Polymerase chain reaction detection of *Kingella kingae* in children with culture-negative septic arthritis in eastern Ontario

Robert Slinger MD[1,2], Ioana Moldovan[1], Jennifer Bowes MSc[1], Francis Chan PhD[1,2]

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BACKGROUND: The bacterium *Kingella kingae* may be an under-recognized cause of septic arthritis in Canadian children because it is difficult to grow in culture and best detected using molecular methods.
OBJECTIVES: To determine whether *K kingae* is present in culture-negative joint fluid specimens from children in eastern Ontario using polymerase chain reaction (PCR) detection methods.
METHODS: *K kingae* PCR testing was performed using residual bacterial culture-negative joint fluid collected from 2010 to 2013 at a children's hospital in Ottawa, Ontario. The clinical features of children with infections caused by *K kingae* were compared with those of children with infections caused by the 'typical' septic arthritis bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*.
RESULTS: A total of 50 joint fluid specimens were submitted over the study period. Ten were culture-positive, eight for *S aureus* and two for *S pyogenes*. Residual joint fluid was available for 27 of the 40 culture-negative specimens and *K kingae* was detected using PCR in seven (25.93%) of these samples. Children with *K kingae* were significantly younger (median age 1.7 versus 11.3 years; P=0.01) and had lower C-reactive protein levels (median 23.8 mg/L versus 117.6. mg/L; P=0.01) than those infected with other bacteria.
CONCLUSIONS: *K kingae* was frequently detected using PCR in culture-negative joint fluid specimens from children in eastern Ontario. *K kingae* PCR testing of culture-negative joint samples in children appears to be warranted.

Key Words: *Septic arthritis;* Kingella kingae*; PCR*

La détection du *Kingella kingae* au moyen de l'amplification en chaîne par polymérase chez les enfants atteints d'arthrite septique à bactériologie négative dans l'est de l'Ontario

HISTORIQUE : La bactérie *Kingella kingae* peut être une cause sous-diagnostiquée d'arthrite septique chez les enfants canadiens, car elle est difficile à développer en culture et qu'elle est plus facile à déceler à l'aide de méthodes moléculaires.
OBJECTIFS : Déterminer si le *K kingae* est présent dans des prélèvements de liquide articulaire à bactériologie négative provenant d'enfants de l'est de l'Ontario, à l'aide des méthodes de détection par amplification en chaîne par polymérase (PCR).
MÉTHODOLOGIE : Les chercheurs ont réalisé la PCR du *K kingae* dans le liquide articulaire à bactériologie négative résiduel prélevé entre 2010 et 2013 dans un hôpital pour enfants d'Ottawa, en Ontario. Ils ont comparé les caractéristiques cliniques des enfants atteints d'une infection causée par le *K kingae* à celles d'enfants infectés par les bactéries habituellement responsables de l'arthrite septique, soit le *Staphylococcus aureus* et le *Streptococcus pyogenes*.
RÉSULTATS : Au total, 50 prélèvements de liquide articulaire ont été soumis pendant la période de l'étude. Dix étaient à bactériologie positive, soit huit au *S aureus* et deux au *S pyogenes*. Il y avait du liquide articulaire résiduel pour 27 des 40 prélèvements à bactériologie négative, et le *K kingae* a été décelé par PCR dans sept d'entre eux (25,93 %). Les enfants infectés par le *K kingae* étaient beaucoup plus jeunes (âge médian de 1,7 an plutôt que de 11,3 ans; P=0,01) et avaient un taux de protéine C-réactive plus faible (médiane de 23,8 mg/L plutôt que de 117,6 mg/L; P=0,01) que ceux infectés par d'autres bactéries.
CONCLUSIONS : Le *K kingae* était souvent décelé par PCR dans les prélèvements de liquide articulaire à bactériologie négative réalisés chez des enfants de l'est de l'Ontario. Il semble justifié d'effectuer une PCR du *K kingae* dans les prélèvements articulaires à bactériologie négative réalisés chez les enfants.

Acute septic arthritis in children can lead to chronic joint damage with long-term impairment of function if not diagnosed and treated appropriately (1). Based on bacterial cultures of joint fluid specimens, *Staphylococcus aureus* is reported to be the most common cause of paediatric septic arthritis. Several other bacteria can also cause joint infections in children, including *Streptococcus pyogenes* (group A streptococcus), *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Salmonella* species and *Streptococcus agalactiae* (group B streptococcus) (1). However, it is reasonably common to have negative cultures with clinically suspected septic arthritis even in the absence of previous antibiotics.

