The role of the local microbial ecosystem in respiratory health and disease

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Respiratory tract infections are a major global health concern, accounting for high morbidity and mortality, especially in young children and elderly individuals. Traditionally, highly common bacterial respiratory tract infections, including otitis media and pneumonia, were thought to be caused by a limited number of pathogens including *Streptococcus pneumoniae* and *Haemophilus influenzae*. However, these pathogens are also frequently observed commensal residents of the upper respiratory tract (URT) and form—together with harmless commensal bacteria, viruses and fungi—intricate ecological networks, collectively known as the ‘microbiome’. Analogous to the gut microbiome, the respiratory microbiome at equilibrium is thought to be beneficial to the host by priming the immune system and providing colonization resistance, while an imbalanced ecosystem might predispose to bacterial overgrowth and development of respiratory infections. We postulate that specific ecological perturbations of the bacterial communities in the URT can occur in response to various lifestyle or environmental effectors, leading to diminished colonization resistance, loss of containment of newly acquired or resident pathogens, preluding bacterial overgrowth, ultimately resulting in local or systemic bacterial infections. Here, we review the current body of literature regarding niche-specific upper respiratory microbiota profiles within human hosts and the changes occurring within these profiles that are associated with respiratory infections.

1. Introduction

The human body harbours a large variety of microbial communities: intricate interacting networks consisting of a myriad of bacteria, fungi, viruses, bacteriophages, archaea and eukaryotes, that colonize different body surfaces, including the skin [1], vagina, oral cavity, gut [2], upper respiratory tract (URT) [3] and lung [4]. The recent advent of high-throughput sequencing methods has made it possible to study these communities and their relationship with health and (chronic) disease in detail. Particular interest has been paid to the bacterial communities or ‘microbiome’ of the gut [5], and its role in metabolism [6], immune maturation, mucosal barrier functions [7] and colonization resistance [8,9]. These studies have led to a paradigm shift where the old ‘one pathogen/one disease’ theory as postulated by Dr Robert Koch in the nineteenth century, has been gradually replaced by the theory that human health is the outcome of a complex, interconnected network of interactions between microbes and their host [10].

Although much less extensively studied, we postulate that the URT microbiome is, analogously to the gut microbiome, a strong determinant of respiratory health. When the microbiome is perturbed, for example by antibiotic treatment or disease state, potential pathogens (hereafter termed ‘pathobionts’) such as *Streptococcus pneumoniae*, can overgrow, spread and ultimately result in acute local or disseminated respiratory infections, such as acute otitis media (AOM), pneumonia, septicaemia or meningitis.
The URT exhibits many important physiological functions such as filtering, humidifying and warming of inhaled air [11] and consists of a communicating system comprising the anterior nares, nasal cavity, nasopharynx, sinuses, Eustachian tube, middle ear cavity, oral cavity, pharynx and larynx, forming the interface between the external environment and the lower respiratory and gastrointestinal tract (figure 1) [12,13]. The mucosal surfaces of these niches are colonized with a wide array of bacteria, mostly members of the phyla Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria and Fusobacteria [3,14,15]. However, large differences in microbial profiles can be observed between niches at lower taxonomic levels. We hypothesize that these differences arise from specific niche characteristics, resulting from variations in humidity, acidity and epithelial cell type. Interestingly, the interplay between these abiotic components and microbiota seems to adhere to known principles in general ecology, such as environmental selection, dispersal, speciation and drift [16], which enables us to describe and potentially even predict ecosystem dynamics in health and disease.

In this review, we will focus on the ecological interactions within the bacterial URT microbiome in health and in acute respiratory diseases. First, we describe the relevant ecological processes that direct bacterial community assembly in the human URT (§2). Then, in §§3–6 we discuss niche-specific variations in microbial composition and elaborate on possible mechanistic explanations for the observed co-occurrence and co-exclusion patterns. Finally, we will describe changes in bacterial community composition in relation to local and disseminated infectious diseases (§§7,8).

2. The human upper respiratory tract niches from an ecological perspective

The human URT is a unified system of skin and mucosal surfaces that stands in direct and continuous contact with the outer world. The nostrils or anterior nares are closest to the outside environment and skin surface, and are lined with skin-like keratinized squamous epithelium, containing sebaceous and serous glands [17]. This niche acts as the vestibule to the nasal cavity, which is characterized by pseudostratified ciliated columnar epithelium containing mucin-secreting goblet cells (respiratory epithelium) [18]. The nasal cavity serves as the collection site for secretions of the frontal, maxillary, ethmoidal and sphenoidal sinuses and constitutes a continuum with the more posteriorly located nasopharynx, which reaches from the choanae anteriorly to the posterior wall of the nasopharynx and is covered with adenoid gland tissue. The lateral wall of the nasopharynx contains the pharyngeal ostium of the Eustachian tube, connecting the nasopharynx to the middle ear. Like the nasal cavity, the lateral nasopharyngeal cavity is carpeted with respiratory epithelium, but patches of stratified squamous epithelium appear with increasing age [18]. The nasopharynx is separated from the oropharynx by the soft palate, which enables
us to breathe through our nose and prevents nasal regurgitation of food [12]. Both the oropharynx and the oral cavity are lined with non-keratinized stratified squamous epithelium [18] and are part of both the respiratory and gastrointestinal tracts.

