton SI, Kleinman K, et al. Epidemiology and risk factors for Staphylococcus aureuscolonization in children in the post-PCV7 era. BMC Infectious Diseases. 2009;9(1):110.

90. Park B, Nizet V, Liu GY. Role of Staphylococcus aureus Catalase in Niche Competition against Streptococcus pneumoniae. Journal of Bacteriology. 2008;190(7):2275-8.

91. Regev-Yochay G, Dagan R, Raz M, Carmeli Y, Shainberg B, Derazne E, et al. Association between carriage of Streptococcus pneumoniae and Staphylococcus aureus in Children. JAMA. 2004;292.

92. Pericone CD, Overweg K, Hermans PWM, Weiser JN. Inhibitory and Bactericidal Effects of Hydrogen Peroxide Production by Streptococcus pneumoniae on Other Inhabitants of the Upper Respiratory Tract. Infection and Immunity. 2000;68(7):3990-7.

93. Margolis E. Hydrogen Peroxide-Mediated Interference Competition by Streptococcus pneumoniae Has No Significant Effect on Staphylococcus aureus Nasal Colonization of Neonatal Rats. Journal of Bacteriology. 2009;191(2):571-5.

94. McNally LM, Jeena PM, Gajee K, Sturm AW, Tomkins AM, Coovadia HM, et al. Lack of Association between the Nasopharyngeal Carriage of Streptococcus pneumoniae and Staphylococcus aureus in HIV-1–Infected South African Children. The Journal of Infectious Diseases. 2006;194(3):385-90.

95. Khan F, Wu X, Matzkin GL, Khan MA, Sakai F, Vidal JE. Streptococcus pneumoniae Eradicates Preformed Staphylococcus aureus Biofilms through a Mechanism Requiring Physical Contact. Frontiers in Cellular and Infection Microbiology. 2016;6:104.

96. Pettigrew MM, Gent JF, Revai K, Patel JA, Chonmaitree T. Microbial Interactions during Upper Respiratory Tract Infections. Emerging Infectious Diseases. 2008;14(10):1584-91.

97. Lewnard JA, Huppert A, Givon-Lavi N, Pettigrew MM, Regev-Yochay G, Dagan R, et al. Density, Serotype Diversity, and Fitness of Streptococcus pneumoniae in Upper Respiratory Tract Cocolonization With Nontypeable Haemophilus influenzae. Journal of Infectious Diseases. 2016;214(9):1411-20.

98. Shak JR, Vidal JE, Klugman KP. Influence of bacterial interactions on pneumococcal colonization of the nasopharynx. Trends in Microbiology. 2013;21(3):129-35.

99. Perez AC, Pang B, King LB, Tan L, Murrah KA, Reimche JL, et al. Residence of Streptococcus pneumoniae and Moraxella catarrhalis within polymicrobial biofilm promotes antibiotic resistance and bacterial persistence in vivo. Pathogens and disease. 2014;70(3):280-8.

100. Brockman KL, Branstool MT, Atack JM, Robledo-Avila F, Partida-Sanchez S, Jennings MP, et al. The ModA2 Phasevarion of nontypeable Haemophilus influenzae Regulates Resistance to Oxidative Stress and Killing by Human Neutrophils. Scientific Reports. 2017;7:3161.

101. Chien Y-W, Vidal JE, Grijalva CG, Bozio C, Edwards KM, Williams JV, et al. Density Interactions between Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus in the Nasopharynx of Young Peruvian Children. The Pediatric infectious disease journal. 2013;32(1):72-7.

102. Thuan Tong T, Mörgelin M, Forsgren A, Riesbeck K. Haemophilus influenzae Survival during Complement-Mediated Attacks Is Promoted by Moraxella catarrhalis Outer Membrane Vesicles. The Journal of Infectious Diseases. 2007;195(11):1661-70.

103. Shukla SD, Sohal SS, O’Toole RF, Eapen MS, Walters EH. Platelet activating factor receptor: gateway for bacterial chronic airway infection in chronic obstructive pulmonary disease and potential therapeutic target. Expert Review of Respiratory Medicine. 2015;9(4):473-85.

