Pharyngeal Colonization by Kingella kingae

Subjects: Allergy

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Kingella kingae colonizes the oropharynx after the second life semester, and its prevalence reaches 10% between the ages of 12 and 24 months, declining thereafter as children reach immunological maturity. *Kingella kingae* colonization is characterized by the periodic substitution of carried organisms by new strains. Whereas some strains frequently colonize asymptomatic children but are rarely isolated from diseased individuals, others are responsible for most invasive infections worldwide, indicating enhanced virulence. The colonized oropharyngeal mucosa is the source of child-to-child transmission, and daycare attendance is associated with a high carriage rate and increased risk of invasive disease. *Kingella kingae* elaborates a potent repeat-in-toxin (RTXA) that lyses epithelial, phagocytic, and synovial cells. This toxin breaches the epithelial barrier, facilitating bloodstream invasion and survival and the colonization of deep body tissues.

Kingella kingae | colonization | pili | carriage | transmission | children | invasive disease

1. Introduction

Shortly after birth, the skin and the upper respiratory, gastrointestinal, and female genital tracts of a newborn child become gradually coated with a variety of bacterial species, including many potentially dangerous organisms [1]. Despite their distant taxonomic position and vast biological differences, bacteria such as *Haemophilus influenzae* type b and *Streptococcus pneumoniae* colonizing the pharyngeal epithelium display homologous components as pili or antiphagocytic polysaccharide capsules [2]. These striking similarities result from a convergent evolution to adapt to the shared environment, adhere to the mucosal layer, avoid being washed out, and subvert the mucosal immune response.

The composition of the human microbiota is in a continuous dynamic state: organisms are acquired, eradicated, and re-acquired several times in a lifetime, and strains within a given species exhibit a remarkable turnover, indicating that the antigenic variability of virulent factors enables the acquisition of heterologous strains [3][4]. Bacterial colonization is asymptomatic in most cases, and the number of colonized but healthy individuals is enormous compared to those with clinical infections. An invasive disease usually occurs when colonizing bacteria breach the mucosal layer and penetrate the bloodstream. This event may result in the hematogenous dissemination and seeding of the organism to distant sites, causing focal infections. The colonized upper respiratory mucosa is also the source of person-to-person transmission of the bacterium through buccal and respiratory secretions, enabling its spread in the population.

2. *Kingella kingae*: An Oropharyngeal Resident

Similar to many other members of the *Neisseriaceae* family of Gram-negative organisms, *K. kingae* also colonizes the upper respiratory epithelium. In a large prospective study in which pairs of oropharyngeal and nasopharyngeal specimens were obtained from asymptomatic daycare center attendees, *K. kingae* grew in 109 out of 624 (17.5%) oropharyngeal samples. In contrast, the bacterium was not isolated from the nasopharynx, indicating a restricted ecological niche [5]. This finding was corroborated in a separate study in which 4472 oropharyngeal and nasopharyngeal specimens were sequentially collected from a cohort of 716 young children. Overall, 388 (8.7%) oropharyngeal cultures, but only a single nasopharyngeal culture, recovered *K. kingae* [6].

The colonization of the oropharynx by *K. kingae* organisms plays a double role. On the one hand, the exposed oropharyngeal surfaces are the natural reservoir of the bacterium from which it may be transmitted by contaminated buccal and upper respiratory secretions, and thus disseminated. On the other hand, the colonized oropharyngeal epithelium is the stepping-stone from which the bacterium translocates to the bloodstream and disseminates to remote body sites. In-depth study of the colonization phenomenon is, therefore, crucial to understanding the transmission of *K. kingae* and the pathogenesis of invasive infections.

3. Mechanism of Colonization

To colonize the oropharynx, *K. kingae* employs type IV pili that anchor the planktonic bacterium to the mucosal surfaces, and thus avoids being removed by saliva and respiratory fluids [7]. Elaboration of these pili is encoded in a chromosomal gene cluster, similar to that found in other Gram-negative pathogens, and in two other genes located in physically separated chromosomal regions, namely *pilC1* and *pilC2* [7][8]. The chromosomal gene cluster consists of a *pilA1* gene that encodes the major pilin subunit and two additional genes, named *pilA2* and *fimB*. The function of *fimB* is unknown, and it does not appear to be required for pilus expression or attachment [7]. The *pilA1* gene sequence shows marked between-strain variation, and the pilA1 subunit exhibits vast differences in antibody reactivity, suggesting that this exposed virulence factor is subjected to selective pressure by the host's immune system [9]. The pilC1 subunit is needed for twitching motility and adherence, whereas pilC2 has only a minor role in motility and no effect on adherence [8]. The expression of pili in *K. kingae* is finely regulated by the *σ54*, *pilS*, and *pilR* genes [9], and most oropharyngeal isolates and those derived from bacteremic patients express pili. In contrast, those samples isolated from skeletal system infections or endocarditis are usually non-piliated [10]. This finding suggests that piliation promotes *K. kingae* colonization and facilitates the initial bloodstream invasion but is disadvantageous for invading deep body structures.