In several countries, including Israel and France, *Kingella kingae*, a fastidious, Gram-negative coccobacilli that is part of the normal pharyngeal flora of young children, has been detected and is presumed to be a pathogen in culture-negative cases (2,3). Improved detection of *K kingae* using traditional and molecular methods has, therefore, been sought. For example, direct blood culture bottle inoculation with joint fluid specimens has been reported to improve detection relative to agar plate and broth culture methods (4). However, molecular techniques, such as polymerase chain reaction (PCR), are considered to be the most sensitive methods for detection of *K kingae* (3,5). In some centres where molecular techniques are used, *K kingae* has been found to be one of the most common causes of paediatric septic arthritis (2,3,5).

Because molecular methods are not readily available in many centres, clinical features to help differentiate infections caused by *K kingae* have been sought. Children with *K kingae* are generally reported to be younger than children with other causes of septic arthritis, typically <4 years of age (2,3). Ceroni et al (6) reported four variables that are the most useful in differentiating *K kingae* infections from infections with the 'typical' pathogens, *S aureus* and *S pyogenes*, which are the most common culture-proven causes

[1]*Department of Laboratory Medicine and Pathology;* [2]*Department of Pediatrics, Children's Hospital of Eastern Ontario, University of Ottawa, Ottawa, Ontario*
Correspondence: Dr Robert Slinger, University of Ottawa, Children's Hospital of Eastern Ontario, 401 Smyth Road, Ottawa, Ontario K1H 8L1. Telephone 613-737-7600 ext 2651, fax 613-738-4832, slinger@cheo.on.ca
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of septic arthritis in children. These variables include: C-reactive protein (CRP) level <55 mg/L; white blood cell count (WBC) <14,000 leukocytes/mm^3; band count with <150 band forms/mm^3; and temperature <38°C (6).

The purpose of the present study was to determine whether *K kingae* could be detected in culture-negative joint samples from children in eastern Ontario using a real-time PCR method for *K kingae*. We also examined the four variables described by Ceroni et al (6) to determine whether they were useful in recognizing *K kingae* joint infections. Finally, we tested culture-negative specimens for *S aureus, S pyogenes*, *S pneumoniae* and *H influenzae* using real-time PCR to determine whether culture methods had failed to detect any of these other organisms.

METHODS

The present study was performed at the Children's Hospital of Eastern Ontario, a tertiary care paediatric hospital located in Ottawa, Ontario, with 165 beds and approximately 70,000 emergency department visits per year. Of note, this is the only hospital that provides paediatric orthopedic care in the region; therefore, all children with possible septic arthritis in the region are likely to have presented to this hospital.

Ethics approval was obtained from the hospital's research ethics board for testing of residual aliquots of joint fluid specimens that were otherwise to be discarded, and for chart review to obtain laboratory results and patient characteristics. Results of PCR testing were not provided to clinicians.

Joint fluid specimens used in the present study were collected from January 2010 to December 2013 and were: bacterial culture-negative; not related to a trauma or previous orthopedic surgery; and had an adequate volume of residual sample remaining for DNA extraction (600 µL) after all cultures had been inoculated. These residual samples were stored at −80°C until used for DNA extraction.

Joint fluids were cultured using standard microbiological methods. Aliquots were inoculated on sheep blood agar, chocolate agar and in an enrichment broth in the appropriate atmospheres. Media were incubated at 37°C and checked daily for five days. An aliquot was inoculated on anaerobic agar media and stored for five days under strict anaerobic conditions; these were checked daily after a 48 h incubation period.

Nucleic acid extraction

DNA was extracted from 600 µL volumes of joint fluid. Samples first underwent bead-beating in ZR BashingBeads Lysis Tubes (0.5 mm beads; Zymo Research Corporation, USA) and agitated using a Disruptor Genie (Scientific Industries Inc, USA). Supernatants (400 µL) were removed and DNA was extracted using an automated nucleic acid extraction device (iPrep, Life Technologies, USA) with a final elution volume of 50 µL.

A published PCR assay for the *rtxA* gene of *K kingae* detection was used as the initial test and a second assay for the *rtxB* gene was applied if the initial *rtxA* assay was positive (2). These assays detect two independent gene targets in the *K kingae* RTX toxin locus, which is a major virulence factor for the organism. A real-time PCR assay for the detection of the *lytA* gene of *S pneumoniae*, the *hpd* gene of *H influenzae*, the *nuc* gene of *S aureus* and the *spy* gene of *S pyogenes* was also performed as described previously (7).

PCR reactions were prepared in 10 µL volumes in 96-well PCR plates using an automated liquid handler (Eppendorf 5070, Eppendorf Canada, Canada). A negative control (no template) was performed with each sample. Thermocycling was performed using a ViiA7 real-time PCR device (Life Technologies, USA) in fast mode. Initially, a temperature of 95°C was maintained for 20 s, followed by 40 cycles of two-temperature cycling at 95°C for 3 s followed by 60°C for 30 s.