104. Shakhnovich EA, King SJ, Weiser JN. Neuraminidase Expressed by Streptococcus pneumoniae Desialylates the Lipopolysaccharide of Neisseria meningitidis and Haemophilus influenzae: a Paradigm for Interbacterial Competition among Pathogens of the Human Respiratory Tract. Infection and Immunity. 2002;70(12):7161-4.

105. Lysenko ES, Ratner AJ, Nelson AL, Weiser JN. The Role of Innate Immune Responses in the Outcome of Interspecies Competition for Colonization of Mucosal Surfaces. PLOS Pathogens. 2005;1(1):e1.

106. Camilli R, Vescio MF, Giufrè M, Daprai L, Garlaschi ML, Cerquetti M, et al. Carriage of Haemophilus influenzae is associated with pneumococcal vaccination in Italian children. Vaccine. 2015;33(36):4559-64.

107. Jefferies JMC, Clarke SC, Webb JS, Kraaijeveld AR. Risk of Red Queen dynamics in pneumococcal vaccine strategy. Trends in Microbiology. 2011;19(8):377-81.

108. Stockmann C, Ampofo K, Pavia AT, Blaschke AJ, Mason EO, Presson AP, et al. Clinical and Epidemiological Evidence of the Red Queen Hypothesis in Pneumococcal Serotype Dynamics. Clinical Infectious Diseases. 2016;63(5):619-26.

109. Yan M, Pamp SJ, Fukuyama J, Hwang PH, Cho D-Y, Holmes S, et al. Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and S. aureus carriage. Cell host & microbe. 2013;14(6):631-40.

110. Pettigrew MM, Laufer AS, Gent JF, Kong Y, Fennie KP, Metlay JP. Upper Respiratory Tract Microbial Communities, Acute Otitis Media Pathogens, and Antibiotic Use in Healthy and Sick Children. Applied and Environmental Microbiology. 2012;78(17):6262-70.

111. Bomar L, Brugger SD, Yost BH, Davies SS, Lemon KP. Corynebacterium accolens Releases Antipneumococcal Free Fatty Acids from Human Nostril and Skin Surface Triacylglycerols. mBio. 2016;7(1).

112. Kanmani P, Clua P, Vizoso-Pinto MG, Rodriguez C, Alvarez S, Melnikov V, et al. Respiratory Commensal Bacteria Corynebacterium pseudodiphtheriticum Improves Resistance of Infant Mice to Respiratory Syncytial Virus and Streptococcus pneumoniae Superinfection. Frontiers in Microbiology. 2017;8(1613).

113. Muñoz-Elías EJ, Marcano J, Camilli A. Isolation of Streptococcus pneumoniae Biofilm Mutants and Their Characterization during Nasopharyngeal Colonization. Infection and Immunity. 2008;76(11):5049-61.

114. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, et al. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature. 2010;465(7296):346-9.

115. Brock Neil R, Shao JQ, Apicella MA. Biofilm formation on human airway epithelia by encapsulated Neisseria meningitidis serogroup B. Microbes and Infection. 2009;11(2):281-7.

116. Weimer KED, Armbruster CE, Juneau RA, Hong W, Pang B, Swords WE. Coinfection with Haemophilus influenzae promotes pneumococcal biofilm formation during experimental otitis media and impedes the progression of pneumococcal disease. The Journal of infectious diseases. 2010;202(7):1068-75.

117. Ferkol T, Schraufnagel D. The Global Burden of Respiratory Disease. Annals of the American Thoracic Society. 2014;11(3):404-6.

118. Durack J, Boushey HA, Lynch SV. Airway Microbiota and the Implications of Dysbiosis in Asthma. Current Allergy and Asthma Reports. 2016;16(8):52.

119. Depner M, Ege MJ, Cox MJ, Dwyer S, Walker AW, Birzele LT, et al. Bacterial microbiota of the upper respiratory tract and childhood asthma. Journal of Allergy and Clinical Immunology. 2017;139(3):826-34.

120. Huang YJ, Sethi S, Murphy T, Nariya S, Boushey HA, Lynch SV. Airway Microbiome Dynamics in Exacerbations of Chronic Obstructive Pulmonary Disease. Journal of Clinical Microbiology. 2014;52(8):2813-23.