In addition to the pili, *K. kingae* elaborates a trimeric autotransporter protein named Knh (*Kingella* NhhA homolog), which is essential for the strong anchoring of the organism to the oropharyngeal epithelium [11]. However, the carbohydrate capsule conceals the Knh element, rendering it inaccessible for attachment to the host's cells. Porsch et al. have proposed that, following an initial weak adherence of the long pili to the epithelial surface, a strong retraction of these filaments displaces the capsule and exposes the Knh protein, which may then firmly stick to the mucosal surface [11].

4. Immunity to Colonization and Infection

The crucial role played by the immune system in preventing oropharyngeal *K. kingae* colonization and subsequent invasive disease is supported by the fact that adults with a variety of immunosuppressive conditions are at increased risk of *K. kingae* disease [12]. In a longitudinal study in which serum antibody levels against *K. kingae* outer-membrane proteins were measured by an ELISA test, IgG levels were high at 2 months of age and gradually diminished thereafter, reaching a nadir level at 6–7 months, then remaining low until the age of 18 months, followed by an increase in 24-month-old children. IgA levels were lowest at 2 months and slowly increased between 4 and 7 months of age. A further increment of both antibody types was measured in children aged ≥24 months [13].

This pattern is consistent with protection from colonization and invasive disease by vertically transmitted immunity and limited exposure to *K. kingae* in early infancy. Vanishing maternal antibodies and increasing social contacts result in exposure to the organism in the second life semester, with corresponding increasing IgG and IGA levels. While the colonization and attack rates of disease are high in the second year, antibody levels remain high and stable. Colonization rates, and the incidence of invasive infections and IgG levels decline in older children as they reach immunological maturity. Because asymptomatic *K. kingae* carriage is common in early childhood, whereas invasive infections are exceptional, it is postulated that pharyngeal colonization is the immunizing event.

Similar to other respiratory pathogens such as pneumococci and *H. influenzae* type b, *K. kingae* elaborates a polysaccharide capsule and secretes an exopolysaccharide. Both components inhibit the host's immune response [14][15][16], enabling colonization of the upper respiratory tract, protecting the organism from phagocytosis by blood leukocytes and tissue macrophages, and facilitating the invasion of deep tissues. The maturation of the T-cell independent arm of the immune system, which is responsible for producing antibodies to polysaccharide antigens, is delayed in humans until the age of 2–4 years [2], explaining the increased susceptibility of young children to both colonization and disease. It should be noted that whereas the prevalence of *K. kingae* colonization reaches its peak and remains steady during the second year of life, the age-related curve of invasive disease is markedly skewed to the left: >75% of affected children are aged <18 months, and >95% are younger than 48 months, indicating that resistance to invasive infections is acquired before immunity to mucosal colonization [12].

5. Colonization and Transmission

A prospective study was conducted in the school year 1993–1994 among two cohorts of young children attending a daycare facility in southern Israel to investigate the dynamics of colonization and transmission of *K. kingae* in children attending out-of-home care facilities [5]. Oropharyngeal specimens were obtained biweekly over 11 months and seeded on the selective blood-agar-vancomycin (BAV) medium [5]. The recovered *K. kingae* isolates were originally studied by pulsed-field gel electrophoresis (PFGE) and ribotyping techniques with multiple restriction enzymes, and immunoblotting with rabbit immune serum [17]. A strict criterion consisting of complete DNA band identity by the three typing methods was employed to characterize the isolates and prove the person-to-person transmission of the strains. More recently, the isolates were retested by PFGE with the highly discriminative *Eag*I

enzyme, which is the currently recommended tool for PFGE analysis of the species. The results of this latter analysis are depicted in **Figure 1**.