The URT is an interesting area from an ecological perspective: the constant exposure to the external environment and its accompanying conditions, and influences of respiration and gastrointestinal processes shape different habitats for a wide palette of microorganisms. Community ecology provides a large number of theoretical frameworks to explain bacterial community assembly, composition, diversity and its underlying processes. Historically, it was believed that microbes were cosmopolitan and arranged in ecosystems that were shaped purely by the force of environmental selection [19]. However, more recently, the idea that microbiological ecosystems are structured by a combination of both selective and chance-driven processes—selection, ecological drift, speciation and dispersal—has been increasingly embraced [20].

Of the processes involved, dispersal (i.e. movement of organisms across space) seems responsible for initial bacterial colonization of the URT. Directly after birth, the skin, gut, nasopharynx and mouth microbial communities are undifferentiated and colonized by bacterial species reflective of delivery mode: in vaginally delivered children Lactobacillus, Prevotella, Atopobium and Sneathia are predominant genera, whereas in infants born by caesarean section typical skin inhabitants such as staphylococci are initially observed [21]. Immaturity of the host's immune system enables tolerance of these initial colonists in the absence of a manifest inflammatory response; instead, early microbial exposure is believed to be vital to consecutive development of the immune system [22], which in turn contributes to local bacterial containment in a later stage through selection. Selection results from between-species differences in functionality or microbial fitness and drives variation in growth or replication rates, further shaping the bacterial community structure, both with regard to composition and relative abundance of species. Environmental pressure formed by constant exposure of the URT to the external environment and its accompanying conditions, as well as differentials in oxygen, pH, humidity, immunological factors, nutrients and epithelial cell type, ultimately shape different (micro)habitats within the URT [18,23].

Within these habitats, additional selection is mediated by interspecies interactions that can vary in both strength and directionality, i.e. can either be mutual, commensal or antagonistic [24]. Existing microbial assemblages are constantly challenged by new colonizers originating from both exogenous (i.e. inhalation of airborne microorganisms and contact with food-associated microbes) [25,26] and endogenous (i.e. draining middle ears and sinuses, regurgitation from stomach content and sputum from the lungs) sources. Apart from dispersal and selection, a third ecological process, speciation, might be involved in the development of a given microbial community. Speciation is the result of genetic isolation and local diversification, the latter of which is caused by adaptation of a bacterial species to its new environment by mutation and recombination (i.e. horizontal gene transfer). The fourth ecological process affecting bacterial community assembly is ecological drift, a chance-driven process that occurs in a community of functionally equivalent species. Speciation and ecological drift might play a role in development of respiratory bacterial communities after initial community assemble and ecological niche-specialization, though little evidence is available to support this hypothesis.

In concert, these four ecological processes structure interaction networks of bacterial species, resulting in ecosystems that can be characterized by their biodiversity. In ecosystem theory, biodiversity is a term used to quantify both species richness (i.e. number of species) and evenness (i.e. distribution of species) and is thought to give rise to ecosystem stability [27,28] and enhance ecosystem functioning [29,30]. In the URT, bacterial richness and evenness vary dramatically between niches, with the highest richness and evenness observed in the oropharynx and oral cavity [3,28]. Contrastingly, the anterior nares harbour a bacterial microbiome exhibiting low biodiversity and are thereby more comparable to other areas covered by skin-like epithelium [1,28].

3. The human upper respiratory tract niches: the anterior nares

The microbial ecosystem of the anterior nares is typically enriched for members of the phyla Actinobacteria (i.e. Corynebacterium and Propionibacterium spp.) and Firmicutes (i.e. Streptococcus in children and Staphylococcus spp. in adults) with a low abundance of anaerobes belonging to the Bacteroidetes phylum [28,31,32]. The number of Proteobacteria strongly varies, with studies reporting high abundance of Moraxellaceae in children [31,33], enrichment for Proteobacteria in adult ICU-patients (i.e. members of the orders Enterobacterales and Pseudomonadales) [34] and low abundance of members of the class Gammaproteobacteria in healthy adults [32].

Corynebacterium, Propionibacterium and Staphylococcus are the most frequently carried genera in the anterior nares, which is independent of ethnic backgrounds and geographical location [31,35]. The microbiome of the adult anterior nares can generally be sub-classified in one of four distinct microbial profiles or 'types', which are characterized by predominance of either Corynebacterium, Propionibacterium, Monoxella or Staphylococcus spp., suggesting that complex synergistic and antagonistic relationships between these bacteria are driving within-niche variation [35]. Interestingly, Corynebacterium- and Propionibacterium-enriched profiles seem to be more ‘tolerant’ to members of other genera, like staphylococci, in contrast to Monoxella-dominated bacterial communities.

The keratinized squamous epithelium of the anterior nares includes sebum-producing glands that seem responsible for the selective enrichment of lipophilic bacteria, like Propionibacterium spp. [14,36]. Propionibacterium spp. are capable of hydrolysing lipid-rich sebum, hence releasing short-chain free fatty acids [37]. As a consequence, the pH is lowered, inducing outgrowth of especially corynebacteria and coagulase-negative staphylococci, whereas the relatively moist and oxygen-rich conditions in the anterior nares will more specifically select for outgrowth of Staphylococcus aureus and corynebacteria [38]. Thus, the simultaneous presence of Propionibacterium and Staphylococcus spp. might be at least partly driven by different features of the local environment, although true synergism between both genera might also occur by the production of coproporphyrin III by Propionibacterium spp., promoting S. aureus biofilm formation [39]. The observation that co-presence between Corynebacterium and S. aureus is species-specific (it holds for Corynebacterium accolens but not for Corynebacterium pseudodiphteriticum) [14],
might explain previous ambiguous reports on either the synergistic or antagonistic interaction between these species in both mechanistic and epidemiological studies [34,35,40,41].

