Distribution of *Kingella kingae* capsular serotypes in France assessed by a multiplex PCR on osteoarticular samples

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Key words: *Kingella kingae*, capsule, typing, epidemiology, pathophysiology

Conflict of interest: authors have no conflict of interest to disclose

Funding: no funding source

Kingella kingae is the leading pathogen of osteoarticular infection (OAI) in <4-year-old children in different countries where improved culture methods and nucleic acid amplification assays are routinely employed (1). The oropharynx is recognized as the portal of entry for *K. kingae*, which can penetrate the bloodstream and invade distant organs, facilitated by several virulence factors such as a recently described polysaccharide capsule (2). On an international collection of 150 invasive and carriage isolates from 10 countries, Porsch et al. (2) have described four capsule types using a multiplex polymerase chain reaction (PCR) approach. Over 95% of invasive isolates in the collection were type 'a' or type 'b', while capsule type 'c' and type 'd' were more commonly observed among carriage isolates (2, 3). This distribution was described on *K. kingae* isolates; however *K. kingae* is a fastidious bacterium and whether the strains harboring a capsule type 'a' or 'b' can be more easily cultivated from clinical samples is unknown. Such difference may lead to biased epidemiological results. Moreover, among this collection, 30 invasive isolates were from France, mainly isolated from 2010 to 2013 (n=24/30, range 1972-2013). To our knowledge, no more recent data are available yet, and whether the epidemiology has changed remains to be determined.

We aimed to describe the *K. kingae* capsule serotypes using a multiplex PCR protocol, as recently described (4), on a fraction of our collection of osteoarticular samples, which were positive with the *K. kingae* specific real-time PCR, used as routine in our laboratory (5).

From July 2013 to April 2018, we found 115 *K. kingae*-positive osteoarticular samples from 105 patients (1 to 3 per patients). Samples origins were: joint fluid (95/105; 90.5%), synovial biopsy (8/105; 7.6%), bone abscess (1/105; 0.9%), and sub-cutaneous collection (1/105; 0.9%). Samples origins were: joint fluid (95/105; 90.5%), synovial biopsy (8/105; 7.6%), bone abscess (1/105; 0.9%), and sub-cutaneous collection (1/105; 0.9%).Capsular PCR allowed identifying unambiguously a capsule type in all of these 115 samples (Figure 1).

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Among the 105 patients, the PCR results showed 71 (67.6%) capsules 'a', 33 (31.4%) capsules 'b', 1 (0.9%) capsule 'c', and no capsule 'd'. When multiple samples were available for one patient, the results were identical for each sample. There was no significant variation between distribution of capsules 'a' and 'b' along the study period or by age or whether the culture was positive or negative (Table 1).

In a large collection of *K. kingae*-positive osteoarticular samples in France, we demonstrated that our capsular PCR is able to identify the capsular serotypes of invasive *K. kingae* in skeletal system specimens. Knowing that *rtx*A/B and *cpn*60 PCRs cannot discriminate *K. kingae* from *K. negevensis* and *Simonsiella muelleri*, respectively, it may be suggested that adding the capsular PCR to the molecular diagnosis may increase its specificity (4). We observed that 99% of *K. kingae* OAI involved a strain harboring a capsule type 'a' or 'b', which is similar to that observed in the Israeli and the international collections of invasive isolates (2, 3). Our results strengthen the hypothesis that the type 'a' and type 'b' capsules may have enhanced pathogenic properties. Polysaccharide capsules are a common target for development of vaccine, such as pneumococcal vaccine; the implication of capsules 'a' and 'b' in *K. kingae* invasive infections could be of high interest for the development of a *K. kingae* vaccine. On the other hand, very few data on the distribution of capsule serotypes among carriage isolates are available yet. In a recent study it was described that types 'a' and 'b' were predominant in a small population of healthy children carrying *K. kingae* in France (4). A better characterization of the distribution of *K. kingae* capsular serotypes among healthy carriers would lead to better understand the role played by the capsule in the potential of the organism to cause invasive infection.

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Legend to Figure

Figure 1. Gel electrophoresis of the *Kingella kingae* multiplex capsular PCR in osteoarticular samples. The molecular weight is related to the capsule serotype: ‘a’, ‘b’, ‘c’ and ‘d’ types are identified from the highest to the lowest molecular weight, respectively (lane 4). Capsule type ‘a’ was identified in 4 samples (lanes 1, 6, 7 and 8), capsule type ‘b’ was identified in 2 samples (lanes 3 and 5), and capsule type ‘c’ was identified in 1 sample (lane 2). Lane 9, sterile water; Lane 10, DNA ladder.

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