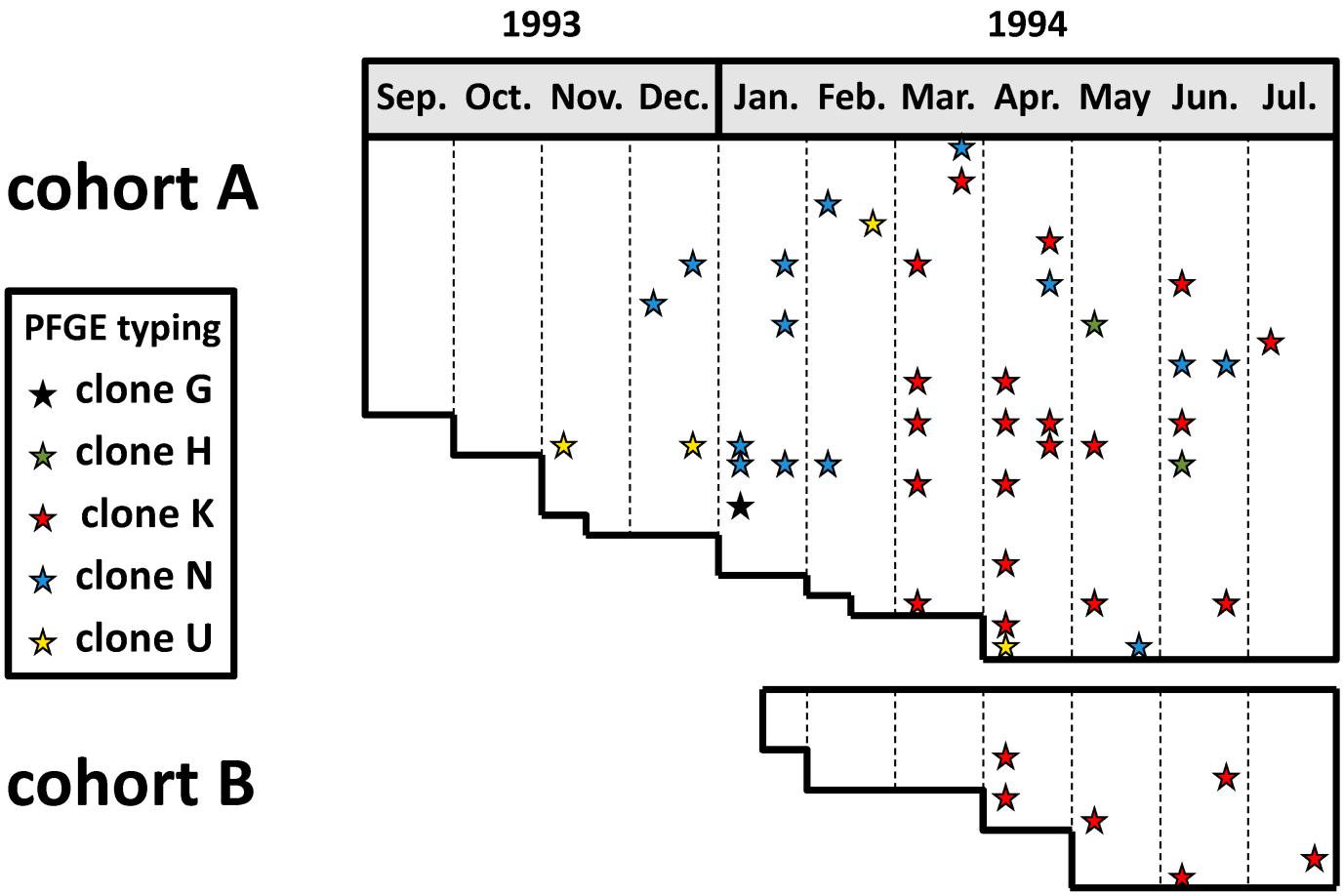

Figure 1. Dissemination of *K. kingae* clones among two cohorts of attendees at an Israeli daycare center. Horizontal lanes: individual attendees. Each star represents a positive pharyngeal culture, while the different colors represent distinct PFGE clones.

Thirty-five of 48 (73%) children carried *K. kingae* organisms at least once, and, on average, 28% of the attendees were colonized at any point in time [5]. Individual attendees exhibited sporadic, intermittent, or continuous carriage, and residing strains were frequently substituted by new strains after weeks or months, showing that similar to other respiratory bacteria, *K. kingae*'s carriage is a dynamic phenomenon with frequent turnover of colonizing organisms [17]. However, it should be pointed out that in the study, a single colony per positive culture was typed, and therefore simultaneous carriage of multiple strains and/or persistence of a previously carried organism at a low and undetectable level cannot be ruled out.

