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Management of an outbreak of invasive *Kingella kingae* skeletal infections in a day care center

Short title: Skeletal infections in a day care center

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Abstract

Background: *Kingella kingae (Kk)* is frequently responsible for invasive skeletal infections in children aged 3–36 months. However, few outbreaks of invasive *Kk* infections in day care centers have been reported. The objective of the present study was to describe (a) the clinical and laboratory data recorded during an outbreak of invasive *Kk* skeletal infections, and (b) the management of the outbreak.

Method: Four children from the same day care center were included in the study May and June 2019. We retrospectively analyzed the children's clinical presentation and their radiological and laboratory data. We also identified all the disease control measures taken in the day care center.

Results: We observed cases of septic arthritis of the wrist (Case 1), shoulder arthritis (Case 2), cervical spondylodiscitis (Case 3,) and knee arthritis (Case 4). All cases presented with an oropharyngeal infection and concomitant fever prior to diagnosis of the skeletal infection. All cases were misdiagnosed at the initial presentation. The mean (range) age at diagnosis was 10.75 months (9–12). The three patients with arthritis received surgical treatment. All patients received intravenous and then oral antibiotics. In Cases 1 and 2, *Kk* was detected using real-time PCR and a ST25-*rtxA*1 clone was identified. The outcome was good in all four cases. Four other children in the day care center presented with scabies during this period and were treated with systemic ivermectin. The Regional Health Agency was informed, and all the parents of children attending the day care center received an information letter. The day care center was cleaned extensively.

Conclusion: Our results highlight the variety of features of invasive skeletal *Kk* infections in children and (given the high risk of transmission in day care centers) the importance of diagnosing cases as soon as possible.

Keywords: septic arthritis; *Kingella kingae*; spondylodiscitis; outbreak; day care center

1. Introduction

The *Kingella* genus belongs to the *Neisseriaceae* family and comprises fives species. The best-known and most comprehensively described species is *Kingella kingae* (*Kk*). These fastidious, encapsulated, Gram-negative coccobacilli mostly live as commensals in the oral cavity in humans and animals [1]. Although the commensal carriage of *Kk* is asymptomatic, this bacterium sometimes disseminates through the bloodstream and becomes an opportunistic pathogen. Within the *Kingella* genus, *Kk* is the leading cause of bone and joint infections (BJIs) in infants aged between 6 and 36 months [2–5]. A hemolytic and cytotoxic toxin belonging to the RTX (repeats-in-toxin) family has been identified as a key virulence factor in the ability of *Kk* ability to spread through the bloodstream and cause BJIs [6,7]. Also, an identical *Kingella* RTX locus has been detected in the newly described species *K. negevensis*, although the pathogenicity of the latter has yet to be demonstrated [8,9].

The initial clinical presentation of a *Kk* skeletal infection is often moderate and may not even be associated with fever or inflammation. An unambiguous diagnosis usually requires the use of molecular biology tools. The outcome of a *Kk* skeletal infection is usually good, with no sequelae. However, oropharyngeal dissemination between infants and adults through close interpersonal contact has been described [10,11]. A cluster of invasive *Kk* infections is defined as the occurrence of at least two epidemiologically connected cases (one of which is confirmed or highly probable) within the same community of children and within the same 4-week period [1].

Here, we describe a cluster of BJIs among children attending the same day care center. Unusually, the BJIs affected the upper limbs and the neck.

2. Patients and method

We retrospectively reviewed demographic, clinical, laboratory, and radiological data from children having presented at Amiens University Hospital's Department of Pediatric Surgery (Amiens, France) with BJIs and having attended the same day care center between May and June 2019. This medical chart review was registered with the French National Data Protection Commission (*Commission nationale de l'informatique et des libertés*, Paris, France). The children's parents gave their informed consent for the present analysis.

We noted the initial clinical signs of infection, the body temperature, the onset of BJI symptoms, the laboratory parameters (white blood cells and the serum C-reactive protein [CRP] level), the radiological, ultrasound and/or computed tomography (CT) features, the type and site of the BJI, the pharmacological and surgical treatments, the complications, and the presence or absence of recurrence. We also analyzed the order of appearance of the sign and symptoms. Standard bacteriological cultures were performed for blood and joint fluid samples, in accordance with the French Society of Microbiology guidelines (SFM REMIC v6.2)[12]. A specific real-time polymerase chain reaction (PCR) assay targeting the malate dehydrogenase gene (as described by El Houmami et al. [13]) was used to probe for *Kk* DNA in the culture-negative samples. All cases were classified according to the Yagupsky et al. criteria [14]. A proven case was defined as a compatible clinical presentation and the detection of *Kk* (using a bacteriological culture or a PCR assay) in a blood or joint fluid sample. A highly probable case was defined as a compatible clinical presentation and the detection of *Kk* (using a bacteriological culture or a PCR assay) in an oropharyngeal sample. A presumed case was defined as a compatible clinical presentation and the occurrence of another case in the previous or following month. The clonal nature of the outbreak was investigated using multilocus sequence typing (MLST) and *rtxA* toxin gene sequencing in joint fluid extract, according to the method of Basmaci et al. [3]. Lastly, we noted (a) all the