121. Stokell JR, Gharaibeh RZ, Hamp TJ, Zapata MJ, Fodor AA, Steck TR. Analysis of Changes in Diversity and Abundance of the Microbial Community in a Cystic Fibrosis Patient over a Multiyear Period. Journal of Clinical Microbiology. 2015;53(1):237-47.

122. Vissing NH, Chawes BLK, Bisgaard H. Increased Risk of Pneumonia and Bronchiolitis after Bacterial Colonization of the Airways as Neonates. American Journal of Respiratory and Critical Care Medicine. 2013;188(10):1246-52.

123. SMPJ P, KM dW-dG, HM J, WAA dSP, GA T-S, AL W, et al. Development of the Nasopharyngeal Microbiota in Infants with Cystic Fibrosis. American Journal of Respiratory and Critical Care Medicine. 2016;193(5):504-15.

124. de Steenhuijsen Piters WAA, Sanders EAM, Bogaert D. The role of the local microbial ecosystem in respiratory health and disease. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 2015;370(1675).

125. Larsen JM, Musavian HS, Butt TM, Ingvorsen C, Thysen AH, Brix S. Chronic obstructive pulmonary disease and asthma-associated Proteobacteria, but not commensal Prevotella spp., promote Toll-like receptor 2-independent lung inflammation and pathology. Immunology. 2015;144(2):333-42.

126. Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proceedings of the National Academy of Sciences. 2011;108(13):5354-9.

127. Klein JO. The burden of otitis media. Vaccine. 2000;19:S2-S8.

128. Coker TR, Chan LS, Newberry SJ, et al. Diagnosis, microbial epidemiology, and antibiotic treatment of acute otitis media in children: A systematic review. JAMA. 2010;304(19):2161-9.

129. Laufer AS, Metlay JP, Gent JF, Fennie KP, Kong Y, Pettigrew MM. Microbial Communities of the Upper Respiratory Tract and Otitis Media in Children. mBio. 2011;2(1).

130. Sakwinska O, Schmid VB, Berger B, Bruttin A, Keitel K, Lepage M. Nasopharyngeal microbiota in healthy children and pneumonia patients. J Clin Microbiol. 2014;52.

131. Chochua S, D'Acremont V, Hanke C, Alfa D, Shak J, Kilowoko M, et al. Increased Nasopharyngeal Density and Concurrent Carriage of Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis Are Associated with Pneumonia in Febrile Children. PloS one. 2016;11(12):e0167725.

132. Mazur NI, Martinón-Torres F, Baraldi E, Fauroux B, Greenough A, Heikkinen T, et al. Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. The Lancet Respiratory Medicine.3(11):888-900.

133. WAA dSP, A H, R H, E B, B S, M-C S-A, et al. Nasopharyngeal Microbiota, Host Transcriptome, and Disease Severity in Children with Respiratory Syncytial Virus Infection. American Journal of Respiratory and Critical Care Medicine. 2016;194(9):1104-15.

134. Singh NK. Pneumococcal vaccine concerns: Shape-shifters and beyond. Vaccine. 2007;25(29):5244-5.

135. Potential Consequences of the Pneumococcal Conjugate Vaccine. New England Journal of Medicine. 2006;355(1):95-6.

136. van Gils EJM, Hak E, Veenhoven RH, Rodenburg GD, Bogaert D, Bruin JP, et al. Effect of Seven-Valent Pneumococcal Conjugate Vaccine on Staphylococcus aureus Colonisation in a Randomised Controlled Trial. PloS one. 2011;6(6):e20229.

137. Spijkerman J, Prevaes SMPJ, van Gils EJM, Veenhoven RH, Bruin JP, Bogaert D, et al. Long-Term Effects of Pneumococcal Conjugate Vaccine on Nasopharyngeal Carriage of S. pneumoniae, S. aureus, H. influenzae and M. catarrhalis. PloS one. 2012;7(6):e39730.

138. Hammitt LL, Akech DO, Morpeth SC, Karani A, Kihuha N, Nyongesa S, et al. Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae and non-typeable Haemophilus influenzae in Kilifi, Kenya: findings from cross-sectional carriage studies. The Lancet Global Health. 2014;2(7):e397-e405.

139. Lewnard JA, Givon-Lavi N, Huppert A, Pettigrew MM, Regev-Yochay G, Dagan R, et al. Epidemiological Markers for Interactions Among Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus in Upper Respiratory Tract Carriage. The Journal of Infectious Diseases. 2016;213(10):1596-605.

