Kingella kingae: a review

Iain Williams

Iain wrote this article during his second-year studies at Lancaster Medical School last year.

INTRODUCTION

Over the last 20 years *Kingella kingae* (*K kingae*) has been recorded in several regions, including Israel, Western Europe and northern America.[1] It was previously thought not to be a significant cause of disease in humans, but newer detection and diagnostic techniques have demonstrated its association with a variety of conditions. It is included in the HACEK group (*Haemophilus spp., Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae*) of gram negative, slow-growing fastidious bacteria that cause endocarditis.[2] It is an emerging pathogen that has been linked to osteomyelitis in children.[3]

Common investigations for osteoarticular infections are usually investigated with techniques such as radioisotope scanning (involving compounds such as technetium-99, gallium-67 and indium-111), magnetic resonance imaging (MRI), X-ray and laboratory tests.[3] The laboratory tests involve analysis of erythrocyte sedimentation rate (ESR), white cell count and blood culture. Due to the difficulty in identifying organisms by culture, antibiotic choice is based on local sensitivities. *K kingae* itself has been best identified using polymerase chain reaction (PCR) analysis since 1998, and this is currently the gold standard for detection.[4]

The aim of this review is to gain an understanding of this relatively new pathogen and to review methods by which it can be identified. The method of research was to critically analyse literature from a database, using specific search criteria.

MICROBIOLOGY

K kingae is a gram negative, slow-growing fastidious bacterium. It has been described as appearing under the microscope in pairs, or short chains, with tapered ends. It is occasionally misidentified as gram positive because it is liable to resist decolourisation. It is difficult to culture, requiring specialist media.[5] When colonies are grown, they produce marked impressions on agar surfaces, leaving identifiable patches upon removal. It produces spreading-corroding growth, which is indicative of long '*type IV pili*',[6] and smooth colony growth, which indicates a variant colony. *K kingae* is an acid-producing bacterium, which uses only glucose and maltose as substrates. It has been described commonly in literature as non-motile, oxidase positive and exhibiting negative catalase urease reactions.[7]

EPIDEMIOLOGY

A 1995 study examined Israeli paediatric services to demonstrate the incidence of invasive *K kingae* infections.[8] All southern Israeli children are born in and receive care at one facility; therefore, the incidence could be calculated from this population. Invasive infections were defined by being able to isolate the organism from normally sterile bodily fluids. Patients were surveyed during a 15-year period, from 1998 to 2001. Seventy-four cases of invasive infection were recorded, 50 being male and 24 being female. Seventy-three patients were below the age of 48 months, while one was a 21-year-old adult. The most common site of infection was the skeletal system, accounting for 48 patients (65%). Occult bacteremia was seen in 22 patients (30%), lower respiratory tract infections in three patients (4%) and endocarditis in the adult patient (1%).

K kingae's seasonal distribution has been noted as similar across several isolated populations. With the Israeli study illustrating 29.4% of cases occurring between January and June, and 70.6% occurring from July to December. Asymptomatic colonisation of the upper respiratory tract is common, and this appears to be the first stage of infection.[6] The posterior pharynx is believed to be the area where colonisation begins, and it is the epithelial cells located here that are susceptible to adherence by the pathogen reference.

Pathophysiology

Bacterial factors that allow adherence to cells, colonisation and seeding, are not completely understood, but an important factor seems to be *type IV pili*. *K kingae* has been observed adhering to a variety of cells in the respiratory tract and synovial joints; cells adhered to include Chang cells, Hep-2 (human larynx) and SW982 cells (human synovial). Some researchers have concluded that the fibres expressed by *K kingae* are, in fact, *type IV pili*, and these are required for adherence. Genes in the cluster *K kingae pilA2-pilA2-fimB* were all found to be transcribed.[6]

Patients with *K kingae* infections usually present with respiratory tract also, among other symptoms concurrent with viral pathology around the oropharyngeal region, suggesting damage to the mucosa helps facilitate invasion into the bloodstream. A haemolysin toxin, capable of lysing epithelial cells, is produced by *K kingae*.[9] When *K kingae* is able to remain in the bloodstream it can then disseminate into bones, joints or the epicardium.[1]

It is usually the long bones that are affected by *K kingae*. Patients are usually known to endure symptoms for a longer duration than those with septic arthritis (9.2+/-9.4 vs 3.2+/-3 days respectively). That being said, chronic conditions involving the bacteria remain uncommon. The long bones that are usually affected include the femur, tibia, ulna, fibula, humorous and radius.[10-12]

Infections involving *K kingae* are not known to resolve spontaneously. Some cases demonstrate lack of clear signs of osteomyelitis, but do show limping, etc.[13] These cases have resolved by the time blood cultures are returned. Good prognosis has also been reported in a case series from 2000, using four cases.[14] Of course, these observations come from case series, and judgement on *K kingae*'s severity must be withheld until larger studies can be undertaken using a longer timeframe. In addition to this, descriptions of *K kingae* as a milder infection have been disagreed with; a recent study (in 2014) demonstrated ten unusually severe cases between 2008 and 2011.[15] It was suggested by this study that due to its mild onset *K kingae* diagnosis can be delayed. This may lead to growth cartilage damage, which is a pertinent concern in children. It appears that there can be a risk in late identification of the bacteria, and therefore detection methods should be optimal.