measures taken in the day care center following the diagnosis of the BJIs, and (b) any other pathogens documented among the day care center children and staff.

3. Results

Four patients were included in the analysis. All had an unremarkable personal medical history. The patients' demographic, clinical, and laboratory data are summarized in Table 1 and the chronological data are depicted in Figure 1. There were three cases of septic arthritis (Cases 1, 2, and 3) and one case of C3–C4 spondylodiscitis (Case 4). The septic arthritis variously affected the right mediocarpal joint (Case 1), the left shoulder (Case 2), and the right knee (Case 3).

All four children were attending the same day care center, which included 22 children and 11 staff. The mean (range) age at diagnosis was 10.75 months (9–12). All patients presented with fever and oropharyngeal symptoms a few days before the onset of acute BJI symptoms, and were therefore given symptomatic medications. One case (Case 1) was associated with enteroviral disease.

None of the patients had a history of trauma. Although all the children had been seen by a pediatrician or in a pediatric emergency department at least once, the BJIs had been either diagnosed late or misdiagnosed.

All patients presented with a limited range of joint motion and either joint swelling (Cases 1, 2, and 3) or torticollis (Case 4). No radiological signs of infection were observed upon presentation. An ultrasound assessment revealed joint effusion in Cases 1, 2, and 3.

In Case 4, the patient had developed fever (39°C) and oropharyngeal symptoms 3 weeks before the first consultation. At the first consultation, torticollis was the only symptom present. The white blood cell count was normal, and the serum CRP level was 10 mg/L. No radiological, scintigraphic, or CT signs of infection were observed. The child was diagnosed

with torticollis due to atlantoaxial rotatory fixation. In view of the persistent symptoms, another CT scan was performed (on day 24) and revealed C3–C4 spondylodiscitis.

In the three cases with arthritis (Cases 1, 2, and 3), emergency arthrocentesis was combined with irrigation with an isotonic saline solution.

Standard bacteriological cultures of blood and joint fluid samples were all negative. No oropharyngeal samples had been taken. Real-time PCR enabled the detection of *Kk* DNA in samples for Cases 1 and 2. The real-time PCR assay was negative for Case #3. Luckily, the DNA in Cases 1 and 2 was sufficiently intact for MLST and *rtxA* sequencing, and a ST25-*rtxA*1 clone was identified.

All patients received the same empiric treatment with intravenous antibiotics (cefazolin 100 mg/kg daily, and gentamycin 5 mg/kg daily) for 3 days, followed by per os treatment with amoxicillin+clavulanic acid (80 mg/kg daily) and rifampicin (10 mg/kg daily). The patients with uncomplicated arthritis (Cases 1 and 3), complicated arthritis with early recurrence (Case 2), and complicated spondylodiscitis (Case #4) were treated for 3, 6, and 12 weeks, respectively. For Cases 1 and 2, the rifampicin was stopped when *Kk* was identified and amoxicillin+clavulanic acid was substituted by amoxicillin.

On the day after discharge, Patient 2 returned to the emergency department for early recurrence of the shoulder arthritis. She had fever (39°C), a limited range of joint motion, and an inflammatory scar. The white blood cell count was 10.500/mm^3 and the serum CRP level was 62 mg/L. An ultrasound examination of the shoulder confirmed the presence of joint effusion. Emergency shoulder arthrotomy was performed, and the immediate surgical outcome was good.

The mean (range) length of follow-up was 141.5 days (120–177). In all cases, the outcomes were good. Pain and limited range of joint motion were not observed. In Case 4, the last X-ray confirmed that the height of the vertebral body had been restored.

The incident was reported to the Regional Health Agency. Following the occurrence of the third case, the head of the day care center sent an information letter to all the parents. The parents met with the center's designated pediatrician and a physician from the Regional Health Agency. The nursery was cleaned extensively. No oropharyngeal samples were collected. A child with a history of ventricular septal defect was given amoxicillin (40 mg/kg b.i.d. for 4 days) and rifampicin (10 mg/kg, b.i.d. for 2 days). No other cases of BJIs were found in the day care center.