140. Francino MP. Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances. Frontiers in Microbiology. 2016;6(1543).

141. Thanabalasuriar A, Kubes P. Neonates, antibiotics and the microbiome. Nat Med. 2014;20(5):469-70.

142. Raymond F, Ouameur AA, Déraspe M, Iqbal N, Gingras H, Dridi B, et al. The initial state of the human gut microbiome determines its reshaping by antibiotics. The ISME Journal. 2016;10(3):707-20.

143. Cox LM, Blaser MJ. Antibiotics in early life and obesity. Nat Rev Endocrinol. 2015;11(3):182-90.

144. Boursi B, Mamtani R, Haynes K, Yang Y-X. The effect of past antibiotic exposure on diabetes risk. European Journal of Endocrinology. 2015;172(6):639-48.

145. Buffie CG, Jarchum I, Equinda M, Lipuma L, Gobourne A, Viale A, et al. Profound Alterations of Intestinal Microbiota following a Single Dose of Clindamycin Results in Sustained Susceptibility to Clostridium difficile-Induced Colitis. Infection and Immunity. 2012;80(1):62-73.

146. Zaura E, Brandt BW, Teixeira de Mattos MJ, Buijs MJ, Caspers MPM, Rashid M-U, et al. Same Exposure but Two Radically Different Responses to Antibiotics: Resilience of the Salivary Microbiome versus Long-Term Microbial Shifts in Feces. mBio. 2015;6(6).

147. Pittman JE, Wylie KM, Akers K, Storch GA, Hatch J, Quante J, et al. Association of Antibiotics, Airway Microbiome, and Inflammation in Infants with Cystic Fibrosis. Annals of the American Thoracic Society. 2017;14(10):1548-55.

148. Budden KF, Gellatly SL, Wood DLA, Cooper MA, Morrison M, Hugenholtz P, et al. Emerging pathogenic links between microbiota and the gut-lung axis. Nat Rev Micro. 2017;15(1):55-63.

149. McCullers JA. Insights into the Interaction between Influenza Virus and Pneumococcus. Clinical Microbiology Reviews. 2006;19(3):571-82.

150. Morris DE, Cleary DW, Clarke SC. Secondary Bacterial Infections Associated with Influenza Pandemics. Frontiers in Microbiology. 2017;8(1041).

151. Avadhanula V, Rodriguez CA, DeVincenzo JP, Wang Y, Webby RJ, Ulett GC, et al. Respiratory Viruses Augment the Adhesion of Bacterial Pathogens to Respiratory Epithelium in a Viral Species- and Cell Type-Dependent Manner. Journal of Virology. 2006;80(4):1629-36.

152. McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. Nat Rev Micro. 2014;12(4):252-62.

153. Plotkowski M-C, Puchelle E, Beck G, Jacquot J, Hannoun C. Adherence of Type I Streptococcus pneumoniae to Tracheal Epithelium of Mice Infected with Influenza A/PR8 Virus. American Review of Respiratory Disease. 1986;134(5):1040-4.

154. Gulraiz F, Bellinghausen C, Bruggeman CA, Stassen FR. Haemophilus influenzae increases the susceptibility and inflammatory response of airway epithelial cells to viral infections. The FASEB Journal. 2015;29(3):849-58.

155. Li N, Ren A, Wang X, Fan X, Zhao Y, Gao GF, et al. Influenza viral neuraminidase primes bacterial coinfection through TGF-β–mediated expression of host cell receptors. Proceedings of the National Academy of Sciences. 2015;112(1):238-43.

156. Dockrell DH, Whyte MKB, Mitchell TJ. Pneumococcal Pneumonia: Mechanisms of Infection and Resolution. Chest. 2012;142(2):482-91.

157. McCullers JA, Bartmess KC. Role of Neuraminidase in Lethal Synergism between Influenza Virus and Streptococcus pneumoniae. The Journal of Infectious Diseases. 2003;187(6):1000-9.

158. Pittet LA, Hall-Stoodley L, Rutkowski MR, Harmsen AG. Influenza Virus Infection Decreases Tracheal Mucociliary Velocity and Clearance of Streptococcus pneumoniae. American Journal of Respiratory Cell and Molecular Biology. 2010;42(4):450-60.