Although the niche characteristics seem similar between adults and children, a relatively higher abundance of Streptococcus, Moraxellaceae and Neisseriaceae was reported in the anterior nares of healthy children compared with healthy adults [31]. As these families are even more abundant in the adjacent nasopharynx, it remains uncertain whether their predominance in the anterior nares of children is merely a reflection of dispersal from their primary niche rather than a true difference in microbial equilibrium in the anterior nares of children compared with adults (figure 1a).

Alternatively, age-related niche-specific local immunity may play a role in keeping resident pathogens at bay. In the anterior nares, both epithelial cells and local immune cells, such as neutrophils and natural killer (NK)-cells, produce antimicrobial peptides (AMPs) and proteins, which form the first-line defence against invading microbes and coordinate downstream immune signalling [42]. Moreover, various fatty acids produced by the epidermis exhibit antimicrobial activity against for example S. aureus [43,44]. Microbial homeostasis in the anterior nares is additionally governed by cellular elements of immune system as illustrated by a study on nasal decolonization of S. aureus in mice, which was shown to be T-cell dependent and reliant on upregulation of proinflammatory cytokines such as IL-17A [45]. Especially the latter process will mature with age, potentially directing resident microbial community structure in an age-dependent manner.

4. The human upper respiratory tract niches: the nasopharynx

In line with the gut and anterior nares, variations in microbial profiles can be observed in the nasopharynx of individuals from early in life onwards. These initial nasopharyngeal bacterial assemblages are typified by predominance of species belonging to the genera Moraxella, Corynebacterium, Dolosigranulum, Streptococcus or Staphylococcus spp. [46] and are likely related to dispersal of bacterial species derived from the maternal skin (Staphylococcus and Corynebacterium spp.) or vaginal microbiome (Staphylococcus, Streptococcus and Dolosigranulum spp.) during the perinatal period. Additionally, the type of infant feeding seems to have an important role in both community establishment and subsequent development: exclusive breastfeeding at six weeks of life was associated with increased Corynebacterium/Dolosigranulum profiles, whereas S. aureus predominance was observed in infants who were exclusively formula fed [26]. Although several of the early profiles disappeared within the first months of life, initial profile signatures were indicative of successive nasopharyngeal microbiome composition and stability. More stable patterns were marked by early high abundance of Moraxella and Corynebacterium/Dolosigranulum spp., whereas the less stable profiles, characterized by high abundance of Streptococcus spp. and acquisition of Haemophilus influenzae over time, were associated with higher rates of parent-reported respiratory infections and wheezing in the first years of life [46]. In adults, apart from a clear absence of Moraxella spp., similar bacterial inhabitants of nasopharyngeal microbiota were identified, although the power is lacking to clearly identify distinct microbiome profiles in this age group [3,23,47–49].

Species observed in the nasopharynx show high overlap with the inhabitants of topographically proximate niches like the anterior nares (mainly Gram-positive aerobes, including Staphylococcus, Dolosigranulum, Corynebacterium and Propionibacterium spp.) and the oropharynx (Streptococcus spp.). Gram-negative anaerobes that are encountered primarily in the oropharynx and oral cavity, such as Prevotella and Veillonella spp., were also observed in low abundance in the nasopharynx of young children [23,26], which might be explained by frequent nasal regurgitation of oral content in this age group.

Mechanistic insights into the observed interactions between putative commensals and pathogens are sparse. Cultivation-based colonization studies typically date from before the era of next-generation sequencing techniques and exclusively focused on a selection of well-known pathobionts, including S. pneumoniae, Moraxella catarrhalis, H. influenzae, S. aureus and haemolytic streptococci. Well recognized is the co-exclusion of S. pneumoniae and S. aureus in the URT of immunocompetent individuals [50], which may partially be mediated by hydrogen peroxide production of S. pneumoniae [51], although CD4+ T-cell involvement is suggested by the apparent lack of this interaction in HIV-infected patients [52]. Exclusion patterns are also observed between S. pneumoniae and H. influenzae and could be driven by competition for adherence to the human platelet-activating factor receptor (PAF-r) [53,54], but also by modulation of the host innate immune system; for example, in animal models, H. influenzae colonization has been demonstrated to induce neutrophil recruitment and potentiate opsonophagocytic killing of co-colonizing pneumococci [55,56]. In return, resistance to H. influenzae-induced opsonophagocytosis is conveyed by natural selection for thicker pneumococcal capsules, consequently enhancing virulence and metabolic costs [57]. Xu & Pichichero [58] have recently shown the additional involvement of the adaptive immune system in bacterial interactions in healthy children, with pneumococcal-specific IgG and IgA antibody responses being enhanced by co-colonization of S. pneumoniae together with H. influenzae or M. catarrhalis.