For all four cases, the clinical presentation was compatible with a *Kk* infection [14]. Two cases (Cases 3 and 4) were categorized as presumed cases, in view of the occurrence of a proven case in the previous or following month.

Four other children in the day care center presented with scabies, and were treated with systemic ivermectin.

4. Discussion

A few outbreaks of *Kk* BJIs in French day care centers have been reported [15–18]. The present report is the first to have described an outbreak with BJIs in three uncommon sites (the wrist, the shoulder, and the C3C4 vertebrae) caused by *Kk.* According to the literature, septic arthritis is the most common presentation (73%) and mostly affects the lower limbs [4]. Spondylodiscitis is a rare presentation of invasive *Kk* infections (5%), and a cervical site is extremely rare [19]. Petrus et al. reported on a case of spondylodiscitis caused by *Kk*; the clinical presentation (torticollis) described was the same as that in our Case 4 [19]. In our case, the late diagnosis was explained by the insidious presentation of the spondylodiscitis: no fever, no inflammatory syndrome, and normal scintigraphy. The relationship with *Kk* was only established after the day care center had held a meeting with all the parents. The relationship between the cases was established in accordance with the criteria of Yagupsky et

al. [1]. Although Case 4 was the first in the series, we did not find *Kk* DNA in the patient's samples – perhaps because the time interval between symptom onset and diagnosis was too long. Nevertheless, the fact that Case 1 was identified only 3 days after Case 4 enabled us to classify the latter as a probable case. In order to detect the *Kk* genome in these culture-negative samples, we used the primers and probe targeting the malate dehydrogenase gene (*mdh*) published by El Houmami et al. [13]. The *mdh* gene represents, to our knowledge, the most sensitive and specific target for detection of *Kk* in comparison with targets used in various studies. Indeed, the *rtxA* and *rtxB* genes (encoding for the RTX toxin) do not distinguish between *Kk* and *K. negevensis* species [9], while it seems that the *mdh* gene is not present in the *K. negevensis* genome. Also, the primers and probe targeting the *groEL* gene (encoding for a "chaperone protein") previously published display several mismatches resulting in a decreased sensitivity with some strains and/or some poor samples [9]. We detected *Kk* DNA in two of the four cases (Cases 1 and 2).

MLST and *rtxA* gene sequencing enabled the identification of the ST25-*rtxA1* clone in both proven cases. This is a strong argument in favor of horizontal, child-to-child transmission. The ST25-*rtxA*1 clone was involved in a French outbreak in 2013 [16]. We feel very lucky to have achieved MLST directly from the samples, because sequencing is generally difficult in samples containing very low DNA loads. Several authors also faced this problem [20,21].

In another case report, the investigators found an ST25-*rtxA*1 strain in an oropharyngeal sample (but not in a knee joint fluid sample) from a patient with a confirmed *Kk* knee abscess [20]. The ST25-*rtxA*1 clone seems to be particularly virulent, and tends not to be associated with oropharyngeal colonization [3,22]. However, on the basis of the available data, we cannot be certain that one of the two cases transmitted the clone to the other. Nevertheless, ST25-*rtxA*1 predominates among French invasive strains; it accounts for between 24% and 30.9% of the isolates [3,22]. However, the concomitant presence of scabies in the day care

center highlighted the promiscuity of the child-to-child interactions and probably increased the risk of disease transmission in general and oropharyngeal transmission in particular.

In the general population of children below the age of 4 years, two European studies have shown that the prevalence of oropharyngeal *Kk* colonization is 10% and varies with the toddler's age [5,23]. However, the prevalence of invasive *Kk* infections was below 1% [23]. In accordance with a report by France's High Commission for Public Health (*Haut Conseil de la Santé Publique*), the day care center was not closed but was extensively cleaned [18]. The day care center's staff and the families in contact with the index cases were not screened for oropharyngeal *Kk*, since this measure and preventive antibiotic prophylaxis have not proven their effectiveness – oropharyngeal colonization by *Kk* does not usually result in symptoms. The possibility was raised of using the positivity of the oropharyngeal sample as an etiological marker of invasive *Kk* infection when no other pathogen was found or when deep sampling was not possible. To prevent the spread of *Kk* in day care centers, we also recommend frequent and through washing of children's hands with soap and water, and keeping nails short.

5. Conclusion

The present case series highlighted the difficulty of establishing a link between the various clinical presentations and the *Kk* outbreak. Given the high risk of transmission in day care centers, it is essential to identify these cases rapidly.

