A transversal pilot study of oropharyngeal carriage of *Kingella kingae* in healthy children younger than 6 months

Vasiliki Spyropoulou, Gabriel Brändle, Albane Bertha Rosa Maggio, Rebecca Anderson della Llana, Abdessalam Cherkaoui, Gesuele Renzi, Jacques Schrenzel, Sergio Manzano, Dimitri Ceroni

Geneva, Switzerland

Background: **The aim of this pilot study was to investigate the extent of oropharyngeal *Kingella kingae* carriage during the first 6 months of life.**

Methods: **We conducted a monocentric transversal pilot study on healthy children younger than 6 months in order to define the oropharyngeal carriage rate. Participants were recruited between December 2013 and September 2015 among children without symptoms or signs of invasive infections.**

Results: **We demonstrated an oropharyngeal carriage rate of 0.67% in children younger than 6 months. Due to the really low carriage rate, it was not possible to draw statistically significant conclusion about any other characteristic of our population.**

Conclusions: **The present study suggests that the oropharyngeal carriage of *Kingella kingae* among a Swiss population of healthy infants younger than 6 months is exceptional. The scarcity of colonization and disease in the early months of life suggests thus that defense against mucosal carriage and invasive infection is above all provided by vertically acquired immunity. Limited exposure of the neonates due to limited social contacts may also represent another factor avoiding neonates' mucosal *Kingella kingae* carriage.**

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Author Affiliations: Child and Adolescent Department (Spyropoulou V, Brändle G); Pediatric Sport Medicine and Obesity Care Program (Maggio ABR); Intensive Care Unit (Anderson della Llana R); Clinical Microbiology Laboratory, Service of Infectious Diseases (Cherkaoui A, Renzi G, Schrenzel J); Genomic Research Laboratory, Service of Infectious Diseases (Schrenzel J); Pediatric Emergency Division (Manzano S); Pediatric Orthopedic Service (Ceroni D), University Hospitals of Geneva, Geneva, Switzerland

Corresponding Author: Vasiliki Spyropoulou, MD, Child and Adolescent Department, University Hospitals of Geneva, Str Willy-Donzé 6, CH-1211 Geneva 14, Switzerland (Email: vasiliki.spyropoulou@hcuge.ch/vanessa.spyropoulou@gmail.com)

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Brief report

Introduction

Kingella kingae (*K. kingae*) is currently considered the major bacterial cause of osteoarticular infections in children younger than 4 years.[1-3] This organism is a frequent component of the oropharyngeal microbiota of young children, transmitted from person to person.[4] There is some evidence that *K. kingae* first colonizes the oropharynx before spreading to distant sites by hematogenous means.[5] Respiratory carriage of *K. kingae* appears thus to be a prerequisite for invasive infections.[6-8]

The carriage rate among children younger than 4 years of age is about 10%, but the oropharyngeal colonization rate is not uniform. Studies have demonstrated that there are sharp differences during this narrow time period, and that the colonization rate is probably the highest in children aged 12-24 months.[9-13] Finally, the colonization rate declines among older children and adults.[9,10] Pharyngeal carriage of *K. kingae* in children younger than 6 months is exceptional,[9,10] coinciding with the lack of morbidity due to this pathogen in early childhood indicating that maternal immunity and limited social contacts provide protection.[11] However, limited data are available in this population, and evidences have been obtained by culture methods that are recognized to have reduced sensitivity compared to nucleic acid amplification assays.

The aim of this prospective epidemiologic pilot study was therefore to determine the respiratory carriage of *K. kingae* among a Swiss population of healthy infants aged younger than 6 months by using a more efficient *K. kingae*-specific molecular detection method.

Methods

From December 2013 to September 2015, we conducted a transversal pilot study in the of Geneva's University

Hospital. Children aged younger than 6 months were recruited and screened for asymptomatic oropharyngeal carriage of *K. kingae*. These children were either hospitalized for clean surgery or non-bacterial infectious diseases, attending our orthopedic outpatient clinic, or visiting the emergency department. Exclusion criteria were the presence of invasive bacterial diseases and the administration of antimicrobial drugs during the previous two months. The study received institutional review board approval (09-309, No 2) and was conducted in accordance with Good Clinical Practice Guidelines and the provisions of the *Helsinki Declaration*. Informed consent from the participants' parents.

Measures

A tonsil swab was collected from all participants; and the following data were collected: age of the participant, gender, primary reason for consultation and presence of children living within their household. Oropharyngeal swabs were subsequently analyzed with a real-time polymerase chain reaction (PCR) assay, specific for *K. kingae*;[12] thus, the primary outcome was defined as a positive result on a PCR assay.

