Age-Dependent Carriage of *Kingella kingae* in Young Children and Turnover of Colonizing Strains

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In a longitudinal study, *Kingella kingae* carriage rate was nil below 6 months of age, 1.5% at 6 months, 9.6% at 12 months, remained stable between 10.4% and 12.0% during the second year of life, and decreased significantly to 5.3% at 30 months. Replacement of carried strains occurred over time.

Key words. *Kingella kingae*; prevalence rate; respiratory colonization; strain turnover; young children.

Use of improved detection methods has resulted in the recognition of *Kingella kingae* as a common etiology bacteremia and skeletal system infections in children aged 6 months to 3 years [1]. *K kingae* is carried asymptotically in the pharynx and disseminates by intimate contact between family members, playmates, and attendees to daycare center facilities [1–4]. In addition, pharyngeal isolates of patients with invasive disease are genotypically identical to those recovered from the blood, suggesting that the colonized mucosa is the portal of entry of *K kingae* organisms to the bloodstream [4]. A prospective study was conducted to investigate the age-related *K kingae* carriage in a large cohort of young healthy children.

MATERIALS AND METHODS

Over a 12-month period starting on August 2005, a cohort of 716 healthy children attending Well-Baby Care clinics in southern Israel were enrolled in a study aimed at evaluating the effect of different immunization schedules with a 7-valent pneumococcal conjugated vaccine [5]. After obtaining a signed informed consent from their parents, children were randomly allocated to 3 sampling groups: 506 children underwent oro- and nasopharyngeal cultures at 2, 4, 6, 7, 12, 13, 18, 19, 24, and 30 months of age (group A), 174 children were sampled at 12, 13, 18, 19, 24, and 30 months of age (group B), and 36 children were sampled at 18, 19, 24, and 30 months of age (group C). Overall, 356 (50%) children attended daycare facilities at some point during the study period. The study was approved by the Soroka University Medical Center and National Ethics Committees.

Bacteriological Methods

Oropharyngeal and nasopharyngeal specimens were obtained separately at each scheduled visit to isolate pneumococci. Starting on July 2006, inoculated swabs were also streaked onto a selective medium to detect *K kingae* [1]. In children in whom *K kingae* was repeatedly isolated, a single randomly chosen colony per visit was thawed, subcultured, and genotyped by pulsed field gel electrophoresis (PFGE), as described [6]. To estimate the genetic relatedness among strains, PFGE restriction patterns were interpreted according to the criteria of Tenover et al [7]. Isolates exhibiting similar (indistinguishable and closely related) profiles were considered to belong to the same clone.

Data Analysis

By the time the first specimens were processed for *K kingae* isolation, the study was already on its way for almost a year and, therefore, in a large fraction of group A children, cultures obtained during the first 4 visits were not examined for presence of the organism. In addition, because of the surveillance schedule, children in groups B and C were only sampled since the ages of 12 and 18 months onwards. However, because the vast majority of children were consistently sampled between the ages of 12 and 30 months disregarding their group allocation, a subanalysis was performed in all those cultured at least...
in 5 of the 6 last visits to determine the *K kingae* prevalence and incidence of carriage in this age interval. The McNemar’s test was used to assess the statistical significance of the differences in the carriage rate.

The turnover of *K kingae* strains over time was assessed by molecular typing of isolates in the subset of colonized children in which the organism was recovered in >1 visit. It was assumed that if prolonged carriage enables eradication of the colonizing strain and its replacement by a different organism, strains isolated within a brief period will exhibit higher genotypic concordance than isolates separated by longer time intervals. Because the intervals between consecutive samplings were unequal (ie, 1 month between visits 3 and 4, 5 and 6, and 7 and 8, 5 months between visits 4 and 5, 6 and 7, and 8 and 9, and 6 months between visits 9 and 10), the proportion of genotypically similar strains was determined independently for pairs of positive cultures separated by short intervals (≤2 months) and for those separated by long intervals (>5 months). In children in whom only 2 visits were positive, a single comparison was possible, while children with ≥2 *K kingae*-positive visits allowed multiple comparisons following the formula \(\sum_{n=1}^{n-1}\), where n is the number of positive visits. The proportion of genotypically similar strain pairs out of the total number of pairs was calculated for short-term and long-term intervals separately, and compared by the \(\chi^2\) test. A *P* value <.05 was considered statistically significant for all calculations.

