Asymptomatic Carriage of Respiratory Pathogens: "The Wolf shall Dwell with the Lamb…and a Little Child shall Lead them" (*Isaiah 11: 6*)

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Abstract: The human respiratory mucosa becomes colonized by a wide array of bacterial species in early life. Residing microorganisms comprise commensal as well as potentially virulent bacterial species that have adapted to and co-evolved with their host. Because of the specific environmental conditions prevalent in the respiratory tract, colonizing organisms have developed functionally similar components that enable them to thrive on the mucosal surfaces and establish chains of person-to-person spread through droplet transmission. Members of the normal respiratory flora exhibit complex relations ranging from cooperation to mutual interference. This normal flora is maintained under strict control by the integrity of the respiratory epithelium and immunological phenomena, whereas residing bacteria show remarkable antigenic variability that guarantees survival of the species through a constant turnover of colonizing strains. The overall composition of carried organisms can be deeply affected by antibiotic exposure and use of conjugate vaccines. Although bacterial carriage is usually asymptomatic, contributory factors such as young age, primary or acquired immunodeficiencies, viral infections, and emergence of hypervirulent clones, may facilitate the development of mucosal or systemic disease.

Keywords: Respiratory colonization, carriage, transmission, invasive infection, immunity, children.

FROM CLINICAL DISEASE TO ASYMPTOMATIC COLONIZATION

Since ancient times mankind has been affected by plagues such as cholera, tuberculosis or malaria that have wiped-out individuals and entire populations, and shaped human history. Consequently, research in the field of infectious diseases has been motivated by the will to fight illness and cure patients. With the discovery that infections are caused by living creatures in the last decades of the 19th century, specific strategies designed to preventing disease and detecting their causative agents soon developed. These impressive advances were followed by development of drugs aimed to selectively kill pathogenic microorganisms, and many diseases that previously caused morbidity and mortality on a large scale were brought under control.

This disease-centered approach lead to a Manichean view in which man represented the good and microbes, the quintessence of evil. This well-entrenched belief overlooked the perplexing fact that some of the most deadly bacterial pathogens, such as pneumococci or *Staphylococcus aureus,* are frequently found as components of the normal flora and are carried without symptoms by a large fraction of healthy individuals, and particularly young children.

Inquiry into this paradox has progressively demolished the traditional view, replacing it by a deeper understanding of a relationship that, although established long ago, keeps evolving by the combined effects of selective pressure on the part of the host and remarkable adaptability on the part of the microorganisms.

MUCOSAL COLONIZERS: AN EXCLUSIVE SOCIAL CLUB

Exposure of a susceptible individual to respiratory bacteria often results in adherence of the organism to the mucosal surfaces, followed by *in-situ* multiplication. In most cases, asymptomatic bacterial colonization, rather than clinical disease, is the outcome of this encounter.

Acquisition of the normal upper respiratory tract flora starts shortly after birth and, over time, the bacterial population increases in density and diversifies in a generally orderly fashion, comprising commensal as well as potentially pathogenic species [1-4]. The prevalence of mucosa-associated bacteria gradually raises between the ages of 6 months and 2 years, decreasing thereafter [1, 4]. This pattern is the net result of multiple interacting factors such as increasing socialization and exposure with age, vanishing maternally derived antibodies, and the inability to clear encapsulated bacteria in early childhood caused by physiological delay in the maturation of the T-cell independent arm of the immune system [1]. Once immunological maturation occurs, bacterial clearance is facilitated and colonization rates, as well as susceptibility to infection, decline.

The upper respiratory environment demands strict requirements for membership in the normal flora club: the ability to attach to the host's mucosal lining and avoid

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washout through surface-exposed molecular mechanisms, utilize available nutrients, and mitigate the local immune response. Colonizing organism should be also able to use droplets of respiratory secretions as a vehicle for dissemination to new susceptible hosts, establishing endless chains of person-to-person transmission in space and time. Therefore, as the result of convergent evolution, organisms as diverse as *Haemophilus influenzae* (a gram-negative pleomorphic coccobacillus) and *Streptococcus pneumoniae* (a gram-positive coccus), show functionally similar components, such as capsules, pili, and adhesins, which enable them to thrive in the shared upper respiratory niche.

The human respiratory microbiota is, by no means, a random mixture of organisms living in a chaotic neighborhood, but an ecosystem organized according to bio-geographic boundaries. For instance, *S. aureus* is an inhabitant of the anterior nares, *S. pneumoniae*, meningococci, and *H. influenzae* tend to concentrate in the nasopharynx (and in lesser numbers in the oropharynx), and *Kingella kingae* colonizes the tonsillar surfaces.