On the other hand, synergistic co-occurrence patterns between species have also been observed, for example between M. catarrhalis and H. influenzae. This interaction is conferred indirectly by secretion of ubiquitous surface proteins carried by outer membrane vesicles (OMVs) of M. catarrhalis, that bind to the third component of complement (C3), hampering complement-mediated killing of H. influenzae [59]. Moreover, Schaar et al. [60] described that these OMVs carry β-lactamase, conveying resistance to amoxicillin-induced killing to susceptible M. catarrhalis, S. pneumoniae and H. influenzae. Intriguingly, although co-exclusion patterns were observed between S. pneumoniae, H. influenzae and M. catarrhalis in in vitro studies, symbiotic relationships between these bacterial community members have been observed in vivo, suggesting a major influence of selection by host epithelium and immune system on these microbe–microbe interactions [61].

The commensal genus Dolosigranulum, which is merely observed in the anterior nares and nasopharynx in both children and adults [3,14,62], has only recently been identified as a regular commensal of the URT. It is a fermenting lactic-acid-producing bacterium that is commonly co-occurring with corynebacteria, which are lipophilic and non-fermenting. We speculate that as the fermentation process of Dolosigranulum spp. generates lactic acid, which reduces the local pH, it might select for Corynebacterium spp. outgrowth. The presence of corynebacteria is strongly related to breastfeeding,
which might be explained by the fact that this genus is a core member of the human breast milk microbiome [63]. Interestingly, microbial communities characterized by high abundance of *Corynebacterium* and *Dolosigranulum* spp. lacked the presence of *H. influenzae* and *S. pneumoniae*, suggesting colonization resistance against these potential pathogens (figure 1b).

Additional host factors plausibly involved in shaping the local ecosystem occur in the mucus layer that forms a physical barrier and segregates resident bacterial flora in the nasopharyngeal lumen from the epithelial cell surface. The central purpose of this barrier is to limit contact between microbiota and host tissue to prevent low-grade local inflammation and thus translocation of pathogens [22]. Within the mucus layer AMPs and secretory IgA accumulate, further restricting exposure of epithelial cells to resident microbiota [64]. Except for direct action of innate immune components on microbial community composition, it has recently been postulated that mucus drives selection of bacterial communities adapted to metabolizing complex sugars contained in mucus [65]. The mucosal barrier is supported by mucosa-associated lymphoid tissue, which harbours antigen-presenting cells, B- and T-cells, and forms the interface between local innate and adaptive immunity.

5. The human upper respiratory tract niches: the oropharynx

The oropharynx links the mouth, the nasopharynx, the larynx, the lower airways and the gastrointestinal tract, and is thereby exposed to a wide variety of microorganisms, of both exo- and endogenous origins. The species pool from which the oropharyngeal niche can sample is therefore generally large, plausibly contributing to the highly diverse bacterial communities observed in both adults and the elderly. The oropharynx is also the ecological niche for potential pathogenic bacteria that may cause local (pharyngitis) or disseminated (pulmonary) disease [66]. Dispersal of oropharyngeal bacterial communities to the lower respiratory tract by (micro-)aspiration or inhalation is presumably a frequently occurring event both in health and disease, owing to the significant overlap between the oropharyngeal microbiome and bacterial communities observed in the healthy lung [67–69].

Metagenomic sequencing has demonstrated that the healthy adult oropharynx is colonized with pathobionts such as *Streptococcus*, *Haemophilus* and *Neisseria* spp. and the Gram-negative, anaerobic putative commensal genera *Veillonella*, *Prevotella*, *Leptotrichia* and *Fusobacterium* [3,70–74]. A recent study in children showed a similar oropharyngeal microbiome composition to that observed in adults, with enrichment of, most notably, *Neisseria*, *Granulicatella*, *Prevotella*, *Porphyromonas*, *Fusobacteriaceae* and certain *Prevotella* spp. [23].

The oropharynx is especially known to harbour several potentially pathogenic members of the genus *Streptococcus*, including *S. pneumoniae* [75], *S. pyogenes* (group A β-haemolytic *Streptococcus*) [76], *S. agalactiae* (group B β-haemolytic *Streptococcus*) [77] and *Streptococcus dysgalactiae* subsp. *equisimilis* (group C and G β-haemolytic streptococci) [78]. Particularly, *S. pyogenes* is known to cause a wide spectrum of diseases, varying greatly in severity from self-limiting pharyngitis to life-threatening sepsis and streptococcal toxic shock syndrome [79]. To this day, no definitive explanation has been found for this great spread in disease severity, although it has been postulated that interactions between different streptococcal species may play a role in pathogenesis [80]. These interactions are mediated by various quorum-sensing systems (i.e. systems regulating communication between bacterial cells by signalling molecules), which were shown to be involved in induction of biofilm formation *in vitro* [81] and virulence modulation *in vitro* and *in vivo* [82,83].

Contrariwise, antagonistic interactions involving streptococcal species have also been demonstrated. For example, *S. viridans*, a Group A α-haemolytic *Streptococcus*, reduces growth of known otitis media pathogens, *S. pneumoniae*, *M. catarrhalis* and *H. influenzae* *in vitro* [84], suggesting a possible role in containment of these pathobionts (figure 1c).

Apart from synergistic and antagonistic interactions between different streptococcal species, interactions between members of the *Streptococcus* genus and other genera have also been described; for example, *S. pyogenes* has been shown to co-occur with *M. catarrhalis* and non-typeable *H. influenzae* [85]. This epidemiological phenomenon could be mediated by enhanced adherence to and invasion of human epithelial cells, as was observed for *S. pyogenes* and *M. catarrhalis* aggregates *in vitro* [86]. Furthermore, *M. catarrhalis* and *H. influenzae* have been shown to reduce the susceptibility of *S. pyogenes* to penicillin by their production of β-lactamase-containing OMVs *in vitro*, potentially contributing to treatment failure in streptococcal tonsillitis [87].

