Original article

**Prevalence of *Kingella kingae* oropharyngeal carriage and predominance of type a and type b polysaccharide capsules among French young children**

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To the Editor,

*Kingella kingae* is the leading pathogen of osteoarticular infection (OAI) in <4-year-old children in different countries [1]. However, only few studies described the healthy carriage of *K. kingae*. In Israel and Switzerland, the highest colonisation rate was around 10% in the children aged of 12-24 months [1, 2]. To our knowledge, no epidemiological data on the healthy carriage is available in France yet. We aimed to determine the rate of and the factors associated with healthy carriage of *K. kingae* in young children in France, as well as the capsular serotype of these strains, known to be associated either with invasive or carriage strains [3].

Between May 2015 and June 2016, 217 healthy children aged from 6 to 36 months were prospectively enrolled. Throat samples were collected, as previously described [4], by 9 paediatricians from 5 different French departments (from Parisian Region: Paris, Seine-et-Marne, Seine-Saint-Denis, Val-De-Marne; and from Meurthe-et-Moselle, located in the Great East Region), after recording the written consent of at least one of their parents. Patients having received antibiotics during the last 7 days were not eligible for inclusion. The protocol was approved by the Saint-Germain-en-Laye Ethics Committee (Comité de Protection des Personnes Ile-de-France XI).

Continuous variables were compared by Mann–Whitney U test. Categorical variables were compared by Fisher exact test or Chi-square. All analyses were performed with R statistical package 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). A P value <0.05 was considered statistically significant.

Demographical and clinical features of the children included in the study are described in Table 1. Of notice, 171 children (78.8%) were included by 2 paediatricians (“2-paed group”) and 46 (21.2%) by the 7 others (“7-paed group”). In the 7-paed group, children were more frequently cared for out-of-home than in the 2-paed group (p<0.001) (Table 1).

To reliably define a *K. kingae* carriage, we firstly identified the positive *rtx*A samples, and then we needed a negative *gro*EL PCR (highly specific of *K. negevensis*) [5], to discriminate *K. kingae* (*rtx*A+/*gro*EL-) from *K. negevensis* (*rtx*A+/*gro*EL+). No samples were found *rtx*A+/*gro*EL+, excluding the possibility of a mixed colonisation. To consolidate the identification of *K. kingae*, we also performed *cpn*60 real-time PCR [6]. While all the *rtx*A-positive samples were *cpn*60-positive, we observed that some *rtx*A-negative samples were *cpn*60-positive. To explore this discrepancy we attempted to sequence the *cpn*60 allele [7]. When the sequences could be confidently read, we observed that the *rtx*A-positive samples exhibited a known *K. kingae cpn*60 allele, while the *rtx*A-negative samples exhibited an allele closely related to a different species (*Simonsiella muelleri*).

*K. kingae* was detected by PCR in 11 (5.1%; 95% confidence interval (95%CI): 2.6-8.9%) out of the 217 children. No *K. kingae* strain could be isolated by culture on a selective medium, as previously described [4]. The peak of prevalence of healthy carriage appeared in children aged 18-23 months (11.4%; 95%CI: 3.2-26.7%) (see web-only Supplementary Figure S1).

For capsular typing, we performed a multiplex PCR allowing distinguishing the 4 types “a”, “b”, “c”, and “d” using a modified protocol (see web-only Supplementary Tables S1). To identify the capsule type based on molecular weight after gel electrophoresis, we used 8 *K. kingae* strains with known capsule type as positive controls (2 per capsule type) (see web-only Supplementary Figure S2A). Among the 11 *K. kingae* positive throat samples, the capsule type was successfully determined in 9 cases (see web-only Supplementary Figure S2B). Capsules a and b were identified in 4 samples each, and simultaneous capsules a and c were identified in one sample, and no amplification product was visualised for the two remaining samples. Whether a lack of PCR sensitivity was observed in an oropharyngeal sample or capsules other than the already known could be elaborated by the species remain to be determined.