References

1. Yagupsky P. Kingella kingae: carriage, transmission, and disease. Clin Microbiol Rev 2015; 28:54–79.

2. Yagupsky P. Kingella kingae: from medical rarity to an emerging paediatric pathogen. Lancet Infect Dis 2004; 4:358–67.

3. Basmaci R, Yagupsky P, Ilharreborde B, et al. Multilocus sequence typing and rtxA toxin gene sequencing analysis of Kingella kingae isolates demonstrates genetic diversity and international clones. PLoS ONE 2012; 7:e38078.

4. Al-Qwbani M, Jiang N, Yu B. Kingella kingae-Associated Pediatric Osteoarticular Infections: An Overview of 566 Reported Cases. Clin Pediatr 2016; 55:1328–37.

5. Amit U, Flaishmakher S, Dagan R, et al. Age-Dependent Carriage of Kingella kingae in Young Children and Turnover of Colonizing Strains. J Pediatric Infect Dis Soc 2014; 3:160–2.

6. Kehl-Fie TE, St Geme JW. Identification and characterization of an RTX toxin in the emerging pathogen Kingella kingae. J Bacteriol 2007; 189:430–6.

7. Chang DW, Nudell YA, Lau J, et al. RTX toxin plays a key role in Kingella kingae virulence in an infant rat model. Infect Immun 2014; 82:2318–28.

8. El Houmami N, Bakour S, Bzdrenga J, et al. Isolation and characterization of Kingella negevensis sp. nov., a novel Kingella species detected in a healthy paediatric population. Int J Syst Evol Microbiol 2017; 67:2370–6.

9. El Houmami N, Bzdrenga J, Durand GA, et al. Molecular Tests That Target the RTX Locus Do Not Distinguish between Kingella kingae and the Recently Described Kingella negevensis Species. J Clin Microbiol 2017; 55:3113–22.

10. Kiang KM. Outbreak of Osteomyelitis/Septic Arthritis Caused by Kingella kingae Among Child Care Center Attendees. Pediatrics 2005; 116:e206–13.

11. Yagupsky P, El Houmami N, Fournier PE. Outbreaks of Invasive Kingella kingae Infections in Daycare Facilities: Approach to Investigation and Management. J Pediatr 2017; 182:14–20.

12. Société Française de Microbiologie. Infections osseuses et articulaires. In: Société Française de Microbiologie Infections osseuses et articulaires. Paris, 6ème édition, REMIC Ed. 2018. p. 313–320.

13. El Houmami N, Durand GA, Bzdrenga J, et al. A New Highly Sensitive and Specific Real-Time PCR Assay Targeting the Malate Dehydrogenase Gene of Kingella kingae and Application to 201 Pediatric Clinical Specimens. J Clin Microbiol 2018; 56: e00505-18.

14. Yagupsky P. Outbreaks of Kingella kingae infections in daycare facilities. Emerging Infect Dis 2014; 20:746–53.

15. Bidet P, Collin E, Basmaci R, et al. Investigation of an Outbreak of Osteoarticular Infections Caused by Kingella Kingae in a Childcare Center Using Molecular Techniques. Pediatr Infect Dis J 2013; 32:558-60

16. El Houmami N, Minodier P, Dubourg G, et al. An Outbreak of Kingella kingae Infections Associated with Hand, Foot and Mouth Disease/Herpangina Virus Outbreak in Marseille, France, 2013. Pediatr Infect Dis J 2015; 34:246-50

17. El Houmami N, Cointat V, Mirand A, et al. An Outbreak of Kingella Kingae Infections Complicating a Severe Hand, Foot, And Mouth Disease Outbreak in Nice, France, 2016. Pediatr Infect Dis J 2017; 36:530-2

18. Haut Conseil de la santé publique. Gestion d'épisodes de cas groupés d'infections invasives à Kingella kingae en collectivités d'enfants. Avis produit par la Commission spécialisée Maladies infectieuses et maladies émergentes

Le 7 septembre 2018. Available at : https://afpa.org/content/uploads/2018/11/hcspa20180907_gesdecasgrodinfkinkinencoldenf.pdf

19. Petrus M, Rance F, Clément JL, et al. Cervical spondylodiscitis caused by Kingella kingae. Apropos of a case. Ann Pediatr (Paris) 1990; 37:170–2.

20. Basmaci R, Ilharreborde B, Doit C, et al. Two Atypical Cases of Kingella kingae Invasive Infection with Concomitant Human Rhinovirus Infection. J Clin Microbiol 2013;51:3137-9.

