30 years of study of Kingella kingae: post tenebras, lux

CERONI, Dimitri, et al.

Abstract
Kingella kingae is a Gram-negative bacterium that is today recognized as the major cause of joint and bone infections in young children. This microorganism is a member of the normal flora of the oropharynx, and the carriage rate among children under 4 years of age is approximately 10%. K. kingae is transmitted from child to child through close personal contact. Key virulence factors of K. kingae include expression of type IV pili, Knh-mediated adhesive activity and production of a potent RTX toxin. The clinical presentation of K. kingae invasive infection is often subtle and may be associated to mild-to-moderate biologic inflammatory responses, highlighting the importance a high index of suspicion. Molecular diagnosis of K. kingae infections by nucleic acid amplification techniques enables identification of this fastidious microorganism. Invasive infections typically respond favorably to medical treatment, with the exception of cases of endocarditis, which may require urgent valve replacement.

Reference

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**Kingella kingae** is a Gram-negative bacterium that is today recognized as the major cause of joint and bone infections in young children. This microorganism is a member of the normal flora of the oropharynx, and the carriage rate among children under 4 years of age is approximately 10%. *K. kingae* is transmitted from child to child through close personal contact. Key virulence factors of *K. kingae* include expression of type IV pili, Knh-mediated adhesive activity and production of a potent RTX toxin. The clinical presentation of *K. kingae* invasive infection is often subtle and may be associated to mild-to-moderate biologic inflammatory responses, highlighting the importance a high index of suspicion. Molecular diagnosis of *K. kingae* infections by nucleic acid amplification techniques enables identification of this fastidious microorganism. Invasive infections typically respond favorably to medical treatment, with the exception of cases of endocarditis, which may require urgent valve replacement.

**Historical review**

*Kingella kingae* is a species of gram-negative aerobic coccobacilli, which belongs to the family *Neisseriaceae*. This organism was initially referred to as *Moraxella* new species 1 (1,2) or group M organisms (1,3). The first description of the bacterium, later to become known as *K. kingae*, was made in the 1960s by Elizabeth King, an American bacteriologist of the US CDC. In 1968, Henriksen and Bovre formally described the new *Moraxella* species, analyzing nine strains (including two obtained from Elisabeth King) that had been isolated from blood, joint fluid, bone, nose and throat (4). All isolates were characterized by β-hemolysis, acid production from glucose and maltose and a lack of catalase activity (4). The authors distinguished this new microorganism from all other known *Moraxella* species, and named it *Moraxella kingii* in honor of Elisabeth King (4). A further taxonomic modification was made in 1974, when the isolate was renamed *Moraxella kingae* (5). This change to the feminine gender reflected the naming of the organism after the female scientist. Although *Kingella* organisms shared several characteristics with *Moraxella* species, DNA homology studies revealed sufficient differences to suggest the need for a separate genus (6). The final change in nomenclature was thus made in 1976, when the organism was assigned to the genus *Kingella* in the family *Neisseriaceae*; it has since been known as *K. kingae* (7,8). In the same year, two additional species, *Kingella oralis* and *Kingella denitrificans*, were added to the *Kingella* genus (9). Finally, in 2005, Lawson et al. reported the isolation and characterization of a hitherto-unknown Gram-negative, rod-shaped *Neisseria*-like organism from an infected wound resulting from a bite from a kinkajou (10). Based on both phenotypic and phylogenetic evidence, it was proposed that the unknown organism be classified as a new species, *Kingella potus*. Currently, the *Kingella* genus therefore consists of four species: *K. kingae*, *K. denitrificans*, *K. potus* and *K. oralis*.

**Microbiologic features**

**Bacterial characteristics**

*K. kingae* is a coccoid, medium-sized rectilinear rod, appearing as pairs or short chains of short bacilli with tapered ends (4,11). *K. kingae* is an aerobic and facultative, anaerobic β-hemolytic bacterium that is Gram negative, with some tendency to resist decolorization (6). The isolate is nonmobile and has no endospores; contrary to the initial assumption, a recent paper suggests that *K. kingae* elaborates a surface-associated polysaccharide capsule (12). On the basis of studies by Henriksen and Bovre (13), many characteristics of *K. kingae* have been recognized; for example, *K. kingae* is oxidase positive and exhibits negative