The microbial consortium is in a constant state of flux: bacteria are acquired, eliminated and re-acquired many times over during life, and subtypes within a given species also show a remarkable turnover [2]. The noteworthy heterogeneity of pneumococcal polysaccharide capsules (more than 90), or those of encapsulated *H. influenzae* (6), or the outer-membrane proteins of *Moraxella catarrhalis* [5] and *K. kingae* [6] indicate that these components, which are crucial for surviving in the respiratory tract, undergo antigenic variation due to high selective pressure. Although a specific immune response would eradicate the resident strain and prevent re-colonization by a similar organism, the antigenic variability of these key factors would enable colonization by a heterologous strain [2, 5, 6].

IT'S A JUNGLE IN THERE

The complex relationship between eukaryotic hosts cells and colonizing prokaryotic microorganisms has its counterpart in the cell-to-cell interaction between the various carried species. When an ecological niche is already occupied by an organism, other bacteria do not seem to be able to replace the resident population [7]. It has been shown that isolation of *viridans* group streptococci in the pharynx is negatively associated with the presence of pneumococci, *Streptococcus pyogenes* and *S. aureus* [8], while carriage of *Neisseria lactamica* protects from meningococcal colonization in children [9]. This bacterial interference is mediated by release of toxic products (such as hydrogen peroxide or bacteriocins), induction of cross-reactive antibodies, recruitment and activation of polymorphonuclear leukocytes [10], and contest for receptors on the respiratory surfaces or essential nutrients [11].

Bacterial interference not only limits the ability of competing species to colonize a common territory, but also exists between different members of a given species. Transformation-competent *S. pneumoniae* kill pneumococci that do not share the same cognate protein through strain-specific antimicrobial peptides known as pneumocins [12]. Interestingly, this "pneumococcal fratricide" releases DNA from lysed organisms, increasing horizontal gene transfer between strains and enhancing genetic diversification [13].

NORMAL FLORA SELECTION BY VACCINES AND ANTIBIOTICS

In recent years, a 7-valent pneumococcal conjugate vaccine has been licensed and added to regular pediatric vaccination programs in many countries, representing the first vaccine designed against a component of the normal respiratory flora. The vaccine has been remarkably effective in reducing the burden of invasive infections, pneumonia, and acute otitis media in young vaccinees [14]. The vaccine reduces nasopharyngeal colonization by serotypes represented in the preparation, and induces herd immunity in unimmunized individuals living in the same household [14, 15]. There is compelling evidence of substitution of vaccine-serotypes by non-vaccine organisms in asymptomatic carriers, as well as among infected individuals, mostly in compromised patients [16]. Because mutual inhibition between *S. aureus* and *S. pneumoniae* carriage has been demonstrated [17], concern has been raised that the widespread use of conjugate pneumococcal vaccines will increase staphylococcal colonization in an era of escalating incidence of serious infections caused by community-associated methicillin-resistant *S. aureus* [18].

The selective pressure exerted by antibiotics has resulted in loss of biodiversity, conferring clear advantage to antibiotic-resistant microbial clones that have disseminated widely among carriers and are causing difficult-to-treat infections [19]. Overall antimicrobial drug consumption, use of antibiotics for seemingly viral syndromes such as the common cold, inadequate low antibiotic dosages, and long duration of treatments have been identified as selectors for resistant organisms [20, 21]. Not only the magnitude of antibiotic exposure matters, but also the characteristics of the drug are important. The long half-life of azithromycin makes this drug an attractive agent for children but its slow elimination results in a prolonged subinhibitory concentration. Children treated with azithromycin carried significantly more resistant strains than those treated with other macrolides, and after 6 weeks, 85% still harbored macrolide-resistant flora [22].

The effects of antibiotics and vaccines on the normal respiratory flora composition indicate that this ecosystem maintains a fragile equilibrium resulting from the long-term co-evolution of colonizing bacteria with the human host. The clinical and epidemiological effects of altering this delicate balance by factors that have not been hitherto in place in the course of human history, such as antibiotics and vaccines, may offset many of the benefits of these medical advances. Because of the serious Public Health implications of selecting resistant organisms, the magnitude of this alarming problem should be closely monitored by initiatives such as the European Antimicrobial Resistance Surveillance System (EARSS), and physicians and the public should be educated on the proper use of antimicrobial drugs [21].