6. The human upper respiratory tract niches: the oral cavity

Apart from the oropharynx, various sites in the oral cavity have been extensively characterized, particularly by the Human Microbiome Project, including the tongue dorsum, hard palate, sub- and supragingival plaque, buccal mucosa, palatine tonsils, keratinized gingiva and saliva. Similar to the oropharynx, the oral microbiome is subject to major, potentially perturbing factors, including hygiene measures (i.e. tooth brushing and mouthwash rinsing), mechanical forces through mastication, enzymatic activity in saliva, and temperature, pH and dietary alterations [88]. Moreover, like the oropharynx, the mouth is in close contact with the surrounding environment and is thus exposed to large numbers of food- and airborne bacteria, as well as indigenous pathogens from other URT niches. Despite these factors, the oral microbiome has been demonstrated to be highly stable over time [28], which might result from the large core microbiome shared among unrelated individuals [89].

The initial oral microbiome composition in children is, like the other niches, greatly influenced by mode of delivery [21], feeding type, with higher abundance of lactobacilli observed in breastfed children [25], and horizontal transfer of microbes [90]. Initiated by the eruption of teeth, local microbial compositions show an increase in Bacteroidetes (i.e. *Veillonellaceae* and *Prevotellaceae* spp.) and a decrease of Proteobacteria (i.e. *Moraxella* spp.) with age, gradually transitioning towards an adult-like microbiome configuration [91,92] distinguished by *Streptococcus* (presumably *oralis*/*mitis*/*pseudoprophilus*), *Veillonella*, *Selenomonas*, *Gemella*, *Fusobacterium*, *Prevotella*, *Lactobacillus* and *Neisseria* spp. [15,88]. Interestingly, saliva is characterized by relatively high amounts of *Prevotella*, *Neisseria* and *Haemophilus* spp., whereas sub- and supragingival plaques and keratinized gingiva are enriched for corynebacteria; this implies that there is
significant overlap between the oral bacterial community composition and the oropharyngeal microbiome, likely due to local dispersion of bacterial assemblages and similar environmental pressure. The antagonistic and synergistic interactions between bacteria observed in the oropharynx will therefore presumably similarly affect the spatial structure of microbial communities in the mouth.

Although not extensively researched for the URT, variations in microbial community structure have also been demonstrated to arise from differences in local availability of resources; for example, the oral cavity is enriched in bacterial carbohydrate-active enzymes responsible for degradation of simple sugars, such as dextran, whereas intestinal flora is capable of metabolising more complex carbohydrates [93].

Acute infection in the oral cavity is a rare event, presumably owing to the tolerogenic local circumstances mediated by dendritic cells (DCs) and various T-cell subtypes. Similar to the nasopharynx, the oropharynx and oral cavity are sustained by specialized mucosa-associated lymphoid tissue, which is imperative for antigen recognition and initiation of an adequate immune response [94]. The equilibrium between oral mucosal tolerance and inflammation is strongly guarded by DCs. DC-induced upregulation of Toll-like receptor (TLR)4 by resident microbes induces the production of immunosuppressive interleukins including IL-10, subsequently activating regulatory T-cells, thereby dampening inflammation and contributing to the tolerable local conditions [95]. Mucosal inflammation is further reduced by saliva-derived secretory IgA that covers the mucosal surfaces of the oral cavity, hindering contact between microbiota and host mucosa, thus preventing microbial attachment and immune activation, a mechanism referred to as ‘immune exclusion’ [96].

However, crosstalk between resident microflora and the host can also have a potentially unfavourable outcome as was illustrated in a murine model, showing that normal oral commensals can activate inflammasome inducing local release of the highly proinflammatory cytokine IL-1β [97].

7. Pathogenesis of respiratory infections: the pathogen perspective

Traditionally, microbiome alterations have been related to diseases with a chronic inflammatory character, such as obesity [98], COPD [99] and asthma [71]. However, the microbiome’s influence on (susceptibility to) acute upper and lower respiratory infections is increasingly gaining recognition.

Uncomplicated URT infections are highly prevalent and cause substantial morbidity worldwide; approximately 75% of children have suffered from AOM by the age of 3 years [100]. By contrast, lower respiratory tract infections are less common, but are accompanied by a high case-fatality rate [101]. In both types of infection, pathobionts generally residing asymptomatically in the URT are commonly involved [3,102,103], underscoring the need to better understand the pathogenesis of these infections.

Historically, the URT niche is regarded as the ecological niche of most potential respiratory pathogens, such as *S. pneumoniae, H. influenzae* and *S. aureus*. Up to 93% of children under 2 years of age are colonized by at least one potential pathogen when measured by conventional culture [61]; therefore, these pathobionts can without doubt be considered part of the residential flora. Colonization with these bacterial species might actually have an ambiguous role in the development of respiratory diseases: on the one hand, ‘pathogen’ carriage is a prerequisite for development of disease; on the other hand, pathobiont colonization does not necessarily lead to infection and has even been suggested to provide resistance against acquisition of new pathogenic strains [104]. As the risk of developing respiratory infections seems specifically related to recent acquisition of a new strain [104,105], a state of bacterial symbiosis, even including potential pathogens, may be protective against short-term infection and inflammation. Nevertheless, carriage can develop towards respiratory disease in a fraction of cases, a process that can be triggered by various exo- or endogenous stimuli [106–108]. Especially, this fact supports the hypothesis that overgrowth of a potential pathogen is not a random event, but instead a co-occurrence of multiple factors leading to lack of containment of the potential pathogen by the resident commensals and local immunity. Both seem more likely to fail following a recent acquisition of a new bacterium or viral co-infection, inducing a combined state of temporary dysbiosis in the absence of adaptive immunity.