**Keywords**

- bacteremia
- colonization
- endocarditis
- invasive infections
- *Kingella kingae*
- oropharyngeal carriage
- osteoarticular infection
- PCR assays
- RTX toxin
catalase, urease and indole reaction. It produces acid from glucose and maltose, but not from other sugars, hydrolyzes indoxyl phosphate and \( \text{t-propyl-}\beta-\text{naphthylamide and, finally, shows}
\)
positive alkaline and acid phosphatase reactions
\[4,11,13,14\]. \textit{K. kingae} grows on trypticase soy agar with added hemoglobin (blood agar) and chocolate agar, but fails to grow on MacConkey or Krüger media [15]. A selective medium consisting of blood agar with 2 \( \mu \text{g/ml of added}
\)
vancomycin has been developed to inhibit the growth of competing Gram-positive flora and facilitate recognition of \textit{K. kingae} in respiratory specimens [16]. A \( \text{CO}_2\)-enriched atmosphere enhances growth [11], but only a small proportion of strains is truly capnophilic (requires \( \text{CO}_2\) for its growth) [17]. \textit{K. kingae} is relatively fastidious in its growth requirements. It is an aerobe that grows optimally at 33–37°C. Growth at room temperature (20°C) is slight. Growth is adequate on both nutrient and blood agar, and this organism has no requirement for X (hemin) and V (nicotinamide adenine dinucleotide) factors.

**Member of the HACEK group**

\textit{K. kingae} is the fifth member of the HACEK group. The acronym HACEK refers to a grouping of Gram-negative bacilli that forms a normal part of the human flora: \textit{Haemophilus} species, \textit{Aggregatibacter actinomycetemcomitans}, \textit{Cardiobacterium hominis}, \textit{Eikenella corrodens} and \textit{Kingella} species. All of these Gram-negative bacteria are part of the normal oropharyngeal flora, which grow slowly, prefer a carbon dioxide-enriched atmosphere and share an enhanced capacity to produce endocarditis, especially in children and young adults. Based on large reviews, HACEK organisms are responsible for approximately 3–10% of cases of native valve-infective endocarditis [18]. Because of their fastidious and slow growth, they are often a cause of culture-negative endocarditis, although modern culture and genetic identification techniques are challenging this paradigm.

**Different strains of \textit{K. kingae}**

More than 450 strains of \textit{K. kingae} have been isolated from healthy carriers; these different strains were gathered in different studies on the carriage of \textit{K. kingae} conducted among children living in southern and central Israel during the last 25 years [19–24]. Among them, 181 invasive \textit{K. kingae} strains have been isolated between 1992 and 2012 from Israeli patients with bacteremia, skeletal system infections and endocarditis [20]. Although a great variety of \textit{K. kingae} strains circulate within the pediatric population, a small subset is responsible for most clinical infections, and only a few exhibit significant associations with particular clinical diseases [20]. A recent study demonstrated that only five strains (B, H, K, N and P) caused 72.9% of all invasive infections [19,20]. Clone K exhibits an optimal balance between transmissibility and invasiveness, and it is significantly associated with bacteremia. Clone N shows remarkable tissue invasiveness and it is responsible for arthritis and osteomyelitis. The genome from septic arthritis strains has been recently sequenced; \textit{K. kingae} strain PYKK081 is recognized to cause infections of the skeletal system in young children [25], but may also be responsible for infective endocarditis in children and adults. \textit{K. kingae} strain 11220434 is another strain that is responsible for acute arthritis [26]. Finally, clone P organisms are strongly associated with bacterial endocarditis [20].

Oropharyngeal carriage of \textit{K. kingae} Normal oropharyngeal flora

The normal flora of the oropharynx contains a large number of regular bacterial inhabitants. The most important group of microorganisms native to this body niche are the \( \alpha\)-hemolytic streptococci. This group includes \textit{Streptococcus mitis}, \textit{Streptococcus mutans}, \textit{Streptococcus milleri} and \textit{Streptococcus salivarius}. It is believed that these bacteria act as antagonists against invasion by pathogenic streptococci. Additionally, cultures from this region usually show large numbers of diphtheroids, \textit{Moraxella} (formerly \textit{Branhamella}) \textit{catarrhalis}, \textit{Neisseria} species and HACEK organisms, such as \textit{Kingella}. Invasive infections in young children are frequently caused by organisms carried asymptomatically in the respiratory tract [27,28]. Similar to \textit{Streptococcus pneumoniae}, \textit{Haemophilus influenzae} type b or \textit{Neisseria meningitidis}, \textit{K. kingae} resides in the mucosal surface and is able to penetrate the bloodstream, disseminate and invade distant organs [28–30]. Colonization of the respiratory tract by these organisms is, therefore, a prerequisite for later invasion; human populations with high rates of carriage of these pathogens are also at increased risk of acquiring disease [28–31].