DETERMINANTS OF BACTERIAL COLONIZATION IN CHILDREN

Because bacterial colonization of the respiratory mucosal surfaces occurs at the interface between humans and their surroundings, the forces that shape the acquisition, composition, trafficking, and elimination of residing organisms are multiple, reflecting host, microbial,

environmental, and socioeconomic determinants (Table **1**). The interaction of this wide array of factors is enormously complex, and assessing the contribution of particular variables requires rigorous epidemiological methodology, use of carefully chosen controls, and multivariate analysis. For instance, separating the individual contribution of age, number of children at home, daycare attendance, use of antibiotics, and season on the carriage of *S. pneumoniae* poses obvious difficulties. Large families of low-socioeconomic level may choose to put their children in daycare at an early age in order to enable mothers to join the workforce. The high attack rate of viral infections in the winter enhances child-to-child transmission of respiratory bacteria, whereas the seasonal increment in antibiotic consumption favors colonization by resistant strains. Studies are also strongly dependent on methodological issues such as testing frequency, sampling site, specimen collection technique, type of fiber in the swab, use of selective media, transport time to the laboratory, or number of colonies examined, which may spuriously affect colonization rates [23].

Table 1. Factors Influencing Carriage of Potential Respiratory Pathogens

Host Factors
Age
Genetic background
Immune response
Vaccination status
Allergic conditions
Microbiological Factors
Strain fitness
Serotype
Antibiotic susceptibility
Co-colonization and bacterial interference
Environmental Factors
Season
Viral respiratory infections
Overall antibiotic exposure
Exposure to specific antibiotics [e.g. long-acting macrolides]
Diet (breast feeding *vs.* bottle feeding)
Xylitol consumption
Sleeping position
Use of pacifiers
Smoke exposure
Hospitalization
Socioeconomic Factors
Housing
Access to health care
Poor hygiene
Family size
Overcrowding living conditions
Number of siblings
Age of siblings
Parental educational level
Living in closed communities
Daycare attendance
Daycare size and type

WHY CHILDREN DO NOT GET SICK

Obviously, mucosal surfaces provide a large portal of entry to the bloodstream and deep tissues to a variety of residing pathogens. In fact, the majority of human infections caused by endogenous flora occur as a two-step process in which colonization is followed by local and/or systemic invasion. However, careful examination of the evidence reveals that clinical disease occurs in only a small minority of individuals that harbor the organism. Most pneumococcal infections develop shortly (usually within 1 month or less) after mucosal colonization by a new serotype is established, suggesting that once colonization-induced immunity builds on, the narrow window of opportunities to cause disease closes [24, 25].

This invasive process involves multiple successive phases: adherent bacteria should pass through the respiratory epithelium, avoid phagocytosis by submucosal macrophages, penetrate the blood, avoid the systemic host's immune response, and localize in a new entirely different biological niche such as the central nervous system or bone tissues. It is plausible that at each of these stations, the number of organisms is gradually reduced until further progression of the disease is aborted or the invasive bacterial population is derived from a single or few survivors that fully completed the perilous journey [26].

A PRECARIOUS ARMISTICE

From the evolutionary point of view, diseases can be regarded as "work accidents" from which, in most cases, microorganisms have nothing to gain. In fact, clinical illness is not only harmful to the host, but may be also detrimental to the parasite. By circulating in the bloodstream or invading deep organs, bacteria lose access to human body surfaces and, therefore, cannot propagate any further. Sick individuals are isolated from other members of the community, treated with antimicrobial drugs, and may even succumb to the disease. Obviously, all these possible scenarios interrupt the chain of person-to-person transmission, resulting in extinction of the pathogen. From the bacterial perspective, Samson's last pray 'Let me die with the Philistines!' (*Judges* 16: 30) does not make much sense, whereas asymptomatic colonization appears as a more preferable relationship for both, host and pathogen.

According to this conventional wisdom, given enough time, all host-parasite relationships would evolve towards commensalism (coexistence with neither detriment or obvious benefit), or better, mutualism (both the host and the microorganism benefit) [27]. Virulence can be, then, considered a transient artifact revealing a recent association between parasites and their hosts that has not reached yet the optimum level determined by the trade off between the ability to cause host's damage and transmissibility. This view, however, does not satisfactorily explain the fierceness exhibited by many of the residing members of the respiratory consortium such as pneumococci or meningococci. This apparent exception to common sense rules may be due to the fact that initiation of an invasive disease is frequently induced by the same virulence factors developed by the bacterium to get a foothold in the host and persist as an innocuous mucosal dweller, or to improve chances of transmission. The expression of a polysaccharide capsule by

S. pneumoniae reduces entrapment of the bacterium in the respiratory mucus layer, enabling access to the epithelial surfaces [28]. Unfortunately, the capsule also protects the organism from phagocytosis and, therefore, promotes invasiveness by improving its chances of survival in bloodstream and tissues.