DIAGNOSIS AND INVESTIGATION INVOLVED IN BONE AND JOINT INFECTIONS IN CHILDREN

The standard diagnostic procedure of acute osteomyelitis depends on a high degree of suspicion since many children do not exhibit findings typical of the disease. An acute haematogenous osteomyelitis may be established if two of the following criteria are present:[3]

- bone aspiration yields pus
- bacterial culture of bone or blood is positive
- presence of classical signs and symptoms
- radiographic changes typical of osteomyelitis occur

In cases of septic arthritis, the child should exhibit clear signs of sepsis, malaise and local signs of inflammation; except in the case of *K kingae*, where children may not exhibit all of the typical signs and not be febrile at the time of diagnosis. When infection is suspected, investigations would typically include radiographs and analysis of aspirated blood.

Radiographs may demonstrate swelling, and in both bone and joint infections are usually used to exclude other pathologies.[16,17] Other diagnostic tools can be employed, and a general analysis of the main investigations into bone and joint infections is detailed below.

It has been established previously what the strengths and weaknesses of the techniques reviewed are, in terms of general use rather than with reference to *K kingae*. These are briefly summarised in table 1.

The British Society of Children's Orthopaedic Surgery suggests a single test cannot be used to confirm or deny bone or joint infection, as it is an accumulation of supportive findings that establishes a diagnosis.[3] Yet a further complication posed by these conditions is how the underlying pathogen is identified, in order to specify treatment. This consideration becomes important in suspected cases of *K kingae*, since it is difficult to culture and signs of infection are not atypical.

TREATMENT

Treatment of *K kingae* can be completed with antibiotic therapy, often without the need for surgery. There is some inconsistency in treatment due to lack of guidelines, although a recent report by the British Society for Children's Orthopaedic Surgery has provided indications for how the infection should be treated. It has been described as highly susceptible to penicillins and cephalosporins,[9,11,18] but clinically the treatment is often changed to ampicillin or cefuroxime.[5] There was no reason given for this, it was merely an observation of clinical practice.

It is common practice to administer antibiotics before samples can be taken and cultured. Identification of *K kingae* can not only aid treatment in an individual patient, but also help when liaising with bacteriological and infectious disease departments. This is due to empirical antibiotic therapy being based on advice from relevant departments on the sensitivities of local populations of organisms.[3] If *K kingae* can be more readily identified, its consideration in treatment may be increased appropriately.

Technique	Strengths	Weaknesses
Bone scan	• whole body evaluation in one test • low radiation • sensitive evaluation	• needs radiopharms, which are not widely available • low specificity • high cost
MRI	• harmless to patients • excellent detail • contrasts are rarely allergenic	• limited availability • can be a lengthy procedure • relatively expensive
X-ray	• very useful for fractures, injury and abnormal bones • quick, non-invasive and painless	• possible damage from radiation after prolonged exposure
Blood tests	• less invasive in comparison to aspiration	• cannot give complete identification of pathogen • cannot give information on antibiotic susceptibility
Culturing	• easy to manipulate • best for morphology, biochemical reaction, toxicity and antagonistic activity • may be relatively invasive depending on where sample is taken from	• many bacteria are very difficult to culture in anything other than very specific conditions
PCR	• highly sensitive • easy to set up • fast turnaround time	• liable to contaminate • high degree of operator skill required • positive result may be hard to interpret, since some pathogens may remain in the blood without current infection

Table 1 Summary of strengths and weaknesses of the techniques reviewed

TECHNIQUES OF DETECTION

Research focused on restricted analysis of techniques. Imaging, culturing and polymerase have been reviewed primarily, since these have been seen to best identify K kingae *as cause of infection.*

MRI and X-ray diagnosis of *K kingae*

MRI may have potential in identifying *K kingae* infection; this is significant since identification by MRI would be quicker and less invasive than culturing. In a study conducted in 2011, 31 children were studied; 21 having confirmed diagnosis of *K kingae* and ten being diagnosed with gram positive cocci.[7] Findings from this study concluded that MRI can produce accurate identification of *K kingae*, since positive predictive value was (0.82) and the negative value was (0.75). This may be due to characteristics present on images of *K kingae* infected bones that are not present on other gram positive bacterial infections. For instance, children in *K kingae* groups presented with localised epiphyseal cartilaginous lesions; these were never found in the gram positive group.

