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Abstract

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Dimitri Ceroni, Victor Dubois-Ferriere, Abdessalam Cherkaoui, Renzi Gesuele, Christophe Combescure, Léopold Lamah, Sergio Manzano, Jonathan Hibbs and Jacques Schrenzel

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Detection of *Kingella kingae* Osteoarticular Infections in Children by Oropharyngeal Swab PCR

**WHAT’S KNOWN ON THIS SUBJECT:** There is evidence that *Kingella kingae*, the major bacterial cause of osteoarticular infection in children <4 years of age, first colonizes the oropharynx before penetrating the bloodstream and invading distant organs. Diagnosis remains challenging because clinical findings at admission may be normal.

**WHAT THIS STUDY ADDS:** Our study demonstrated for the first time that a simple technique of detecting of *K. kingae* DNA in the oropharynx can provide strong evidence that this microorganism is responsible for the OAI, or even stronger evidence that it is not.

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**abstract**

**OBJECTIVE:** The purpose of this study was to investigate if oropharyngeal swab polymerase chain reaction (PCR) could predict osteoarticular infection (OAI) due to *Kingella kingae* in young children.

**METHODS:** One hundred twenty-three consecutive children aged 6 to 48 months presenting with atraumatic osteoarticular complaints were prospectively studied. All had a clinical evaluation, imaging, and blood samples. Blood and oropharyngeal specimens were tested with a PCR assay specific for *K. kingae*. OAI was defined as bone, joint, or blood detection of pathogenic bacteria, or MRI consistent with infection in the absence of positive microbiology. *K. kingae* OAI was defined by blood, bone, or synovial fluid positivity for the organism by culture or PCR.

**RESULTS:** Forty children met the OAI case definition; 30 had *K. kingae* OAI, 1 had another organism, and 9 had no microbiologic diagnosis. All 30 oropharyngeal swabs from the *K. kingae* case patients and 8 swabs from the 84 patients without OAI or with OAI caused by another organism were positive. The sensitivity and specificity of the oropharyngeal swab PCR assay for *K. kingae* were 100% and 90.5%, respectively.

**CONCLUSIONS:** Detection of *K. kingae* DNA in oropharyngeal swabs of children with clinical findings of OAI is predictive of *K. kingae* OAI. If these findings are replicated in other settings, detection of *K. kingae* by oropharyngeal swab PCR could improve the recognition of OAI.

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**KEY WORDS**

*Kingella kingae*, osteoarticular infection, polymerase chain reaction, diagnosis, children

**ABBREVIATIONS**

OAI—osteoarticular infection

PCR—polymerase chain reaction

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Acute osteoarticular infection (OAI) remains an important concern in children because of potentially devastating consequences for bone development and articular function. In recent years, the diagnosis of *Kingella kingae* OAI has increased, probably because of improvements in culture techniques and the use of molecular methods. *K. kingae* is considered as the major bacterial cause of OAI in children <48 months. However, diagnosis remains challenging because clinical findings at admission may be within the normal range of values and because this fastidious microorganism is difficult to isolate on solid media.

*K. kingae* is a component of the oropharyngeal flora of young children and is transmitted from person to person. Although pathogenesis of *K. kingae* invasive infections remains unclear, there is evidence that *K. kingae* first colonizes the oropharynx before penetrating the bloodstream and invading distant organs. A cytotoxin (RTX) made by *K. kingae* may play a crucial role in colonization of the respiratory tract, in breaching the epithelium, and in damaging bones and joints. Real-time polymerase chain reaction (PCR) assays targeting the RTX toxin genes confirm the presence of *K. kingae* and have demonstrated their importance in the diagnosis of *K. kingae* infection. These molecular methods based on the RTX toxin gene sequence have been shown to be accurate for the diagnosis of *K. kingae* and are now considered the gold standard for diagnosing *K. kingae* OAI. We hypothesized that *K. kingae* should be present in oropharyngeal flora in children with *K. kingae* OAI and should be detectable in oropharyngeal swabs by a PCR assay targeting *K. kingae'*s RTX toxin gene. If so, this simple and noninvasive test could become a helpful diagnostic tool for this disease.

Thus, the objectives of this study were 2-fold: to determine the carriage rate of *K. kingae* in the oropharyngeal flora of children presenting with *K. kingae* OAI and to investigate if an oropharyngeal swab PCR assay might be a valid diagnostic tool for detecting *K. kingae* OAI.

**METHODS**

From January 2008 through January 2012, we enrolled 125 children aged 6 to 48 months in a prospective clinical study at the Children’s Hospital. Subjects were recruited from children presenting to our emergency department with a suspicion of OAI. The study had received institutional review board approval (09-029R, Mat-Ped 09-008R) and was conducted in accordance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki.