For statistical analysis of bacterial detection methods, it was decided a priori that specimen results would be considered as true positives if the culture was positive or if the two PCR assays for the organism were positive.

For the clinical analysis, age and the first recorded values of erythrocyte sedimentation rate, CRP, WBC, band count and temperature were compared between children with *K kingae* septic arthritis and those infected with the 'typical' organisms, *S aureus* and *S pyogenes*, following the classificaton method used by Ceroni et al (6). All culture-positive cases were included in the analysis of 'typical' organisms, even if a fluid sample was unavailable for *K kingae* PCR. The Kruskal-Wallis test was used to compare the median values for these variables between the two groups and Fishers exact test was used to compare the proportions between the two groups for the four criteria reported to differentiate *K kingae* septic arthritis from cases caused by 'typical' organisms (6); P<0.05 was considered to be statistically significant.

RESULTS

A total of 50 nontraumatic/nonsurgical joint fluid specimens were submitted over the study period. Ten samples were culture-positive, eight for *S aureus* (all methicillin-susceptible *S aureas*) and two for *S pyogenes*.

Among the 40 culture-negative samples, 13 were excluded because the residual volumes were inadequate for PCR. Twenty-four of 27 (89%) specimens were obtained before administration of antibiotics. *K kingae* was detected using PCR in seven of 27 (26%) samples, including four knee and three hip specimens. *K kingae* was the only pathogen detected by PCR but missed by culture because PCR tests were negative for *S aureus*, *S pyogenes*, *S pneumoniae* and *H influenzae* in all 27 culture-negative specimens. One of the children found to be PCR-positive for *K kingae* received antibiotics before collection of joint fluid samples for culture. Five of the seven *K kingae*-positive samples had joint cell counts measured, and the mean joint cell count was 97,998 cells/mm^3 (range 49,390 cells/mm^3 to 161,100 cells/mm^3).

Treatment for all seven cases consisted of intravenous cefazolin (150 mg/kg/day) followed by high-dose oral cephalexin (100 mg/kg/day to 150 mg/kg/day). The median total length of therapy was 29 days (intravenous plus oral). No relapses were recorded.

The clinical and laboratory features of children found to have *K kingae* and those with 'typical' organisms are reported in Table 1. The median age of children with *K kingae* of 1.7 years and was significantly lower than the median age (11.3 years) of the 'typical' organism group (P=0.01). Six of seven children with *K kingae* were <4 years of age.

The median CRP level (23.8 mg/L) for the *K kingae* group was also significantly lower than the 'typical' organism group median CRP level (117.6. mg/L; P=0.01). The median erythrocyte sedimentation rate and WBC were not significantly different between the two groups.

The proportion of patients with *K kingae* and 'typical' bacterial organisms who fulfilled Ceroni et al's (6) criteria for recognition of *K kingae* is shown in Table 2. A significantly higher proportion of children with *K kingae* had a CRP level <55 mg/L compared with those with 'typical' organisms (P=0.04). The proportion of patients with fever was lower in the *K kingae* group; however, this was not statistically significant (P=0.07). All patients with *K kingae* had a WBC <14,000/mm^3, as did 80% of those with a 'typical' infection; therefore, this criterion was not discriminatory. None of the patients in either group had band counts >150 cells/mm^3.

TABLE 1
Comparison of age and inflammatory marker measurements of children with *Kingella kingae* septic arthritis with those with typical bacterial organisms

	K kingae (n=7)	Typical organisms† (n=10)
Age, years*	1.7 (0.86–6.20)	11.3 (1.54–14.16)
ESR, mm/h	38.5 (23–50)	49 (28–60)
CRP, mg/L*	23.8 (5.9–81.8)	117.6 (32.8–258)
WBC, cells/mm^3	8100 (6400–18,000)	11,100 (4900–15,700)

*Data presented as median (range). *Statistically significant difference (P<0.05). †*Staphylococcus aureus *and* Staphylococcus pyogenes *were defined as typical organisms. CRP C-reactive protein; ESR Erythrocyte sedimentation rate; WBC White blood cell count*

TABLE 2
Proportion of patients with *Kingella kingae* and typical bacterial organisms who met each of the Ceroni criteria for recognition of *K kingae* (6)

	K kingae (n=7)	Typical organisms† (n=10)
Temperature <38°C	83	40
CRP <55 mg/L*	83	20
WBC <14,000 cells/mm^3	100	80
Bands <150 cells/mm^3	100	100

*Data presented as %. *Statistically significant difference (P<0.05). †*Staphylococcus aureus *and* Staphylococcus pyogenes *were defined as typical organisms. CRP C-reactive protein; WBC White blood cell count*

The median number of criteria present in patients with *K kingae* was four, while the median number in the typical group was two. This difference approached, but did not reach statistical significance (P=0.05).