Statistics

Descriptive analyses (arithmetic mean, standard deviation and range) were used to describe participant characteristics and results of PCR assays on oropharyngeal swabs. Main purpose was the assessment of *K. kingae* carriage among children aged less than 6 months. To determine the prevalence of carriage among the target population, we calculated that a sample size of 1520 subjects would have been warranted. We based our estimations on the less than 1% carriage rate found in children younger than 6 months. We chose an absolute precision of 0.05% (half of the estimated prevalence) according to the method described by Lwanga and Lemeshow.[14] As we disposed of limited financial resources, we decided to start with a pilot study (150 cases; 10% of the minimal sample size) in order to avoid time and money being wasted. Finally, the colonization rate in infants was compared with local data established in a population of young children aged between 6 and 48 months.[13]

Results

A total of 150 children aged under 6 months were enrolled in this study and screened for asymptomatic oropharyngeal carriage of *K. kingae*. Eighty-nine infants were male (59.3%) and 61 were female (40.7%). Considering all the participants together, the average age was 2.5±1.7 months (range: 0.10 to 6 months). The most frequent primary reasons for consultation were viral upper respiratory infections, followed by gastrointestinal disorders, neurologic symptoms and routine orthopedic evaluations. The remaining medical conditions for consultation were fever without focus, hematopoietic, dermatological, endocrinological or urological affections and finally social problems. Fifty-nine participants (39.3%) had siblings aged under 4 years, 36 (24%) lived with children aged over 4 years, whereas the remaining 55 (36.7%) participants had no siblings.

Only one positive result out of 150 analyzed samples was noted, and the prevalence of oropharyngeal *K. kingae* carriage was thus estimated to be 0.67% among this infants' population. Due to the really low carriage rate, it was not possible to draw statistically significant conclusion about any other characteristic of our population, including the gender and the number of children sharing home with them.

Discussion

The present pilot study seems to confirm that the colonization rate is very low in infants younger than 6 months. The organism was detected in only 1 of 150 (0.67%) obtained samples. This asymptomatic oropharyngeal carriage of *K. kingae* rate in infants aged less than 6 months (0.67%) differs significantly from reported by Anderson de la Llana et al (8.7%) in children aged between 6 and 48 months and analysed in the same geographic location with the same detection method.[13]

K. kingae was not recovered from any oropharyngeal cultures obtained from healthy infants aged less than 6 months in two studies.[9,10] However, in a nationwide study comprising Israeli children with invasive *K. kingae* infections, the same research team noted that 1% of participants were younger than 6 months, which thus demonstrating that oropharyngeal carriage is seldom in this age group but not excluded.[15]

The scarcity of colonization and disease in the early months of life suggests that defense against mucosal carriage and invasive infection is provided by vertically acquired immunity. Vertical acquired immunity refers to antibody mediated immunity conveyed to the fetus by his mother during pregnancy and breastfeeding. A longitudinal investigation revealed that the average immunoglobulin (Ig) G levels were high at the age of 2 months, and slowly decreased thereafter reaching the lowest point at 6 to 7 months.[16] The IgG persisted at low levels until the age of 18 months, and exhibited finally a progressive increment in children older than 24 months.[16] A similar longitudinal investigation demonstrated that IgA antibodies (found in the breast milk and transferred to the gut of the infant) were almost undetectable at 2 months, slowly increased between 4 to 7 months of age, and exhibited a further increment in children over 2 years of age.[16]

Limited exposure to social contacts is probably another explanation for reducing mucosal *K. kingae* carriage in this population. In fact, infants during the first 6 months are rarely exposed to social contacts, especially with children aged between 6 and 48 months who are considered the main reservoirs of *K. kingae*. On this subject, Brändle et al[17] have demonstrated that the oropharyngeal colonization rate was similar in children aged between 6 and 48 months and in adults sharing the household with young children, whereas oropharyngeal colonization was absent in those without close contacts with young children. Colonization of infants younger than 6 months or adult populations seems therefore to require close contact with children aged between 6 and 48 months. The main limitation of this study is the fact that it is a pilot study with a limited number of participants. Definition of the real incidence of oropharyngeal carriage in this age group and influence of sharing of household with children aged between 6 and 48 months require future large scale exploratory studies.

In conclusion, the present pilot study seems to confirm that the oropharyngeal carriage of *K. kingae* among the Swiss population of healthy infants younger than 6 months is exceptional. The scarcity of colonization and disease in the early months of life suggests that defense against mucosal carriage and invasive infection is above all provided by vertically acquired immunity. Limited exposure of the young infants due to limited social contacts represents probably another factor reducing mucosal *K. kingae* carriage in this population.

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Ethical approval: This study was approved by Institutional Review Board of Regional Research Ethics Committee (CCER), Geneva, Switzerland (09-309, No 2), and was conducted in accordance with Good Clinical Practice guidelines and the provisions of the *Helsinki Declaration*.

Competing interest: The authors have no conflict of interest to disclose.

Contributors: Spyropoulou V collected data, carried out statistical analyses, and drafted the initial manuscript. Brändle G collected data, and reviewed and revised the manuscript. Maggio ABR carried out statistical analyses, and critically reviewed and revised the manuscript. Anderson della Llana R and Manzano S critically reviewed and revised the manuscript. Cherkaoui A, Renzi G and Schrenzel J performed bacteriological analyses, and critically reviewed and revised the manuscript. Ceroni D conceived and designed the study, and collected data. All authors approved the final manuscript as submitted. Ceroni D is the guarantor.

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