Recently, a broad-spectrum RTX toxin has been identified in *K. kingae*, a respiratory commensal that is emerging as a common cause of septic arthritis and bacteremia in young children [29]. The toxin lyses respiratory epithelial cells, leucocytes, and synovial cells, and appears to have been originated in *Moraxella bovis* or in a common donor organism, and transferred horizontally to *K. kingae*. It is plausible that the toxin has been maintained in the recipient species to increase the area of pharyngeal colonization. Because of its broad-spectrum cytolytic activity, the toxin may incidentally contribute to the pathogenesis of invasive *K. kingae* infections by promoting penetration into the blood, inducing immune evasion by killing phagocytic cells, and damaging skeletal tissues [29].

Before the widespread use of the *H. influenzae* conjugate vaccine, capsular type b accounted for 95% of all invasive isolates of the species. This particular polysaccharide capsule has antiphagocytic properties that confer *H. influenzae* the ability to endure in the bloodstream and invade remote sites such as the meninges. Given the fact that invasive infections have frequently a deadly outcome, they frequently represent a terminal path for both host and parasite. It has been suggested that the type b capsule have been conserved because it provides resistance to desiccation when the bacterium is in transit between hosts, ensuring transmission [30]. From these examples, it appears that co-evolution does not necessarily favor gradual loss of virulence and establishment of a peaceful-coexistence between host and bacteria, and organisms exhibiting high virulence can be also positively selected [27].

Colonizing ability, however, does not necessarily correlates with invasiveness, and pneumococcal serotypes that are frequently harbored in the nasopharynx, such as 3, 6A, 19F, or 23F, are relatively underrepresented among invasive isolates, whereas serotypes 1, 4, 5, and 7F, are likely to cause disease, but are rarely carried in the population [31]. This observation indicates that organisms that are well adapted to a mucosal-associated lifestyle may not survive when transferred to the blood or meninges because colonization of these normally sterile niches may require a different biological specialization.

WHY CHILDREN DO GET SICK

Translocation of respiratory organisms from mucosal surfaces, where they are harmless, to sites where they cause tissue damage but are physically isolated and unable to be transmitted appears to defy any rational understanding. Three different, but not mutually exclusive explanations, have been proposed:

- Obviously, in many cases, physiological immaturity (young age) [32], or an innate (such as primary immunodeficiency) [33] or acquired predisposing condition (such as corticosteroid administration), a chronic debilitating disease [34], or a viral infection [35], may result in increased host vulnerability.
- Because, at any given time, the large respiratory surfaces are colonized by enormous numbers of potentially virulent microorganisms, there is probability that, just by chance alone, bacteria may penetrate the mucosa and cause an invasive infection (the "stochastic explanation") [26].
- Because horizontal transfer of genetic traits in bacteria, the large population of colonizing species frequently comprises genetically heterogeneous organisms. However, isolates from normally sterile sites (such as blood or cerebrospinal fluid) are almost invariably monoclonal. It has been suggested that random mutations occurring amongst colonizing organisms produce a highly virulent clone (the "within-host evolution" theory). These infrequent genetic events may be responsible for the invasiveness of seemingly innocuous commensal bacteria, and explain the rare occurrence of disease in normal hosts and the fact that these infections usually involve a genetically uniform population [26].

CONCLUSIONS

The upper respiratory tract of young children harbors a multifarious bacterial flora that includes some of the most virulent human pathogens. Successful mucosal colonization is crucial for the survival of respiratory organisms by establishing chains of person-to-person transmission, and an indispensable step in the causation of clinical disease. Studies conducted over the last decades are gradually revealing the mechanisms involved in keeping the dynamic equilibrium between the host and mucosa-associated organisms, as well as between the different bacterial populations residing on the respiratory surfaces. Antibiotic use and abuse have selected resistant traits that have spread among the colonizing flora and resulted in the emergence of difficult-to-treat infections, whereas introduction of conjugate pneumococcal vaccines has induced replacement of traditional serotypes included in the preparation by non-vaccine strains. Avoidance of unnecessary antibiotics and careful monitoring of changes in the composition of the upper respiratory flora caused by vaccination are mandatory to protect the biodiversity and equilibrium of this complex ecosystem.

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