Oropharyngeal carriage of \textit{K. kingae} K. kingae is a member of the normal commensal flora of the oropharynx; it may be present on tonsilar cultures, but it has never been isolated from nasopharyngeal cultures, implying a restricted anatomical niche [22]. Children usually...
first acquire *K. kingae* after the age of 6 months; the colonization rate then increases among infants aged between 12 and 24 months, and finally declines among older children and adults [22,27]. The fact that the colonization rate declines in older children and adults probably suggests an age-related immune response that eradicates the organism from the pharynx in older people [22,27]. The carriage rate among children less than 4 years of age and older children is approximately 10 and 6%, respectively. The colonization rate is probably largest between the ages of 12–24 months, reaching 9–12% [27,32–34]. There are no sex differences in the rate of carriage of *K. kingae* (Box 1) [27].

**Transmission in the community**

The results of many studies strongly support child-to-child transmission of *K. kingae* among young children [21,24,35], and that 50% of children carry this microorganism for long periods (at least twice during a 12-month follow-up period) [36]. Because, on average, young children put their hands or objects in their mouth once every 3 min, commensal respiratory organisms such as *K. kingae* that are disseminated by large droplets are likely to spread to young children who share toys coated with saliva [23,37]. In addition, many viral infections that occur in daycare centers (DCCs) can increase child-to-child transmission of *K. kingae* through contaminated respiratory secretions [23]. DCC attendees frequently exhibit the same strain continuously or intermittently for weeks or even months, which is then replaced by a new strain, suggesting that this carriage is a dynamic process with a frequent turnover of strains [21]. It is important to highlight that a few strains recovered at the level of the oropharynx of DCC attendees are genotypically indistinguishable from organisms from patients with invasive infections, thus suggesting that some children may be asymptomatic carriers of potentially virulent *K. kingae* strains [21]. The correlation between asymptomatic carriage and the subsequent incidence of invasive infections, such as osteoarticular infections (OAI s), remains unclear. A recent study demonstrated that the probability for asymptomatic children carrying *K. kingae* in the oropharynx to develop an OAI due to this pathogen is lower than 1%. Oropharyngeal carriage of *K. kingae* does not imply the subsequent development of invasive infection, but suggests that other cofactors may play a role in the pathogenesis of invasive *K. kingae* infections [32].

**Box 1. Asymptomatic oropharyngeal carriage of Kingella kingae.**

- *Kingella kingae* is a member of the normal commensal flora of the oropharynx
- Its anatomical niche is the tonsillar bed
- Children usually first acquire *K. kingae* after the age of 6 months (maternal-derived immunity)
- The colonization rate increases among infants aged between 12 and 24 months (9–12%)
- The colonization rate declines among older children and adults (age-related immune response that eradicates the organism from the pharynx in older people)
- Oropharyngeal carriage is a prerequisite for later invasive *K. kingae* infections

**Mechanisms of invasive infections**

**Concomitant viral infections**

Although the current knowledge of the pathogenesis of *K. kingae* remains incomplete, available evidence suggests that interactions occur with viral infection. Concomitant upper respiratory tract infection and stomatitis, including varicella-induced oral ulcers, are frequently present in affected patients [38–43]. It seems that *K. kingae* organisms colonizing the oropharynx penetrate a mucosal layer previously damaged by a viral disease [18]. The bacterium might then progress throughout the airways, causing lower respiratory tract infection and/or invade the bloodstream [18]. Transient benign bacteremia may follow and the bacterium might be seeded in the endocardium, joint space, bone or intervertebral discs, resulting in a focal suppurative infection [18]. The reasons for the striking predilection of *K. kingae* for these localizations remain unknown [18].

**Type IV pili expression**

An essential step in both colonization of the respiratory tract and seeding of distal sites is adherence to tissues, including the respiratory epithelium and the synovium [44,45]. *K. kingae* expresses type IV pili that are essential for mediating adherence to respiratory epithelial and synovial cells, presumably facilitating the colonization of the respiratory tract and the seeding of joints [44]. Previous work described the presence of two *K. kingae* colony types called spreading/corrodning and nonspreading/noncorrodning, which correlate with high- and low-density piliation, respectively. Kehl-Fie *et al.* observed an additional spontaneously occurring colony type that correlated with the absence of pili and a lack of bacterial adherence [46]. This nonpiliated colony type is similar in size to the nonspreading/noncorrodning colony type, but is slightly more domed and lacks any fringe, and is virtually identical to the colonies produced by *pilA1* mutants [46]. In the same study, Kehl-Fie *et al.*
found that a high percentage of respiratory and nonendocarditis blood isolates and a low percentage of joint fluid, bone and endocarditis blood isolates expressed pili [46]. Additionally, they observed that only piliated isolates were capable of adherence to respiratory epithelial and synovial cells. They also discovered that the major pilus subunit PilA1 displays a relatively high degree of strain-to-strain variability in sequence [46]. Recent work demonstrated that K. kingae type IV pili are regulated by a transcription factor called σ-54 and by a two-component sensor/regulator system referred to as PilS/PilR [45]. A recent study described a novel adherence mechanism that allows K. kingae to adhere efficiently to human epithelial cells while remaining encapsulated and thus more resistant to immune clearance [12]. These authors discovered a novel surface protein called Knh that mediates K. kingae adherence of organisms lacking pili; thus, pilus retraction is necessary for maximal Knh-mediated adherence in the presence of the capsule [12].