Overall, five distinct PFGE clones were detected in the daycare center during the study period. Four of these clones, namely H, K, N, and U, appeared sequentially in the facility and gradually colonized multiple attendees, while the prevalence of previously carried strains decreased [17] (**Figure 1**). These observations suggest that prolonged colonization induces an immune response that is strain-specific and eradicates or diminishes the density

of the carried strain but does not prevent colonization by an antigenically different organism. Remarkably, although these four highly invasive clones were responsible for over half of the invasive diseases in Israel between 1991 and 2012 [18], none of the colonized daycare attendees developed a clinical *K. kingae* infection during the follow-up period.

In a secondary analysis performed on the aforementioned southern Israel cohort study [19], the temporal dynamics of *K. kingae* carriage were investigated in children among whom the bacterium was isolated on >1 occasion [6]. The proportion of PFGE-similar strains was determined for pairs of positive cultures separated by ≤2 months (short-term intervals) and for those separated by ≥5 months (long-term intervals). The fraction of pairs of similar strains out of the total number of pairs was assessed by PFGE analysis for short-term and long-term intervals, and then compared. Of the short-term interval paired isolates, 17 of 19 (89.5%) yielded genotypically similar clones, while only 20 of 91 (22.0%) long-term interval pairs yielded similar clones ($p < 0.001$), indicating that, over time, colonization enables the eradication of the carried organism, facilitating the later acquisition of a different strain [6].

6. Detection of *K. kingae* Colonization

Because of the abundance and complexity of the upper respiratory and buccal microbiota, the isolation and identification of *K. kingae* in oropharyngeal specimens are notoriously tricky. Although the organism frequently colonizes the oropharynx of young children, its presence in Petri dishes is concealed by the rapid overgrowth of other members of the residing bacterial flora [20].

To facilitate the culture recovery and identification of the species, a selective and differential medium consisting of blood agar with 2 mcg/mL of added vancomycin (BAV medium) has been developed [20]. BAV plates, streaked with oropharyngeal secretions, are incubated for 48 h at 35 °C under aerobic conditions in a 5% CO_2-enriched atmosphere [20]. The aerobic conditions suppress the development of anaerobic species; the glycopeptide antimicrobial drug inhibits the growth of Gram-positive bacteria; and the added CO_2 enhances the growth of capnophilic *K. kingae* organisms, whereas the blood component facilitates the visualization of hemolytic colonies [20] (**Figure 2**).