DISCUSSION

K kingae was frequently detected in culture-negative joint fluid specimens in children in eastern Ontario. Of note, joint cell counts in *K kingae* PCR-positive specimens were elevated and in a range consistent with a diagnosis of septic arthritis (>25,000 cells/mm^3), supporting the pathogenicity of the organism (8). It has recently been demonstrated by genetic typing of *K kingae*, that a small number of sequence types associated with septic arthritis are internationally distributed and responsible for the majority of infections worldwide. Given these findings, it is perhaps not surprising that this organism was found in joint specimens from children in eastern Ontario (9).

Despite the fact that six of the seven *K kingae* cases had not received antibiotics before specimen culture, the organism was not detected by culture in any specimens over the study period, consistent with previous reports regarding the poor sensitivity of bacterial culture. Culture detection is reported to be more sensitive if blood culture bottles are directly inoculated with joint fluids; however, PCR methods have been demonstrated to have greater sensitivity than the direct blood culture bottle method (3,5).

Because cultures were negative, antimicrobial susceptibility testing could not be performed in the present study. *K kingae* is generally considered to be susceptible to penicillin and cephalosporins but inherently resistant to vancomycin. All children in the present study appeared to respond well to first-generation cephalosporin (cefazolin/cephalexin) treatment. However, in vitro studies suggest that oxacillin and clindamycin do not provide adequate antimicrobial coverage for *K kingae* (10).

Because antibiotics, such as cloxacillin or vancomycin, which have poor or no activity against *K kingae*, may be used in septic arthritis cases to provide empirical coverage for methicillin-susceptible *S aureus* and methicillin-resistant *S aureus*, respectively, there are advantages to identifying *K kingae* so that appropriate therapy is prescribed. In addition, resistance to penicillin in *K kingae* through a TEM-1 beta-lactamase has recently been reported in several countries (11). These penicillin-resistant isolates are susceptible in vitro to third-generation cephalosporins, such as ceftriaxone, but susceptibility to cefazolin has not been evaluated.

As has been reported by others, *K kingae* was found in younger children compared with 'typical' organisms in our region. We also observed a significant difference in median CRP level, and the CRP threshold of <55 mg/L used in Ceroni et al's (6) criteria appeared to help discriminate *K kingae* from 'typical' organisms. However, we did not observe differences between the groups using the criteria of WBC <14,000 leukocytes/mm^3 or a band shift with <150 band forms/mm^3. Differentiation of the groups based on the temperature criteria (<38°C) approached but did not reach statistical significance. Because the variables used by Ceroni et al (6), other than CRP, were not helpful in our setting, a larger multi-centre study to create a new clinical prediction tool for *K kingae* for use in centres where PCR is not readily available may be needed.

Of note, *K kingae* has been reported twice previously in Canadian children. One involved an 18-month-old child with *K kingae* endocarditis, while the other described a case of *K kingae* spondylodiscitis; *K kingae* was detected in culture in both cases (12,13). No cases of molecular detection of *K kingae* in children have been reported.

Our study had some limitations; one was the small sample size. Despite this, differences in age and CRP levels between the groups were so markedly different that statistical significance was reached. Another study limitation was that some culture-negative specimens were unavailable for PCR testing due to inadequate residual specimen volumes, and some of these samples may have contained *K kingae*. In addition, it is possible that the PCR assay used may have failed to detect *K kingae* in some specimens tested. For the 20 culture-negative/*K kingae* PCR-negative cases, the median age was 9.5 years, and only two cases involved children <4 years of age; therefore, we do not believe it is likely that many of these cases were due to *K kingae* not detected by PCR. Finally, joint fluid samples were frozen at −80°C until tested rather than being tested immediately; however, because DNA is well-preserved at this temperature, we do not believe this delay had a significant effect on PCR results.

CONCLUSION

Through use of real-time PCR, we have shown for the first time, to our knowledge, that *K kingae* is an important cause of septic arthritis in children seen at a Canadian paediatric hospital. *K kingae* should be clinically suspected in younger children, usually <4 years of age, who have CRP levels typically <55 mg/L. However, because some children with *K kingae* infections will be older or have higher CRP levels, we believe that, wherever possible, *K kingae* joint fluid PCR testing should be performed in culture-negative cases of suspected septic arthritis.

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