**Study Group Selection**

One hundred twenty-three children with joint pain, limp, or restricted limb movement, with no history of trauma were enrolled. Their parents provided written consent. To be part of the study, children had to have biological, radiologic, and PCR-based protocol investigations.

**Evaluation of Study Patients**

All children had a clinical evaluation, blood samples, radiologic investigations, and an oropharyngeal swab. Results of oropharyngeal swab PCR assay were obtained after 24 to 48 hours and were not known to pediatric clinicians and radiologists during initial management.

When patients had clinical or laboratory findings suggestive of OAI, a MRI study was performed, and images were analyzed for evidence of OAI based on the literature. If imaging suggested OAI, arthrocentesis or bone aspiration was performed under fluoroscopy guidance. Analysis of aspirates included a Gram-stain, a real-time PCR assay specific to the *K. kingae*, and a broad range PCR assay. If patients did not have consistent clinical and biological signs of OAI, a follow-up examination was scheduled 48 hours after initial consultation, and investigations were ordered at that time depending on clinical evolution.

**Case Definitions**

OAI was defined as a positive culture or organism-specific PCR result from blood, joint fluid, or bone aspirate, or MRI findings consistent with infection in the absence of a positive culture. The diagnosis of OAI caused by *K. kingae* was established when blood or osteoarticular aspiration cultures were positive, or when PCR specific for this microorganism was positive on blood, synovial fluid, or bone aspiration. The diagnosis of OAI caused by other microorganisms was defined as bacterial growth other than *K. kingae* on culture of synovial fluid, blood, or bone aspirate, or by bone broad-range PCR. The diagnosis of presumed OAI was defined as an MRI with conclusive evidence of infection but either a negative culture of joint fluid or bone, or no culture obtained. The diagnosis of OAI was excluded when there was no growth on culture of a biological sample, no evidence of OAI on MRI, improvement without treatment, and/or another cause of limb movement limitation identified.

**Clinical Laboratory Testing**

Data obtained for all patients included age, gender, temperature, involved bone or joint, and laboratory data including white blood cell count and differential, platelet count, erythrocyte sedimentation rate, serum C-reactive protein, and PCR assays on oropharyngeal swabs and peripheral blood. Laboratory, clinical, and imaging data were collected and used for therapeutic and clinical purposes but were not analyzed in this study.
The joint fluid or bone aspirate sample was sent to the laboratory for Gram-staining, cell count, and immediate inoculation onto Columbia blood agar (incubated in CO₂-enriched atmosphere), CDC anaerobe 5% sheep blood agar (incubated under anaerobic conditions), chocolate agar (incubated in CO₂-enriched atmosphere), and brain-heart broth. The media were incubated for 10 days. Blood cultures were processed by using the Bactec system (Becton Dickinson).

**PCR**
All biological materials (oropharyngeal swab, synovial fluid, bone biopsy specimen, and peripheral blood) were analyzed with a real-time PCR assay specific to the *K. kingae*. The assay is designed to detect 2 gene targets from the *K. kingae* RTX toxin locus, rtxA and rtxB. DNA was extracted with a MagNAPure LC instrument using the MagNAPure LC DNA isolation kit II (Roche Molecular Biochemicals) according to the manufacturer’s instructions. TaqMan Universal PCR Master Mix with AmpErase UNG (Applied Biosystems) was used with 0.5 μL of input DNA and nuclelease-free water (Promega). Each PCR analysis was performed in duplicate. If the *K. kingae* PCR assay was negative, osteoarticular samples were submitted to broad-range PCR assay.

**Statistical Analysis**
The ability of PCR-based protocol on oropharyngeal swabs for the diagnosis of *K. kingae* was assessed by sensitivity, specificity, and the accuracy (proportion of well-classified subjects). The 95% confidence intervals of these proportions were obtained by the Clopper-Pearson method.

**RESULTS**

**Cases**
Between January 2008 and January 2012, 123 patients met criteria for the study; 40 had microbiologic or MRI evidence of OAI. Among these 40 patients with OAI, 30 (75%) had a proven *K. kingae* OAI identified by PCR assays performed on bone aspirates, joint aspirates, or blood (Table 1). In all these 30 patients, cultures remained negative for *K. kingae* and other microorganisms. One (2.5%) had culture positive for *Haemophilus influenzae* type b, and the remaining 9 (22.5%) had no microbiologic diagnosis. Eighty-three patients had no evidence of OAI.