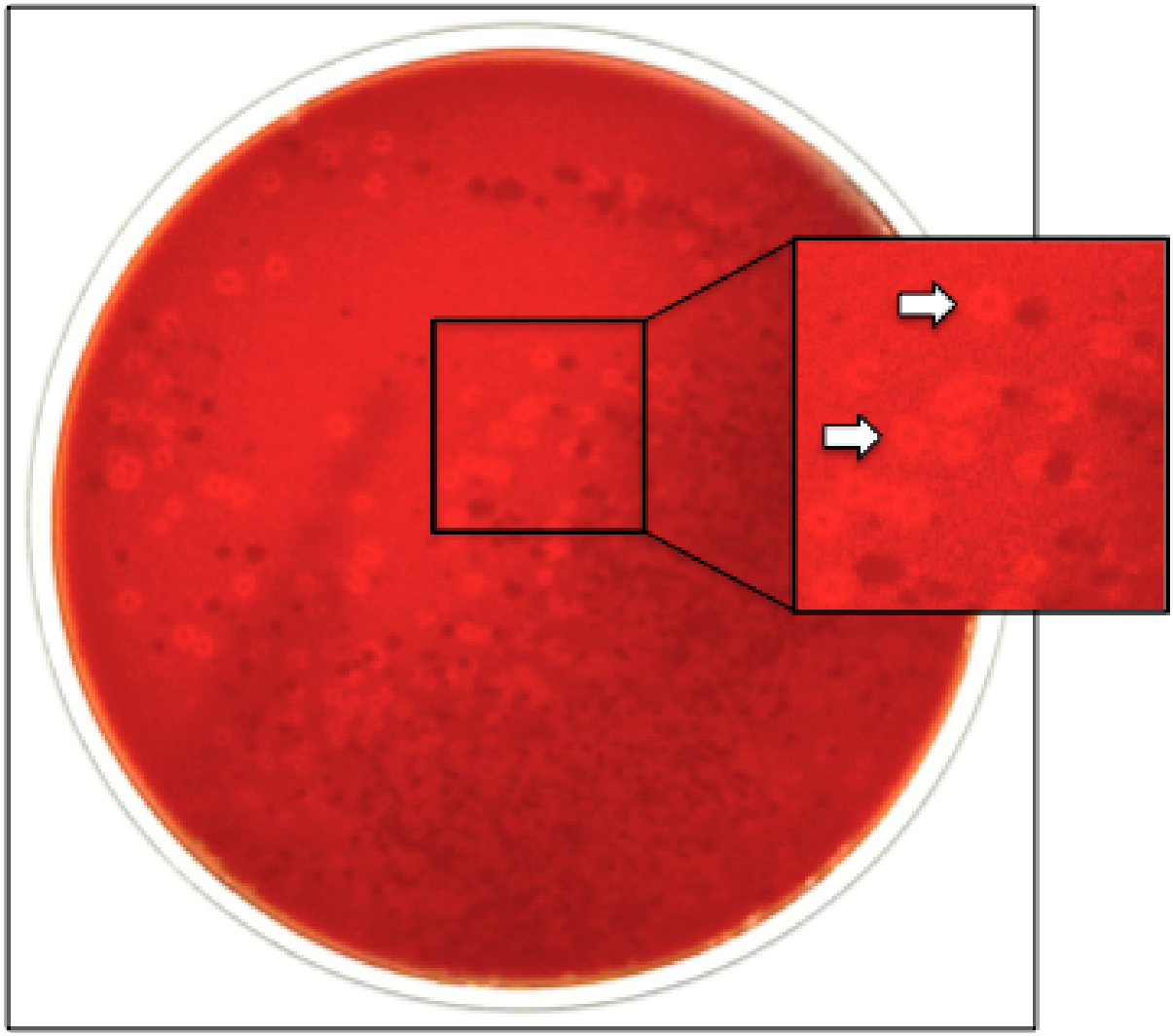

Figure 2. Oropharyngeal specimen seeded onto selective BAV medium exhibiting growth of β-hemolytic *K. kingae* colonies.

When the capability of the BAV and the traditional blood agar media for the primary isolation of *K. kingae* from oropharyngeal cultures were compared in a prospective study, the BAV plate identified 43 of 44 (97.7%) carriers [20]. In contrast, the comparator detected only 10 (22.7%) ($p < 0.001$ by the Chi-square test) [20]. The BAV medium and a Columbia-agar-based variant [21] have been successfully used in the investigation of the acquisition, prevalence, and transmission of *K. kingae* in the pediatric population and the investigation of outbreaks of invasive disease in daycare facilities [5][6][19][21][22]. It should be pointed out that chocolate agar media are not suitable for *K. kingae* detection in primary cultures since they do not reveal the presence of β-hemolytic colonies. The use of chocolate agar probably contributed to the failure to identify respiratory *K. kingae* carriers in a cluster of infections among attendees at a North Carolina daycare center [23].

In recent years, nucleic acid amplification tests (NAATs) targeting the 16S rRNA or species-specific genes have been introduced into clinical practice to detect fastidious bacteria in normally sterile body fluids and tissues. NAATs enable bacterial identification within hours instead of days and in patients receiving antibiotic therapy [24]. This revolutionary approach is gaining increasing popularity as a sensitive and convenient culture-independent tool for

diagnosing invasive *K. kingae* infections [24][25][26][27][28][29][30] and for identifying *K. kingae* carriers in prevalence studies [22][31]. However, because only single genes are amplified, NAATs do not discriminate between different *K. kingae* strains and, thus, have a limited value in investigating complex disease outbreaks [22].

Tests that amplify *K. kingae*-specific genes have a higher sensitivity than those targeting the broad spectrum 16S rRNA gene [24]. The three species-specific genes that are targeted by the current assays are the *rtx* operon that encodes the RtxA toxin [32], chaperonin 60 (the *cpn60* gene, also known as *groEL*) [24], and the malate dehydrogenase (*mdh*) gene [28]. The *rtx*-based tests do not discriminate between *K. kingae* and the hemolytic and recently described *Kingella negevensis* species that also colonizes the pediatric oropharynx [28], and those that amplify the *cpn60* target show suboptimal sensitivity due to variability in the gene sequence among *K. kingae* strains [28]. The novel molecular assay that targets the *mdh* gene exhibits an optimal sensitivity and specificity and will probably replace the older tests [28].

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