21. Basmaci R, Ilharreborde B, Bidet P, et al. Isolation of Kingella kingae in the oropharynx during K. kingae arthritis in children. Clin Microbiol Infect 2012; 18:134-6.

22. Basmaci R, Bidet P, Yagupsky P, et al. Major Intercontinentally Distributed Sequence Types of Kingella kingae and Development of a Rapid Molecular Typing Tool. J Clin Microbiol 2014;52:3890-7.

23. Ceroni D, Dubois-Ferrière V, Anderson R, et al. Small Risk of Osteoarticular Infections in Children With Asymptomatic Oropharyngeal Carriage of Kingella Kingae. Pediatr Infect Dis J 2012; 31:983-5

Table 1: Demographic, clinical, and laboratory data

Case	Sex	Age *	History	First signs of infection	Oropharyngeal symptoms	Diagnosis	Site	Fever	Edema	Restricted motion	US	X-ray/CT	Scintigraphy	WBC	CRP	*Kk* identified	Surgery
#1	F	12	Enterovirus infection 2 weeks previously	Fever 39.5°C	Yes	Arthritis	Right Wrist	37.7° C	Yes	Yes	e	Normal	np	11,200	58	Yes	Puncture+ Arthrocentesis
#2	F	11	0	Fever 39°C	Yes	Arthritis	Left Shoulder	38.6° C	Yes	Yes	e	Normal	np	13,600	70	Yes	Puncture+ Arthrocentesis+Arthrotomy
#3	M	9	0	Fever 39°C	Yes	Arthritis	Right Knee	37.1° C	Yes	Yes	e	Normal	np	16,660	52	No	Puncture
#4	F	11	0	Fever 39°C	Yes	Spondylodiscitis	C3-C4	No	No	Yes	No e	Normal CT scan	Negative	9,700	15	No	No

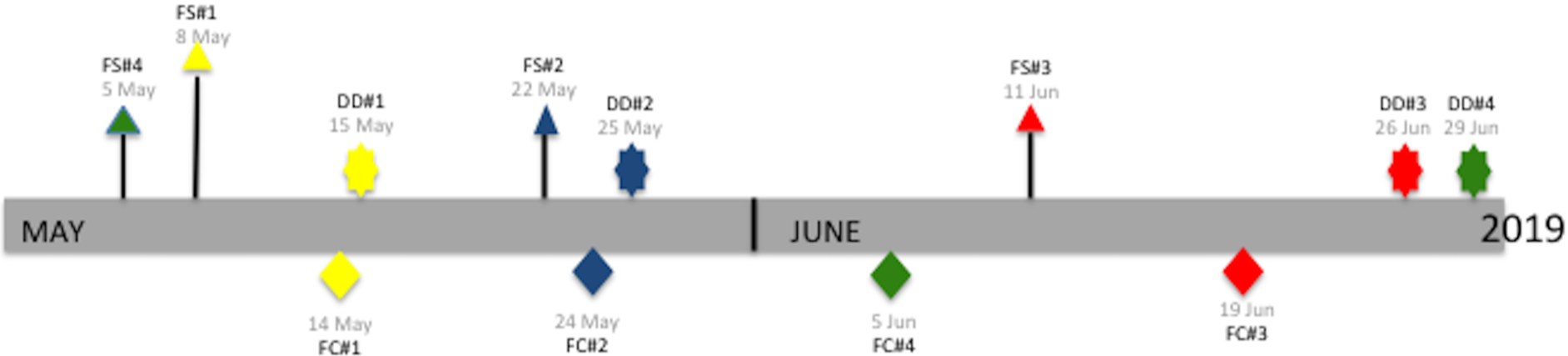
* Age is expressed in months.

f: female; m: male; US: ultrasound; CT: computed tomography; e: effusion; np: not performed; WBC: white blood cell count (cells/mm^3); CRP: C-reactive protein (mg/L); *Kk: Kingella kingae*

Figures:

Figure 1: Chronology of each case

FS: date of the first sign (triangle) , DC: date of the first consultation (losange), DD: date of diagnosis (star), yellow signs: case#1, blue signs: case#2, red signs : case#3, green signs : case#4


FS#1
8 May
FS#4
5 May
DD#1
15 May
FS#2
22 May
DD#2
25 May
FS#3
11 Jun
DD#3
26 Jun
DD#4
29 Jun
MAY
JUNE
2019
14 May
FC#1
24 May
FC#2
5 Jun
FC#4
19 Jun
FC#3