1   **Distribution of *Kingella kingae* capsular serotypes in France assessed by a multiplex**

2   **PCR on osteoarticular samples**

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21   *Kingella kingae* is the leading pathogen of osteoarticular infection (OAI) in <4-year-

22   old children in different countries where improved culture methods and nucleic acid

23   amplification assays are routinely employed (1). The oropharynx is recognized as the portal of

24   entry for *K. kingae*, which can penetrate the bloodstream and invade distant organs, facilitated

25   by several virulence factors such as a recently described polysaccharide capsule (2). On an

26   international collection of 150 invasive and carriage isolates from 10 countries, Porsch et al.

27   (2) have described four capsule types using a multiplex polymerase chain reaction (PCR)

28   approach. Over 95% of invasive isolates in the collection were type 'a' or type 'b', while

29   capsule type 'c' and type 'd' were more commonly observed among carriage isolates (2, 3).

30   This distribution was described on *K. kingae* isolates; however *K. kingae* is a fastidious

31   bacterium and whether the strains harboring a capsule type 'a' or 'b' can be more easily

32   cultivated from clinical samples is unknown. Such difference may lead to biased

33   epidemiological results. Moreover, among this collection, 30 invasive isolates were from

34   France, mainly isolated from 2010 to 2013 (n=24/30, range 1972-2013). To our knowledge,

35   no more recent data are available yet, and whether the epidemiology has changed remains to

36   be determined.

37   We aimed to describe the *K. kingae* capsule serotypes using a multiplex PCR protocol, as

38   recently described (4), on a fraction of our collection of osteoarticular samples, which were

39   positive with the *K. kingae* specific real-time PCR, used as routine in our laboratory (5).

40   From July 2013 to April 2018, we found 115 *K. kingae*-positive osteoarticular samples from

41   105 patients (1 to 3 per patients). Samples origins were: joint fluid (95/105; 90.5%), synovial

42   biopsy (8/105; 7.6%), bone abscess (1/105; 0.9%), and sub-cutaneous collection (1/105;

43   0.9%). Samples origins were: joint fluid (95/105; 90.5%), synovial biopsy (8/105; 7.6%),

44   bone abscess (1/105; 0.9%), and sub-cutaneous collection (1/105; 0.9%).Capsular PCR

45   allowed identifying unambiguously a capsule type in all of these 115 samples (Figure 1).

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46   Among the 105 patients, the PCR results showed 71 (67.6%) capsules 'a', 33 (31.4%)

47   capsules 'b', 1 (0.9%) capsule 'c', and no capsule 'd'. When multiple samples were available

48   for one patient, the results were identical for each sample. There was no significant variation

49   between distribution of capsules 'a' and 'b' along the study period or by age or whether the

50   culture was positive or negative (Table 1).

51   In a large collection of *K. kingae*-positive osteoarticular samples in France, we

52   demonstrated that our capsular PCR is able to identify the capsular serotypes of invasive *K.*

53   *kingae* in skeletal system specimens. Knowing that *rtx*A/B and *cpn*60 PCRs cannot

54   discriminate *K. kingae* from *K. negevensis* and *Simonsiella muelleri*, respectively, it may be

55   suggested that adding the capsular PCR to the molecular diagnosis may increase its specificity

56   (4). We observed that 99% of *K. kingae* OAI involved a strain harboring a capsule type 'a' or

57   'b', which is similar to that observed in the Israeli and the international collections of invasive

58   isolates (2, 3). Our results strengthen the hypothesis that the type 'a' and type 'b' capsules

59   may have enhanced pathogenic properties. Polysaccharide capsules are a common target for

60   development of vaccine, such as pneumococcal vaccine; the implication of capsules 'a' and

61   'b' in *K. kingae* invasive infections could be of high interest for the development of a *K.*

62   *kingae* vaccine. On the other hand, very few data on the distribution of capsule serotypes

63   among carriage isolates are available yet. In a recent study it was described that types 'a' and

64   'b' were predominant in a small population of healthy children carrying *K. kingae* in France

65   (4). A better characterization of the distribution of *K. kingae* capsular serotypes among

66   healthy carriers would lead to better understand the role played by the capsule in the potential

67   of the organism to cause invasive infection.

68   **References**

69   1.   Yagupsky P. 2015. *Kingella kingae*: carriage, transmission, and disease. Clin

70   Microbiol Rev 28:54-79.

3

71 2.   Porsch EA, Starr KF, Yagupsky P, St Geme JW, 3rd. 2017. The Type a and Type b

72   Polysaccharide Capsules Predominate in an International Collection of Invasive

73   *Kingella kingae* Isolates. mSphere 2: e00060-17

74 3.   Starr KF, Porsch EA, Seed PC, Heiss C, Naran R, Forsberg LS, Amit U, Yagupsky P,

75   Azadi P, St Geme JW, 3rd. 2016. *Kingella kingae* Expresses Four Structurally Distinct

76   Polysaccharide Capsules That Differ in Their Correlation with Invasive Disease. PLoS

77   Pathog 12:e1005944.

78 4.   Basmaci R, Deschamps K, Levy C, Mathy V, Corrard F, Thollot F, Bechet S, Sobral

79   E, Bidet P, Cohen R, Bonacorsi S. 2018. Prevalence of *Kingella kingae* oropharyngeal

80   carriage and predominance of type a and type b polysaccharide capsules among

81   French young children. Clin Microbiol Infect in press.

82 5.   Ilharreborde B, Bidet P, Lorrot M, Even J, Mariani-Kurkdjian P, Liguori S, Vitoux C,

83   Lefevre Y, Doit C, Fitoussi F, Pennecot G, Bingen E, Mazda K, Bonacorsi S. 2009.

84   New Real-Time PCR-Based Method for *Kingella kingae* DNA Detection: Application

85   to Samples Collected from 89 Children with Acute Arthritis. J Clin Microbiol

86   47:1837-1841.

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88 **Legend to Figure**

89 **Figure 1. Gel electrophoresis of the *Kingella kingae* multiplex capsular PCR in**

90 **osteoarticular samples.** The molecular weight is related to the capsule serotype: 'a', 'b', 'c'

91 and 'd' types are identified from the highest to the lowest molecular weight, respectively (lane

92 4). Capsule type 'a' was identified in 4 samples (lanes 1, 6, 7 and 8), capsule type 'b' was

93 identified in 2 samples (lanes 3 and 5), and capsule type 'c' was identified in 1 sample (lane

94 2). Lane 9, sterile water; Lane 10, DNA ladder.

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1   **Table 1. Distribution of capsule types among 105 patients with *K. kingae* osteoarticular**

2   **infection**

	All (n=105)	Capsule 'a' (n=71)	Capsule 'b' (n=33)	Capsule 'c' (n=1)
Year				
2013	24	19 (79.2)	5 (20.8)	0
2014	13	8 (61.5)	5 (38.5)	0
2015	0	-	-	-
2016	19	10 (52.6)	9 (47.4)	0
2017	39	26 (66.7)	12 (30.8)	1 (2.6)
2018	10	8 (80.0)	2 (20.0)	0
Age				
<1 year	30	20 (66.7)	10 (33.3)	0
1 year	60	41 (68.3)	18 (30.0)	1 (1.7)
2 years	12	7 (58.3)	5 (41.7)	0
3 years	2	2 (100.0)	0	0
4 years	1	1 (100.0)	0	0
Culture				
Positive	11	7 (63.6)	4 (33.4)	0
Negative	94	64 (68.1)	29 (30.9)	1 (1.0)

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