Next-generation sequencing of the nasopharyngeal microbiome of healthy children and children suffering from mild URT infections, revealed that colonization with and density of a potential pathogen (*S. pneumoniae, M. catarrhalis* and *H. influenzae*) is related to a less evenly distributed microbiome, suggesting reduced containment by the commensal flora [33,109]. This is supported by the observations that increased abundance of the three AOM pathogens is accompanied by decreased presence of the commensals *Lactococcus, Anoxybacillus, Corynebacterium* and *Dolosigranulum* spp. Also, in contrast to the increased abundance of *Haemophilus and Streptococcus* spp. and members of the Pasteurellaceae during AOM, an increased abundance of *Dolosigranulum* and *Corynebacteria* spp. was associated with absence of AOM [33]. In addition, both biodiversity and bacterial density of the nasopharyngeal microbiome are lower in children diagnosed with AOM compared with healthy children, altogether suggesting ecological dysbiosis to be key to development of these infections [110]. This is further underlined by a study on the tonsillar crypt microbiome of children and adults suffering from recurrent tonsillitis, Proteobacteria (mainly *Haemophilus* spp.) were associated with disease, whereas Bacteroidetes (especially *Prevotella* spp.) abundance was associated with absence of disease [111]. Particularly, this latter finding was in conjunction with a study on the oropharyngeal microbiome in (elderly) pneumonia patients, which showed that lack of members of the Bacteroidetes phylum including *Prevotella* spp. as well as other Gram-negative anaerobic species like *Leptotrichia* and *Veillonella* spp. is associated with pneumonia [112].

The hypothesis that the abundance of pathogenic species can be modulated by the resident bacterial flora has been comprehensively addressed in a study on the association between sinus microbiome changes and chronic rhinosinusitis (CRS). In patients suffering from CRS, the investigators observed increased abundance of *Corynebacterium tuberculostearicum* and reduced abundance of bacterial members belonging to the families Lactobacillaceae, Enterococcaceae, Aerococcaceae and Streptococcaceae. Strikingly, mice depleted from their resident sinus microbiome developed a more severe CRS phenotype upon administration of *C. tuberculostearicum* compared with mice with an intact sinus microbiome [113], suggesting resident bacterial community members are not mere bystanders, but actually have an important role in containing pathogens.
Unfortunately, there are no studies available yet using next-generation sequencing methods to determine a possible association between URT microbiota and acute rhinosinusitis, laryngitis or pharyngitis. Conventional studies, however, suggest that acute rhinosinusitis is caused most frequently by viral agents, although a secondary bacterial infection by, among others, S. pneumoniae and H. influenzae has been observed in a limited number of cases [114]. Similarly, the majority of infectious laryngitis cases have a viral origin, potentially predisposing to bacterial laryngitis, caused by pathogens such as H. influenzae type b, S. pneumoniae, S. aureus, β-haemolytic streptococci, M. catarrhalis and Klebsiella pneumoniae [115]. Cultivation-based approaches in pharyngitis patients demonstrated that β-haemolytic streptococci other than group A or C, S. pyogenes (group A Streptococcus), S. equisimilis and Streptococcus anginosus were isolated more frequently compared with healthy controls [116]. However, the possible (protective) role of commensals in these pathogenic processes has so far been largely ignored.

Imbalance of the oral microbiome has been investigated, especially in relation to the development of periodontal disease, caries and cardiovascular health [117], showing a central role for keystone pathogens Porphyromonas gingivalis, Treponema dentium and Tannerella forsythia [118,119]. We postulate that an imbalanced oral microbiome might also have an effect on respiratory health through physiological episodes of micro-aspiration [66]. Compelling evidence for the importance of oral health and micro-aspiration is generated by a study in residents of nursing homes, showing that neglect of oral health measures leads to a dramatic increase in incidence of and mortality by pneumonia [120].

We therefore speculate that the healthy URT microbiome in general provides us with colonization resistance, which is defined as (i) resistance against acquisition and establishment of a new pathogen or (ii) containment of potentially pathogenic bacteria residing amidst harmless commensals in a balanced microbiome [121].

8. Pathogenesis of respiratory infections: the ecosystem perspective

Colonization resistance against foreign pathogens can be provided by occupation of otherwise vacant URT niches by indigenous flora. As a result, an invading pathogen has to compete with the commensal flora for adhesion receptors on the mucosal surface as well as nutrients, limiting the chances that the intruding species is indeed able to adhere, replicate, disseminate and cause disease. Direct evidence that this mechanism also takes place in the URT is sparse, although it has been demonstrated that following antibiotic treatment, which leads to a reduction of bacterial community density and diversity, the risk of (a new) AOM is temporarily increased [33,122].