RESULTS

During the study period, a total of 4472 oropharyngeal and 4472 nasopharyngeal cultures were obtained from the cohort. A single nasopharyngeal culture and 388 (8.7%) oropharyngeal cultures were positive for *K kingae* (*P* < .001).

Overall, 283 of 716 (39.5%) children carried *K kingae* at least once, of which 64 were colonized twice, 13 had 3 positive visits, and 3 children had 4.

None of 28 and 131 cultures obtained at 2 and 4 months, respectively, grew the organism, while 3 of 200 (1.5%) and 5 of 253 (2.0%) cultures were positive at 6 and 7 months, respectively. The prevalence of *K kingae* among 624 children sampled at least in 5 occasions between the ages of 12 and 30 months remained relatively stable between 9.6% and 12.0% (*P* > .05), and decreased significantly to 5.3% at 30 months (*P* < .001) (Figure 1). Overall, 258 of these 624 children carried *K kingae* at least once between the ages of 12 and 30 months (incidence rate: 41.3%).

The 80 children in whom *K kingae* was isolated in multiple visits yielded a total of 179 isolates, of which 173 (96.6%), derived from 78 children, could be retrieved and typed. Seventeen of 19 (89.5%) short-term interval and 20 of 91 (22.0%) long-term interval paired isolates yielded genotypically similar clones (*P* < .001). In 2 of the 16 children with ≥3 positive visits, a *K kingae* strain that had been apparently eradicated was isolated again in a later culture.

DISCUSSION

The present study was designed to investigate the respiratory carriage of *K kingae* in the population segment at the highest risk for invasive infections caused by the organism [8]. The study results confirm that the tonsils are the natural reservoir of *K kingae*, whereas nasopharyngeal colonization is exceptional [9].

Almost 40% of the enrolled children carried *K kingae* at least once during the surveillance period, and >10% were colonized in multiple opportunities. These figures are lower than those found in a previous survey in which attendees to a daycare center in southern Israel were sampled every 2 weeks during an 11-month period [9]. In that study, 35 of 48 (72.9%) children carried *K kingae* at least once, and one half had ≥2 positive cultures [9]. It should be pointed out that while transmission of respiratory organisms is greatly facilitated in daycare facilities, only one half of the children in the present study attended out-of-home care [1], a substantial fraction missed the initial surveillance cultures, and the population was sampled discontinuously, resulting in full 6-month gaps during the final stages. Obtaining cultures at such long intervals could have overlooked events of short-term colonization. Therefore, the figures found in the present study should be considered a minimal estimate.
The study results show that the *K. kingae* carriage is strongly dependent on the child’s age. This epidemiological curve shows a striking parallel with the age-related incidence of invasive *K. kingae* infections, which shows a peak during the second year of life and a sharp decline thereafter. In a nationwide study comprising 291 previously healthy Israeli children with invasive *K. kingae* infections, only 4 (1%) were younger than 6 months, 110 (38%) were aged 6–11 months, 148 (51%) were aged 12–23 months, 25 (9%) were aged 24–35 months, and 4 (1.4%) were older than 3 years [8].

In the present study, *K. kingae* organisms isolated on multiple occasions showed a noteworthy turnover of colonizing strains. Genotypic concordance was lost over time, and sequential carriage of as many as 4 different clones was detected. Because only a single *K. kingae* colony from each positive pharyngeal culture was typed, unrecognized colonization by multiple clones and persistence of “old” strains at a low level, rather than complete replacement, cannot be definitely excluded. It should be pointed out, however, that re-isolation of a clone that was previously carried and lost was uncommon. This pattern, instead of a random temporal distribution of PFGE genotypes, suggests clearance or at least quantitative reduction in the colonizing density over time. It is possible that prolonged carriage induces strain-specific immunity that facilitates elimination of the carried organism, but does not prevent acquisition of an antigenically different strain [10]. This possibility is supported by the demonstration of strain-to-strain variability of *K. kingae* outer-membrane proteins as well as the PilA1 gene encoding the major pilus subunit, suggesting that immunogenic surface-exposed bacterial components involved in pharyngeal colonization undergo antigenic variation to evade the immune response [10, 11].

In summary, *K. kingae* colonizes the oropharynx of healthy young children, whereas nasopharyngeal colonization is exceptional. The carriage rate is age dependent and parallels the age-related incidence of invasive infections. Carriage is characterized by a frequent turnover of colonizing strains, similar to that observed in other pathogens of respiratory origin [12].

**REFERENCES**