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

4 Romain Basmaci^{a,b}; Kizzy Deschamps^c; Corinne Levy^{d,e,f}; Vincent Mathy^g; François
5 Corrad^d, Franck Thollot^h; Stéphane Béchet^{d,f}; Elsa Sobral^{d,g}; Philippe Bidet^{b,g}; Robert
6 Cohen^{d,e,f,i}; Stéphane Bonacorsi^{b,g}

7
8 **Affiliations:**

9 ^aService de Pédiatrie-Urgences, Hôpital Louis Mourier, AP-HP, F-92700 Colombes, France;

10 ^bIAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, F-75018 Paris,
11 France;

12 ^cService de Pédiatrie Générale, Hôpital Robert-Debré, AP-HP, F-75019 Paris, France

13 ^dAssociation Clinique Thérapeutique Infantile du Val de Marne (ACTIV), Saint Maur des
14 Fossés, France;

15 ^eCentre de Recherche Clinique du Centre Hospitalier Intercommunal de Créteil, France

16 ^fUniversité Paris Est, IMRB-GRC GEMINI, Créteil, France

17 ^gService de Microbiologie, Hôpital Robert-Debré, AP-HP, Centre National de Référence
18 associé *Escherichia coli*, F-75019 Paris, France;

19 ^hAFPA, Association Française de Pédiatrie Ambulatoire; Saint-Germain-en-Laye, France

20 ⁱUnité Court Séjour, Petits Nourrissons, Service de Néonatalogie, Centre Hospitalier
21 Intercommunal de Créteil, France;

22 **Corresponding author:** Romain Basmaci, Service de Pédiatrie-Urgences, Hôpital Louis
23 Mourier, 178 rue des Renouillers 92700 Colombes, France, romain.basmaci@aphp.fr, tel: +33
24 (0)1.47.60.63.64, fax: +33(0)1.47.60.63.76

25 **Key words:** *Kingella kingae*, carriage, epidemiology, day-care center, capsule

26 To the Editor,

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

35

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

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

48

49 Demographical and clinical features of the children included in the study are described
50 in Table 1. Of notice, 171 children (78.8%) were included by 2 paediatricians (“2-paed

51 group”) and 46 (21.2%) by the 7 others (“7-paed group”). In the 7-paed group, children were
52 more frequently cared for out-of-home than in the 2-paed group ($p < 0.001$) (Table 1).

53 To reliably define a *K. kingae* carriage, we firstly identified the positive *rtxA* samples,
54 and then we needed a negative *groEL* PCR (highly specific of *K. negevensis*) [5], to
55 discriminate *K. kingae* (*rtxA*+/*groEL*-) from *K. negevensis* (*rtxA*+/*groEL*+). No samples were
56 found *rtxA*+/*groEL*+, excluding the possibility of a mixed colonisation. To consolidate the
57 identification of *K. kingae*, we also performed *cpn60* real-time PCR [6]. While all the *rtxA*-
58 positive samples were *cpn60*-positive, we observed that some *rtxA*-negative samples were
59 *cpn60*-positive. To explore this discrepancy we attempted to sequence the *cpn60* allele [7].
60 When the sequences could be confidently read, we observed that the *rtxA*-positive samples
61 exhibited a known *K. kingae* *cpn60* allele, while the *rtxA*-negative samples exhibited an allele
62 closely related to a different species (*Simonsiella muelleri*).

63 *K. kingae* was detected by PCR in 11 (5.1%; 95% confidence interval (95%CI): 2.6-
64 8.9%) out of the 217 children. No *K. kingae* strain could be isolated by culture on a selective
65 medium, as previously described [4]. The peak of prevalence of healthy carriage appeared in
66 children aged 18-23 months (11.4%; 95%CI: 3.2-26.7%) (see web-only Supplementary Figure
67 S1).

68 For capsular typing, we performed a multiplex PCR allowing distinguishing the 4 types “a”,
69 “b”, “c”, and “d” using a modified protocol (see web-only Supplementary Tables S1). To
70 identify the capsule type based on molecular weight after gel electrophoresis, we used 8 *K.*
71 *kingae* strains with known capsule type as positive controls (2 per capsule type) (see web-only
72 Supplementary Figure S2A). Among the 11 *K. kingae* positive throat samples, the capsule
73 type was successfully determined in 9 cases (see web-only Supplementary Figure S2B).
74 Capsules a and b were identified in 4 samples each, and simultaneous capsules a and c were
75 identified in one sample, and no amplification product was visualised for the two remaining

76 samples. Whether a lack of PCR sensitivity was observed in an oropharyngeal sample or
77 capsules other than the already known could be elaborated by the species remain to be
78 determined.