159. Wu N-H, Yang W, Beineke A, Dijkman R, Matrosovich M, Baumgärtner W, et al. The differentiated airway epithelium infected by influenza viruses maintains the barrier function despite a dramatic loss of ciliated cells. Scientific Reports. 2016;6:39668.

160. Ghoneim HE, Thomas PG, McCullers JA. Depletion of alveolar macrophages during influenza infection facilitates bacterial super-infections. Journal of immunology (Baltimore, Md : 1950). 2013;191(3):1250-9.

161. Shahangian A, Chow EK, Tian X, Kang JR, Ghaffari A, Liu SY, et al. Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. The Journal of Clinical Investigation. 2009;119(7):1910-20.

162. Small C-L, Shaler CR, McCormick S, Jeyanathan M, Damjanovic D, Brown EG, et al. Influenza Infection Leads to Increased Susceptibility to Subsequent Bacterial Superinfection by Impairing NK Cell Responses in the Lung. The Journal of Immunology. 2010;184(4):2048-56.

163. Klouwenberg PK, Tan L, Werkman W, van Bleek GM, Coenjaerts F. The Role of Toll-like Receptors in Regulating the Immune Response against Respiratory Syncytial Virus. 2009;29(6):531-50.

164. Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, van Loo G, et al. Identification of Oxidative Stress and Toll-like Receptor 4 Signaling as a Key Pathway of Acute Lung Injury. Cell. 2008;133(2):235-49.

165. Karlström Å, Heston SM, Boyd KL, Tuomanen EI, McCullers JA. Toll-Like Receptor 2 Mediates Fatal Immunopathology in Mice During Treatment of Secondary Pneumococcal Pneumonia Following Influenza. The Journal of Infectious Diseases. 2011;204(9):1358-66.

166. Hasegawa K, Mansbach JM, Camargo CA. Infectious pathogens and bronchiolitis outcomes. Expert Review of Anti-infective Therapy. 2014;12(7):817-28.

167. Mansbach JM, Hasegawa K, Henke DM, Ajami NJ, Petrosino JF, Shaw CA, et al. Respiratory syncytial virus and rhinovirus severe bronchiolitis are associated with distinct nasopharyngeal microbiota. Journal of Allergy and Clinical Immunology.137(6):1909-13.e4.

168. Weinberger DM, Klugman KP, Steiner CA, Simonsen L, Viboud C. Association between Respiratory Syncytial Virus Activity and Pneumococcal Disease in Infants: A Time Series Analysis of US Hospitalization Data. PLOS Medicine. 2015;12(1):e1001776.

169. Smith CM, Sandrini S, Datta S, Freestone P, Shafeeq S, Radhakrishnan P, et al. Respiratory Syncytial Virus Increases the Virulence of Streptococcus pneumoniae by Binding to Penicillin Binding Protein 1a. A New Paradigm in Respiratory Infection. American Journal of Respiratory and Critical Care Medicine. 2014;190(2):196-207.

170. Wolf AI, Strauman MC, Mozdzanowska K, Whittle JRR, Williams KL, Sharpe AH, et al. Coinfection with Streptococcus pneumoniae Modulates the B Cell Response to Influenza Virus. Journal of Virology. 2014;88(20):11995-2005.

171. Bellinghausen C, Gulraiz F, Heinzmann ACA, Dentener MA, Savelkoul PHM, Wouters EF, et al. Exposure to common respiratory bacteria alters the airway epithelial response to subsequent viral infection. Respiratory Research. 2016;17(1):68.

172. Wylie KM, Mihindukulasuriya KA, Sodergren E, Weinstock GM, Storch GA. Sequence Analysis of the Human Virome in Febrile and Afebrile Children. PloS one. 2012;7(6):e27735.

173. Pitkäranta A, Roivainen M, Blomgren K, Peltola J, Kaijalainen T, Räty R, et al. Presence of viral and bacterial pathogens in the nasopharynx of otitis-prone children. International Journal of Pediatric Otorhinolaryngology. 2006;70(4):647-54.