Evidence of radiography being able to indicate bony destruction in osteomyelitis associated with *K kingae* was found.[3] It was posed that this may be due to greater sub-acute process; that being said, definitive results on its effectiveness in detecting *K kingae* are not available. Although highlighting a possible avenue for further research, both studies used a relatively small sample size. Reproduction of results would be beneficial, as would clear criteria for what constituted an X-ray or MRI image of *K kingae* infection.

Culture detection of *K kingae*

It has been established that *K kingae* is difficult to culture.[1] However, the technique can be improved, and thus the yield can be increased, by inoculating clinical specimens into aerobic blood-culture vials. Following this, *K kingae* grows readily when sub-cultured onto solid media. It has been argued that this method should be used since its effectiveness has been proved in studies conducted in France and Israel.[8] Although, even if the bacterium is isolated, complete analysis including antibiotic susceptibility could take up to three days.[12]

It has been argued by that blood culture vials should not be used for culturing joint fluid.[19] This is due to manufacturers' recommendations, as well as the problem of the fluid used in blood culture vials could not be used for further analysis. While it is pertinent to take into account conventional practice and the recommendations of manufacturers, this study does not consider the practical data recorded by the 1995 Israeli paediatric services study.[12,20] While there may be a place for this innovative technique, it does not outperform other techniques such as PCR. This was found by two studies (in 2003 and 2005), where it was recorded that 12 (67%) of 18 patients (with infection) were positive for *K kingae* when using culturing, in contrast to 100% positivity when using PCR.[10,18]

PCR analysis

PCR has been used effectively to identify *K kingae* in joint and bone infection. The first case of PCR being used to detect *K kingae* was in 1998.[21] Joint fluid was tested, and a primer was used to target a constant sequence found in *K kingae* genes. The target for amplification was 16rRNA, and the 412 nucleotide sequence was found to be 99.7% and 99.5% similar to the available sequence of *K kingae*. This led to effective diagnosis of *K kingae,* and similar analysis has been recorded subsequently using larger cohorts, thus providing better evidence base.[22] The detection rates recorded are similar when targeting 16rRNA, but there have been improvements on sensitivity when using different targets.

The cpn60 gene has also been targeted in PCR, in order to identify *K kingae*.[4] This study used fluid samples from 89 children with suspected septic arthritis to test whether this would be a viable target for PCR analysis. Thirty-six (40%) of the samples had a produced cultures with bacterial growth. *Staphylococcus aureus* was the main isolate 53% in the positive culture, while K *kingae* was found in 19%. In culture negative specimens, 52% were found to be positive for *K kingae* when using PCR, therefore indicating its superiority in detecting the pathogen above simple culturing. Overall out of the 60 children who were found to be positive for septic arthritis, 31 (52%) were found to have *K kingae;* this overtook *Staphylococcus aureus* to be the main cause of septic arthritis. Sequence variations occurred a lot during the study, with that in mind the research proposed better sequencing of the pathogen's genome to improve detection.

One even more recent target for identification using PCR is a toxin named RTX. A 2011 study found that this technique was highly sensitive, being able to produce a ten-fold increase in sensitivity compared to PCR targeting cpn60.[9] All 30 specimens in this study possessed the RTX gene. Positive identification using RTX has also been observed in studies (finding *K kingae* in 28 cases).[23] Limitations of both of these studies are the smaller cohort sizes. Therefore, larger sample sizes would strengthen the case for using RTX, although it does stand to be a good target for PCR analysis.

Care must always be taken when using PCR analysis. This has been highlighted by the authors of a 1998 study, who found DNA from pneumococcal bacteria in samples from healthy children both with and without culture proven infections.[22] Furthermore, Yagupsky's work on PCR detection used broad spectrum targets.[1,8,20] A large piece of fragment DNA was used (800 base pairs), lowering the sensitivity. Yagupsky's work using culture proven septic arthritis caused by other organisms other than *K kingae* could not be completed, due to broad spectrum technique failing to detect *K kingae* due to presence of other organisms.[24]

Contamination has long been recognised as being liable to occur with PCR, so it is not surprising to see this issue arise. Essentially its sensitivity, which is usually construed as a positive, can be its downside also since such a small amount of DNA can be detected. This small amount of DNA may or may not be the one targeted. Secondary rounds of amplification can be done, using primers to isolate the target DNA.