Apart from environmental differences in the URT linked to human physiology, bacteria are also able to modify their environment themselves. Short-chain fatty acid (SCFA) produced by fermenters such as Bacteroidetes and Prevotellales spp., has been shown to directly inhibit pathogenic growth and expression of virulence genes of pathogens in the gut and is able to indirectly modulate bacterial growth conditions by acidification of the environment [123]. Direct inhibition of competing microbes by production of antimicrobial compounds is exemplified by the production of hydrogen peroxide by S. pneumoniae, which is bactericidal for S. aureus [51]. Moreover, some bacterial species produce peptides with antimicrobial efficacy, which are generally referred to as bacteriocins [124]. This mechanism is illustrated by the commensal streptococcal species S. salivarius, which presumably inhibits pathogenic S. pyogenes growth by production of bacteriocins. Although the URT stands in direct contact with the outside world, we postulate the large surface of the nasal cavity contains niches covering a range of oxygen levels and carbon dioxide levels additionally shaping the ecosystem’s habitat (figure 2b).

Biodiversity has been linked to higher resilience against disturbances, such as antibiotic treatment and invasion of new pathogens [125]. Diverse microbial communities might contribute to colonization resistance through various mechanisms, for example by heightening the chance that all resources are used which would otherwise be readily available for pathogens [126]. The maintenance of biodiversity in ecological communities has been related to the presence of so-called keystone species, loss of which would result in extinction of many other (micro-)organisms, hence decreasing the community’s biodiversity (figure 2i) [127,128]. A clear example is the family Christensenellaceae which was recently identified as a potential keystone taxon of the gut microbiome because its presence or absence directly modulated the host’s phenotype [129]. Although keystone species involved in maintenance of the community structure of the URT microbiome have not been identified yet, stability of bacterial communities over time has been linked to the presence of certain putative commensals, such as Monoxella, Dolosigranulum and Corynebacterium spp., resulting in a more healthy host phenotype [46]. Contrarily, early colonization with pathogens such as S. pneumoniae, H. influenzae and M. catarrhalis has been shown to result in a long-term increased risk of pneumonia and bronchiolitis in healthy neonates [130].

Colonization resistance might be diminished by bacterial community structures that induce a low-grade, asymptomatic inflammation, which facilitates invasion by pathogenic bacteria. Studies have shown that colonization with Proteobacteria such as non-typeable H. influenzae is accompanied by mucosal inflammation, characterized by increased epithelial thickness of the large airways and production of early pro-inflammatory cytokines [131]. Contrarily, colonization with commensal Prevotella spp. was associated with low cytokine production, reduced recruitment of neutrophils and leucocytes, and no detectable lung tissue pathology. The more severe inflammation caused by non-typeable H. influenzae colonization is presumably mediated in a TLR4-dependent manner [132]. The ligand for TLR4 is lipopolysaccharide (LPS), a major proinflammatory constituent of the outer cell membrane of Gram-negative bacteria [133]. The immunostimulatory capacity of LPS has been related to its structure: hepta- or hexa-acylated LPS observed in Gammaproteobacteria such as H. influenzae induces a strong TLR4-mediated inflammatory response in contrast to tetra- and penta-acylated LPS, which is typically found in Bacteroidetes members such as Prevotella spp. and has very little TLR4-stimulatory activity [132]. Bacteroidetes-associated LPS even antagonises hexa-acylated LPS-mediated TLR4-signalling [134], dampening downstream signalling, thereby contributing to mucosal homeostasis [135] and colonization resistance versus pathogens (figure 2c).

Apart from variations in the proinflammatory capacity related to the bacterial composition of the human microbiome, mucosal inflammation can also be induced by viral infection,
Figure 2. Hypothetical model on the relationship between the URT microbiome and pathogenesis of infectious respiratory diseases. This figure shows the potential mechanistic explanations for the influence of the URT microbiome on colonization resistance, reduction of which is presumed to be at the core of the pathogenesis of acute respiratory infections, such as AOM, pharyngitis and tonsillitis. In (a), we show the optimal situation in which keystones species, for example Bacteroidetes members for the oropharynx, are at the base of a diverse ecological system of bacterial interactions. (b) Depicts a situation in which synergistic or antagonistic interspecies interactions modulate microbial composition and biodiversity, consequently regulating colonization resistance. For example, the release of metabolic by-products, such as short chain fatty acids (SCFAs) from fermenting bacteria, results in acidification of the environment and inhibition of growth of acidophobic bacteria, such as *S. aureus*. Furthermore, production of bactericidal products such as hydrogen peroxide or bacteriocins results in exclusion of certain bacterial species. In (c), we demonstrate that a Proteobacteria-rich microbiome is associated with a low-grade inflammation through activation of Toll-like receptor 4 (TLR-4) by hepta- or hexa-acylated lipopolysaccharide (LPS), which might forego invasion of pathogenic species. This immunostimulatory effect is reduced and even counteracted when the TLR-4 receptor is activated by tetra- and penta-acylated LPS, of members of the phylum Bacteroidetes, such as *Prevotella* spp. (d) Demonstrates the situation in which viral infection results in exposure of fibrinogen (not shown) through virus-induced epithelial cell damage. Additionally, viral infection, but also the presence of potential pathogenic bacterial species such as *H. influenzae* results in upregulation of adhesion receptors. Moreover, viral infection reduces epithelial integrity, thus contributing to bacterial translocation. Finally, the presence of influenza virus increases the availability of host-derived sialic acid-rich mucins, resulting in increased pneumococcal replication. For colour-codes of various bacterial community members, see the legend of figure 1. ICAM-1, intercellular adhesion molecule 1; CEACAM-1, carcinoembryonic antigen-related cell adhesion molecule 1; PAF-r, platelet-activating factor receptor; IL, interleukin; TNF, tumour necrosis factor. Δ: change in/of.