174. Moore HC, Jacoby P, Taylor A, Harnett G, Bowman J, Riley TV, et al. The Interaction Between Respiratory Viruses and Pathogenic Bacteria in the Upper Respiratory Tract of Asymptomatic Aboriginal and Non-Aboriginal Children. The Pediatric Infectious Disease Journal. 2010;29(6):540-5.

175. Jartti T, Jartti L, Peltola V, Waris M, Ruuskanen O. Identification of Respiratory Viruses in Asymptomatic Subjects: Asymptomatic Respiratory Viral Infections. The Pediatric Infectious Disease Journal. 2008;27(12):1103-7.

176. Rosas-Salazar C, Shilts MH, Tovchigrechko A, Schobel S, Chappell JD, Larkin EK, et al. Differences in the Nasopharyngeal Microbiome During Acute Respiratory Tract Infection With Human Rhinovirus and Respiratory Syncytial Virus in Infancy. The Journal of Infectious Diseases. 2016;214(12):1924-8.

177. Bittinger K, Charlson ES, Loy E, Shirley DJ, Haas AR, Laughlin A, et al. Improved characterization of medically relevant fungi in the human respiratory tract using next-generation sequencing. Genome Biology. 2014;15(10):487.

178. Kieninger E, Singer F, Tapparel C, Alves MP, Latzin P, Tan H-L, et al. High Rhinovirus Burden in Lower Airways of Children With Cystic Fibrosis. Chest. 2013;143(3):782-90.

179. Reponen T, Vesper S, Levin L, Johansson E, Ryan P, Burkle J, et al. High environmental relative moldiness index during infancy as a predictor of asthma at 7 years of age. Annals of Allergy, Asthma & Immunology. 2011;107(2):120-6.

180. van Woerden HC, Gregory C, Brown R, Marchesi JR, Hoogendoorn B, Matthews IP. Differences in fungi present in induced sputum samples from asthma patients and non-atopic controls: a community based case control study. BMC Infectious Diseases. 2013;13(1):69.

181. Roux D, Gaudry S, Dreyfuss D, El-Benna J, de Prost N, Denamur E, et al. Candida albicans impairs macrophage function and facilitates Pseudomonas aeruginosa pneumonia in rat\*. Critical Care Medicine. 2009;37(3):1062-7.

182. Xu H, Sobue T, Thompson A, Xie Z, Poon K, Ricker A, et al. Streptococcal co-infection augments Candida pathogenicity by amplifying the mucosal inflammatory response. Cellular Microbiology. 2013;16(2):214-31.

183. Delhaes L, Monchy S, Fréalle E, Hubans C, Salleron J, Leroy S, et al. The Airway Microbiota in Cystic Fibrosis: A Complex Fungal and Bacterial Community—Implications for Therapeutic Management. PloS one. 2012;7(4):e36313.

184. Cui L, Lucht L, Tipton L, Rogers MB, Fitch A, Kessinger C, et al. Topographic Diversity of the Respiratory Tract Mycobiome and Alteration in HIV and Lung Disease. American Journal of Respiratory and Critical Care Medicine. 2015;191(8):932-42.

185. Brown RL, Clarke TB. The regulation of host defences to infection by the microbiota. Immunology. 2017;150(1):1-6.

186. Deasy AM, Guccione E, Dale AP, Andrews N, Evans CM, Bennett JS, et al. Nasal Inoculation of the Commensal Neisseria lactamica Inhibits Carriage of Neisseria meningitidis by Young Adults: A Controlled Human Infection Study. Clinical Infectious Diseases. 2015;60(10):1512-20.

187. Simonyte Sjödin K, Vidman L, Rydén P, West CE. Emerging evidence of the role of gut microbiota in the development of allergic diseases. Current Opinion in Allergy and Clinical Immunology. 2016;16(4):390-5.

188. Popova M, Molimard P, Courau S, Crociani J, Dufour C, Le Vacon F, et al. Beneficial effects of probiotics in upper respiratory tract infections and their mechanical actions to antagonize pathogens. Journal of Applied Microbiology. 2012;113(6):1305-18.

189. Esposito S, Rigante D, Principi N. Do children’s upper respiratory tract infections benefit from probiotics? BMC Infectious Diseases. 2014;14(1):194.

190. Hall AB, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in disease. Nat Rev Genet. 2017;advance online publication.