**Test Characteristics of the Oropharyngeal Swab PCR Assay**
Oropharyngeal swab PCR assays were performed among all children in the study, but the 9 children with MRI evidence of OAI in the absence of microbiologic confirmation were excluded from statistical analysis of the oropharyngeal swab PCR. This left 114 children included in the statistical analysis, including 30 children with *K. kingae* OAI and 84 other children. RTX toxin PCR was positive in oropharyngeal specimens from 38 children. All 30 patients with *K. kingae* OAI had positive oropharyngeal swab PCRs, as did 8 (7%) of the other 84 patients tested. Among these 8 patients, none had evidence of OAI. Statistical analysis of diagnostic performance of the oropharyngeal swab PCR assay for *K. kingae* OAI showed that sensitivity and specificity were 100% [88.4–100] and 90.5% [82.1–95.8], respectively, with 95% confidence intervals in square brackets. The accuracy of the test was estimated to 93% [86.6–96.9].

**Cases Excluded From Statistical Analysis**
Nine patients met the case definition of OAI based on MRI findings but without

### TABLE 1

<table>
<thead>
<tr>
<th>Child Age, mo</th>
<th>Site of Infection</th>
<th>Type of Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>Rachis</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>Rachis</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>Hand (metacarpal)</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>Knee</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>Hand (metacarpal)</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>Proximal femur</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>Knee</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>Distal radioulnar joint</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>Distal radius</td>
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<td>10</td>
<td>15</td>
<td>Knee</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>Hip</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>Proximal humerus</td>
</tr>
<tr>
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<td>16</td>
<td>9</td>
<td>Knee</td>
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<tr>
<td>17</td>
<td>22</td>
<td>Hip</td>
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<td>Knee</td>
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<tr>
<td>19</td>
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<td>20</td>
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<td>Hip</td>
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<td>Ankle (calcaneus)</td>
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<td>Knee</td>
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<td>Knee</td>
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<td>29</td>
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<td>Hip</td>
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<td>30</td>
<td>18</td>
<td>Ankle and tal bone</td>
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</tbody>
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microbiologic confirmation. These cases were excluded from the analysis above because microbiologic diagnoses of the affected joints were ambiguous. Five of these patients presented radiologic and clinical findings of spondylodiscitis but were not subjected to disc-vertebral puncture. The other 4 cases showed imaging findings consistent with OAI, but aspiration cultures and PCR assays were negative, possibly because of difficult access to the site of infection or low bacterial load. It is noteworthy, however, that all 9 of these patients had positive oropharyngeal swab PCR assays and were treated for *K. kingae* OAI, with clinical improvement.

**DISCUSSION**

Our study confirms the hypothesis that oropharyngeal carriage of *K. kingae* is high among children with bone and joint infections with this organism. Invasive infections in young children are frequently caused by organisms carried asymptptomatically in the respiratory tract. Microorganisms residing in the mucosal surface, such as *Streptococcus pneumoniae, H influenzae* type b, or *Neisseria meningitidis* are able to penetrate the bloodstream, disseminate, and invade distant organs.14,15 Colonization of the respiratory tract by these organisms is therefore a prerequisite for later invasion. Previous studies suggested that the pathogenesis of invasive infection caused by *K. kingae* follows the same pattern and demonstrated that evidence of respiratory carriage of the organism should be found in children with OAI due to *K. kingae*.9,10 Another study revealed that patients with invasive *K. kingae* infections had genotypically identical isolates recovered from pharynx and bloodstream.9 The current study, in which PCR assays were performed on oropharyngeal swabs of children with suspected OAI, demonstrated that PCR of oropharyngeal swabs was always positive in children with proven *K. kingae* OAI. It is important to underline that the presence of *K. kingae* in oropharyngeal flora is not always followed by an invasive infection, because the asymptomatic respiratory carriage rate in young children has been estimated at between 8% and 12%.16,17 Investigations of asymptomatic respiratory carriage have not demonstrated a correlation between the pattern of carriage of *K. kingae* and the occurrence of invasive disease. Nevertheless, a few reports advised antibiotic administration to prevent further cases during outbreaks of *K. kingae* disease among day care center attendees, because respiratory carriage is presumed to constitute a high risk to develop an invasive infection.18–20

Because the initial presentation of children with *K. kingae* OAI is insidious and requires a high index of suspicion,16,19 it is easy for physicians to underestimate the likelihood of this disease in young children. Because there is no noninvasive method for diagnosing OAI due to *K. kingae*, many physicians are reluctant to collect osteoarticular samples for bacteriologic testing when the clinical and biological features are not highly suggestive of OAI.

The second objective of this study was to assess if indirect detection of *K. kingae* in oropharyngeal swabs could help clinicians identify *K. kingae* in children with clinical evidence of OAI. The results of the study demonstrated that an oropharyngeal swab PCR assay for detecting *K. kingae* OAI had very high sensitivity (100%), although its specificity was only 90.5%. Although detection of *K. kingae* in oropharyngeal swabs does not mean always that a child has a *K. kingae* OAI, negative results may exclude *K. kingae* in children with osteoarticular complaints. The result of the PCR assay on oropharyngeal swabs should be integrated into the clinical context. Our patients had a relatively high pretest probability of disease. The specificity of this test for detecting *K. kingae* OAI will probably fall if it is used on children without clinical evidence to support OAI. On the other hand, it is encouraging to note the very high sensitivity, even among children with a high pretest probability of disease. Bayes theorem suggests that if it has a high sensitivity in this population, a negative test will exclude *K. kingae* OAI in most other populations as well.