thereby facilitating adhesion and invasion by pathogenic bacterial species. Viral infections may induce direct damage to ciliated cells, diminishing mucociliary clearance [136,137], resulting in denudation of the epithelium [136], exposing fibronectin, to which pathogens such as *S. pneumoniae*, and to a lesser extent *S. aureus* and *S. pyogenes*, can adhere [138]. Except for fibronectin, other surface molecules or cell receptors, including intercellular adhesion molecule 1 (ICAM-1), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1) and PAF-r are upregulated in response to viral infection, also enhancing bacterial adhesion [139]. Eventually, viral infection can diminish epithelial integrity and promote bacterial translocation [140]. In addition to facilitating adherence and translocation of bacterial cells, viruses can directly affect a plethora of components of the innate immune system in particular, such as impairment of neutrophil function [141], NK cell activation and recruitment [142] and alteration of cytokine profiles [143,144], which might all contribute to reduced colonization resistance against pathobionts, thereby predisposing to bacterial superinfection. Third, influenza virus-induced inflammation leads to increased expression of sialic acid-rich mucins such as Muc5AC, which enhances pneumococcal proliferation [145]. Besides evidence from in vitro studies, there is also evidence from in vivo studies showing that a broad panel of respiratory viruses enhance pathobiont colonization in both adults and children [61,146].

The reverse relationship, i.e. pathobiont presence enhancing viral acquisition and replication, might also occur: for example, *H. influenzae* has been shown to induce expression of ICAM-1 and TLR3-receptors, which enhanced rhinovirus binding and stimulated rhinovirus-induced chemokine production [147]. Furthermore, a recent in vitro study has shown that *H. influenzae* similarly increases viral replication of respiratory syncytial virus, which was related to the release of proinflammatory cytokines IL-6 and IL-8, suggesting specific bacterial community members can aggravate (inflammatory response to) viral infection [148]. Furthermore, pre-incubation of human bronchial epithelial cells with *S. pneumoniae* leads to increased susceptibility to infection with human metapneumovirus (figure 2d) [149].

9. Conclusion and future directions
In this review, we have made an attempt to summarize the current body of evidence regarding the role of the healthy respiratory microbiome in URT health. We postulate that its composition is orchestrated by biotic components such as
microbe–microbe and microbe–host interactions, and by
abiotic components, notably acidity and oxygen availabil-
ity. We propose that an imbalanced URT microbiome is key to
pathogenesis of acute URT infections like AOM and pharyn-
gitis, but may also play a role in the development of lower
respiratory tract infections such as pneumonia. We believe
that the healthy URT microbiome provides us with local col-
onization resistance, which is influenced by the microbiome
composition, biodiversity, host factors and viral infection.
We postulate that a lack of putative commensals might lead
to outgrowth and disentainment of pathogens, such as
S. pneumoniae, H. influenzae, S. pyogenes and M. catarrhalis,
consequently resulting in respiratory illness.

Research effort regarding the role of the URT microbiome
in relation to respiratory health is currently lacking longitu-
dinal study approaches to demonstrate potential cause–effect
relationships between the presence or absence of specific
(groups of) bacterial genera or species and risk of infectious dis-
eases, that might provide us with information of predictive and
potentially preventive value. In addition, experimental in vitro
models and animal challenge models could help to obtain
mechanistic insight into the role of specific (groups of) bacteria
in ecosystem behaviour and host immunity [49]. The drawback
of such models is a lack of generalizability to the human host
and the complexity necessary to provide the complete picture
of ecosystem function. However, in combination, these types
of studies could ultimately help to identify keystone species
that are of major importance to the modulation of the total struc-
ture of microbe–microbe interactions resulting in respiratory
health [129].

Once such species are identified, studies need to be
undertaken to determine whether dysbiotic ecosystems could be
modulated in such a manner that the resulting conditions
resemble for beneficial commensals. A second preventative
strategy could be to study the possibility to reconstitute ‘miss-
ing microbes’ by administration of pre- and probiotics [130].
Although pre- and probiotics could potentially have a signifi-
cant preventative value, it could be of additional value to study
whether these measures might also exert a therapeutic
effect. To advance the field of therapeutics, we could addi-
tionally study the use of small spectrum antimicrobial agents,
ideally targeting a single species or even one specific pathogenic
strain, minimizing the bystander effects current antimicrobials
have. The first steps in this direction have been taken recently:
researchers employed the CRISPR-Cas genome engineering
system to generate RNA-guided nucleases targeting specific
antibiotic resistance or virulence genes of pathogenic bacteria
living in complex microbial communities, exerting an efficient
and specific antimicrobial effect, without disturbance of
indigenous bacterial communities [151].

Further studies investigating cause and effect relation-
ships regarding the above mechanisms are of the utmost
importance and prelude studies on modulation of the com-
plex bacterial ecology in the URT in an attempt to treat and
prevent respiratory infections in the distant future.

Competing interests. E.A.M.S. declares to have received unrestricted
research support from Pfizer, grant support for vaccine studies
from Pfizer and GlaxoSmithKline and fees paid to the institution
for advisory boards or participation in independent data monitoring
committees for Pfizer and GSK. No other authors reported financial
disclosures.

Funding. This research has received funding from the Wilhelmina
Children’s Hospital Fund, ZonMW (grant no. 91209010).

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