Demographical and clinical characteristics of the 11 *K. kingae* healthy carriers, compared to their 206 non-carriers counterparts are described in Table 1.Healthy carriers were more frequently cared for out-of-home than non-carriers (63.6% vs. 21.4%, respectively; p=0.004), especially in day-care centre (63.6% vs. 17.0%, respectively; p=0.002). Thus, the prevalence of carriage was higher in children cared for out of home than in children cared for at home (13.7% vs. 2.4%, respectively; p=0.004). Of interest, although non-significant, 6 out of 7 (85.7%) carriers who were cared for out of home attended at least 4 days per week (Table 1), leading to a prevalence of carriage of 15.8% (6/38; 95%CI: 6.0-31.3%) among children attending a day-care centre at least 4 days per week. Those results appeared similar to those previously described in other countries [2, 8].

Although non-significant, we observed a higher carriage rate during spring (8/117; 6.8%) and autumn (2/32; 6.3%) than during winter (1/68; 1.5%) (p = 0.23).

While a high sensitivity of the oropharyngeal culture method had been observed in children with *K. kingae* septic arthritis [4] no *K. kingae* strain could be isolated from the oropharynx of the healthy children in the current study. Although Ceroni et al. [9] have demonstrated that the colonisation density of *K. kingae*, based on real-time *rtx*A PCR results, among healthy paediatric carriers is not inferior to that observed in children with skeletal system infections, the lack of specificity of this PCR cannot rule out that ill children present a higher bacterial load than asymptomatical children.

Several limitations could be identified in our study. Firstly, the low number of carriers identified may lead to decrease the representativeness of our results. Secondly, the rate of children who were cared for out-of-home in the 2-paed-group (78.8% of the study population) was lower than that in the 7-paed-group, the latter being close to that observed in France [10] (17.0%, 47.8% and 45%, respectively). This may have led to underestimate the carriage rate in our study.

The first study on the *K. kingae* healthy carriage in France revealed a similar carriage rate compared with other countries; and day-care centre attendance appeared as an important associated factor. Capsule types a and b, associated with invasive infection, were commonly observed in our healthy population. Further studies are required to describe the genotypes and the antibiotic susceptibility patterns of the *K. kingae* carriage strains.

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**Contribution:**

Romain Basmaci contributed to the conception and the design of the work, performed analysis and interpretation of data, drafted the work and finally approved the submitted version.

Kizzy Deschamps performed analysis and interpretation of data, drafted the work and finally approved the submitted version.

Corinne Levycontributed to the conception and the design of the work, critically revised the manuscript for important intellectual content and finally approved the submitted version.

Vincent Mathy performed analysis and interpretation of data, drafted the work and finally approved the submitted version.

François Corrard contributed to the design of the work, critically revised the manuscript for important intellectual content and finally approved the submitted version.

Franck Thollot contributed to the design of the work, critically revised the manuscript for important intellectual content and finally approved the submitted version.

Stéphane Béchet performed analysis of data, critically revised the manuscript for important intellectual content and finally approved the submitted version.

Elsa Sobral performed analysis of data, critically revised the manuscript for important intellectual content and finally approved the submitted version.

Philippe Bidet contributed to the conception and the design of the work, critically revised the manuscript for important intellectual content and finally approved the submitted version.

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Stéphane Bonacorsi contributed to the conception and the design of the work, critically revised the manuscript for important intellectual content and finally approved the submitted version.

All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Supplementary materials**

**Supplementary Table S1.** Primer mix solution used in the multiplex PCR for *Kingella kingae* capsule typing

**Supplementary Figure S1. Distribution of children per age group.** 95% CI, 95% confidence interval

**Supplementary Figure S2.** **Composite picture of the gel electrophoresis of the multiplex PCR for *Kingella kingae* capsule typing.** PCR was performed on (A) two *Kingella kingae* strains per capsule type which were used as positive control to identify capsule type: a, b, c, and d (from left-hand side to right-hand side) and on (B) DNA extract from oropharyngeal samples harboring a positive *rtx*A PCR. Mix is the positive control mix showing molecular weight of each capsule type, from capsule « a » with the highest molecular weight to capsule « d » with the lowest molecular weight

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