CONCLUSION

K kingae remains an intriguing pathogen, which has become a leading cause of skeletal infections in children under five years of age. It is well understood on a microbiological level, although due to its role as a cause of disease in humans being only recently recognised there is more work to be done in improving treatment and detection.

Novel detection methods have been demonstrated for identification of *K kingae*. The use of MRI is one interesting possibility for further research, since its speed and lack of invasiveness make it a potentially highly effective diagnostic tool. It would be another recommendation that a large retrospective on patient imaging with confirmed *K kingae* could be performed, in the hope of providing clinical criteria for diagnosis.

Culturing of specimens remains a relatively poor method for detecting *K kingae*, and the most effective method used for growth retains difficulties. It is PCR that has been shown to prove involvement of *K kingae* with best precision, and the key for further development lies in the target for amplification. Several targets have been suggested, and it appears the most promising gene would be RTX. PCR stands to be the best method available for the confirmation of *K kingae* in osteoarticular infections. For specific guidelines into the detection of *K kingae,* these methods must be reviewed against other criteria such as efficacy, cost, availability and invasiveness.

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Commentary . . .

David Telford and Monika Pasztor are consultant microbiologists at Royal Lancaster Infirmary.

Kingella kingae is a species of Gram-negative, aerobic coccobacillus; quite demanding culturally and not easy to grow. It isn't really that new, having been first described in 1960 when it was called *Moraxella kingae*. Iinitially it was thought to have no pathogenic potential, comprising part of the normal upper respiratory flora.

It was subsequently lumped together with a group of other culturally fastidious, Gram-negative organisms and given the name HACEK. This is an acronym composed from the initials of the genera of the group: ***H****aemophilus,* ***A****ggregatibacter,* (previously *Actinobacillus*), ***C****ardiobacterium,* ***E****ikenella* and ***K****ingella*. They are all constituents of normal oropharyngeal flora and were thought to be significant causes of endocarditis; underdiagnosed because of the difficulties in culture. More recent reviews indicate that they are probably quite rare; however, accountable for only 1.4-3% of all cases of this disease.

Most members of the HACEK group have also emerged as unusual causes of skin, soft-tissue, bone and joint infections. *Kingella* was not recognised as a significant cause of infection in children until the 1990s, when culture techniques had improved enough for it to be recognised. Its pathogenic potential has widened to include septic arthritis, osteomyelitis, spondylodiscitis, bacteraemia, endocarditis and occasionally lower respiratory tract infections and meningitis, often with quite subtle, low-grade features and preceded by a recent history of stomatitis or upper respiratory infection.

> **“Our lab database only throws up two isolates since 2009”**

Kingella is too rare to inform routine empiric antimicrobial choice, but it will probably be covered by our empiric choices. There are, however, a huge number of these obscure organisms, many with highly variable and unpredictable sensitivities. Collectively, they constitute the significant reason for invasive sampling before starting antibiotics – even in children. Then, when it's starting to look as though the initial antibiotics are failing, something will be coming through to direct a change. Also, a reference centre would probably want a washout or aspirate for PCR. Unusually for Gram-negative organisms, they are sensitive to penicillin; so sensitive to all the other basic penicillins, though beta-lactamase producing strains are being described. More advanced beta-lactams such as Co-amoxyclav or Cefuroxime would be appropriate here. Low MICs are also seen to Erythromycin, Gentamicin, Chloramphenicol, Tetracycline, Rifampicin and Ciprofloxacin but not Clindamycin. The organism is too rare to permit a robust opinion on which antibiotic delivers the best clinical outcomes. Our advice would be select good oral bioavailability and good biofilm activity based on sensitivity testing.

With regard to PCR, I get uneasy about exquisitely sensitive tests in these situations. Genomic methodologies are developed by enthusiasts who believe in their applicability. They also work in tertiary facilities that tend to accumulate cases. But in very low prevalence situations it's easy to get to a point where false positives outnumber true positives. There's a potential for very poor positive predictive value but the rarity makes it impossible to amass a critical mass of well-evaluated cases for test evaluation purposes.

There's no doubt that modern culture (high-quality enrichment culture and CO_2 enriched incubators over the past 10-20 years) has increased the pickup but I don't think we're missing many nowadays. Our lab database only throws up two isolates since 2009, this one and an eye swab of doubtful clinical significance. A recent review of antimicrobial sensitivities took 12 years to accumulate 145 isolates in Israel; so a dozen per year in a population of eight million with sophisticated medical facilities. If my ratios are right, that's about half a case per year for our 300,000; so two in five years seems reasonable.

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