To assess the diagnostic performances of a PCR assay on oropharyngeal swabs for the diagnosis of *K. kingae* OAI, we needed a gold standard for diagnosis of OAI. Currently, the gold standard for diagnosis of OAI remains a positive culture or PCR from blood or an osteoarticular aspiration. However, negative assays are frequent in OAI, it is not clear whether this is because another organism is causative or because the gold standard assay itself is not sufficiently sensitive. Use of a relatively insensitive gold standard tends to bias evaluations of new assays against specificity. As the main interest of using an oropharyngeal swab PCR would be to rule out a *K. kingae* OAI this presence of positives unconfirmed by culture does not decrease the utility of using the oropharyngeal swab PCR as a screening test.

Finally, we have been concerned about the complete failure of the culture methods for isolating *K. kingae* that we used in this study. The primary isolation of *K. kingae* from joint, bone, or blood samples appears to strongly depend on the methodology used.4 In fact, the recovery of *K. kingae* from purulent specimens seeded onto solid culture media is suboptimal and results most of the time in a frustrating...
proportion of negative cultures. The yield of cultures has been significantly improved by inoculating clinical specimens into aerobic blood culture vials from a variety of automated blood culture systems such as BACTEC (Becton Dickinson, Cockeysville, MD), BacT/Alert (Organon Teknika Corporation, Durham, NC), Isolator 1.5 Microbial Tube (Wampole Laboratories, Cranbury, NJ), or Hemoline DUO (bio-Mérieux Lyon, France). However, no controlled study has been performed to identify the best blood culture system for this purpose. When positive blood culture bottles are subcultured onto blood-agar or chocolate-agar plates, K. kingae usually grows without any difficulty, demonstrating that routine solid media are able to support its nutritional requirements. Hypothesis for explaining this contradictory phenomenon suggests that exudates exert an inhibitory effect on the bacterium and that dilution of purulent samples in a large volume of broth may decrease concentration of such inhibitory factors, thus improving the recovery of this fastidious organism. Nevertheless, even when blood culture vials are used for culturing synovial fluid aspirates, K. kingae is isolated in almost 50% of young children with proven septic arthritis, which indicates that PCR detection of this organism should be routinely used to improve the bacteriologic diagnosis. In addition, even when blood cultures are obtained and processed with the automated Bactec system, these specimens may be negative in patients with focal joint or bone infections, because bacteremia due to K. kingae is probably very short with low bacterial loads. Therefore, we decided, in the current study, to favor the use of specific PCR to detect K. kingae and to perform classic culture methods for isolate osteoarticular infection due to classic germs, such as Staphylococcus aureus or Streptococcus pyogenes.

CONCLUSIONS

To the best of our knowledge, there is no description in the literature of noninvasive methods for ruling out or diagnosing OAI caused by K. kingae. Our study demonstrated for the first time that a simple technique of detecting of K. kingae RTX toxin genes in the oropharynx provides strong evidence that this microorganism is responsible for the OAI, or even stronger evidence that it is not. Such a noninvasive approach to diagnosis will both improve patient safety and reduce health care costs by reducing the need for invasive diagnostic procedures. Although these results need to be validated by other prospective studies, one can legitimately expect that the use of novel microbiologic methods will improve the indirect identification of K. kingae. This is particularly useful because most children with K. kingae OAI respond promptly to conservative treatment with appropriate antibiotics and do not require invasive surgical procedures. Therefore, we believe that the results of the oropharyngeal swab PCR assay might become, in the near future, an early decision-making tool for treating K. kingae OAI in young children. By using a combination of oropharyngeal swab PCR tests, the predictive score for K. kingae OAI, and radiologic investigations when appropriate, it should become possible to achieve a higher accuracy of OAI diagnosis at lower cost, in less time, and with less danger and distress for the patient.

In general, a negative assay should be sufficient to rule out Kingella. In these patients, invasive testing remains necessary for diagnosis, and empirical therapy need not include Kingella coverage. A positive test, on the other hand, is highly predictive of Kingella in 6- to 48-month-old children in our catchment area. Among these patients, antibiotic coverage of Kingella should be included in empirical therapy. If our findings can be replicated elsewhere, we suggest that it may be time to reconsider the universal requirement for traditional invasive diagnosis of young children with OAI and oropharyngeal K. kingae.

REFERENCES


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