79 Demographical and clinical characteristics of the 11 *K. kingae* healthy carriers, compared to
80 their 206 non-carriers counterparts are described in Table 1. Healthy carriers were more
81 frequently cared for out-of-home than non-carriers (63.6% vs. 21.4%, respectively; $p=0.004$),
82 especially in day-care centre (63.6% vs. 17.0%, respectively; $p=0.002$). Thus, the prevalence
83 of carriage was higher in children cared for out of home than in children cared for at home
84 (13.7% vs. 2.4%, respectively; $p=0.004$). Of interest, although non-significant, 6 out of 7
85 (85.7%) carriers who were cared for out of home attended at least 4 days per week (Table 1),
86 leading to a prevalence of carriage of 15.8% (6/38; 95%CI: 6.0-31.3%) among children
87 attending a day-care centre at least 4 days per week. Those results appeared similar to those
88 previously described in other countries [2, 8].

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

91

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

99 Several limitations could be identified in our study. Firstly, the low number of carriers
100 identified may lead to decrease the representativeness of our results. Secondly, the rate of

101 children who were cared for out-of-home in the 2-paed-group (78.8% of the study population)
102 was lower than that in the 7-paed-group, the latter being close to that observed in France [10]
103 (17.0%, 47.8% and 45%, respectively). This may have led to underestimate the carriage rate
104 in our study.

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

110

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127

128 **Contribution:**

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

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

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

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

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

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

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

146 Philippe Bidet contributed to the conception and the design of the work, critically revised the
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148 Robert Cohen contributed to the conception and the design of the work, critically revised the
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150 Stéphane Bonacorsi contributed to the conception and the design of the work, critically
151 revised the manuscript for important intellectual content and finally approved the submitted
152 version.

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

155

156 **Supplementary materials**

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

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

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

168

169 **References**

170

171 [1] P. Yagupsky, Clinical microbiology reviews 28(1) (2015) 54-79.

- 172 [2] R. Anderson de la Llana, V. Dubois-Ferriere, A. Maggio, A. Cherkaoui, S. Manzano, G.
173 Renzi, J. Hibbs, J. Schrenzel, D. Ceroni, *Pediatr Res* 78(5) (2015) 574-9.
- 174 [3] E.A. Porsch, K.F. Starr, P. Yagupsky, J.W. St Geme, 3rd, *mSphere* 2(2) (2017).
- 175 [4] R. Basmaci, B. Ilharreborde, P. Bidet, C. Doit, M. Lorrot, K. Mazda, E. Bingen, S.
176 Bonacorsi, *Clin Microbiol Infect.* 18(5) (2012) E134-6.
- 177 [5] N. El Houmami, J. Bzdrenga, G.A. Durand, P. Minodier, H. Seligmann, E. Prudent, S.
178 Bakour, S. Bonacorsi, D. Raoult, P. Yagupsky, P.E. Fournier, *J Clin Microbiol* 55(10) (2017)
179 3113-3122.
- 180 [6] B. Ilharreborde, P. Bidet, M. Lorrot, J. Even, P. Mariani-Kurkdjian, S. Liguori, C. Vitoux,
181 Y. Lefevre, C. Doit, F. Fitoussi, G. Pennecot, E. Bingen, K. Mazda, S. Bonacorsi, *J Clin*
182 *Microbiol* 47(6) (2009) 1837-1841.
- 183 [7] R. Basmaci, P. Yagupsky, B. Ilharreborde, K. Guyot, N. Porat, M. Chomton, J.M.
184 Thiberge, K. Mazda, E. Bingen, S. Bonacorsi, P. Bidet, *PLoS One.* 7(5) (2012) e38078.
- 185 [8] U. Amit, R. Dagan, P. Yagupsky, *Pediatr Infect Dis J* 32(2) (2013) 191-3.
- 186 [9] D. Ceroni, R.A. Llana, O. Kherad, V. Dubois-Ferriere, P. Lascombes, G. Renzi, L. Lamah,
187 S. Manzano, A. Cherkaoui, J. Schrenzel, *Pediatr Infect Dis J* 32(4) (2013) 212-4.
- 188 [10] F. Angoulvant, R. Cohen, C. Doit, A. Elbez, A. Werner, S. Bechet, S. Bonacorsi, E.
189 Varon, C. Levy, *BMC Infect Dis* 15 (2015) 236.