RTX toxin production
The pathogenesis of K. kingae, including invasion of the bloodstream and osteoarticular damage, has been related to the production of a potent cytotoxin, RTX. This toxin has been shown to be responsible for in vitro cytotoxicity on respiratory epithelial, synovial and macrophage-like cells, with sensitivity levels up to fourfold higher for synovial and macrophage-like cells than for respiratory cells [47]. The RTX locus from K. kingae strains is comprised of the tolC, rtxA, rtxC, rtxD and rtxB genes, which are necessary for the production and secretion of an active RTX toxin [47]. Disruption of the K. kingae RTX locus results in a loss of cytotoxicity for respiratory epithelial, synovial and macrophage cell lines [47]. The K. kingae rtxA, rtxC and rtxB genes encode proteins that have more than 70% identity with their Moraxella bovis homologs. The two remaining genes in the locus – tolC and rtxD – encode proteins with substantial homology to their M. bovis homologs, but even greater homology to their N. meningitidis counterparts [47]. The results of the homology analysis therefore suggest that the K. kingae RTX locus was acquired via horizontal gene transfer from common donor organisms, such as M. bovis or N. meningitidis [47]. All K. kingae clinical strains possess the rtxA toxin gene, making this gene a relevant target to diagnose K. kingae infection by PCR. However, there is a polymorphism of the rtxA gene, which encodes the RTX toxin [48], and a few studies have demonstrated that the most virulent strains of the species harbored a 33-bp duplication or triplication in their rtxA sequence, suggesting that this genetic trait could represent a genetic determinant of virulence [48,49]. Finally, Bendaud et al. reported that K. kingae forms biofilms in a microtiter plate assay [50]. Biofilm formation is likely to play an important role in the ability of K. kingae to colonize the pharynx and the oral cavity and therefore might be crucial in the pathogenesis of septic arthritis, osteomyelitis, endocarditis and other localized infections caused by K. kingae [50].

Epidemiology
Prevalence of invasive infections due to K. kingae
Invasive K. kingae infections particularly affect children between 6 and 48 months of age, and over 60% of episodes occur below the age of 2 years [16,51–55]. The annual incidence of invasive infections due to K. kingae ranged from 9.4 to 27.4 per 100,000 children aged under 4 years [36,56], and peaked in the 6–11-month-old group (40.3 cases/100,000) [57]. An interesting study has demonstrated that antibody concentrations to K. kingae outer membrane proteins were high at 2 months of age, reached nadir values at 6–7 months of age, remained low until the age of 18 months and gradually increased in older children [57,58]. This pattern represents a mirror image of the age-related incidence of invasive K. kingae infections. The low attack rate in the first 6 months of life is suggestive of maternally derived immunity [57,58]. The increased incidence of invasive infections in 6–24-month-old children coincides with the age at which antibody values are lowest. Finally, increasing antibody levels among older children probably represents cumulative exposure to K. kingae antigens, resulting in the decline of clinical infections [57,58]. Therefore, occurrence of invasive infections beyond the fourth year of age occurs predominantly among children with chronic health conditions, such as underlying immunodeficiency or chronic inflammatory diseases (e.g., amyloidosis or systemic lupus erythematosus) [59].

Rate of invasive infections due to K. kingae considering respiratory carriage
In an epidemiological study conducted between 1998 and 2002, the prevalence of respiratory carriage of K. kingae (detected on throat swabs
sent for isolation of Streptococcus pyogenes) did not differ between the February–May (4.3%) and October–December (2.0%) periods in young children, even if a difference (not statistically significant) in carriage rate was noted [27]. Another study showed that monthly respiratory carriage demonstrated peaks in December and April [22]. Nevertheless, it is important to note that the period of the year when the prevalence of the organism in the respiratory tract seems maximal does not coincide with that of invasive infection [27]. Whereas almost three-quarters of patients with invasive K. kingae disease are diagnosed between July and December, the oropharyngeal carriage usually shows an opposite, albeit not significant, trend [27]. The same authors postulated that exposure of children to antibiotics (for streptococcal pharyngitis or respiratory infections) probably accounts for the unexpected low carriage rate found in the October–December period [27]. It appears then that the striking epidemiological features of invasive K. kingae infections cannot be explained on the basis of the characteristics of the respiratory carriage of the organism, and therefore, additional factors should be present. Most of the time, signs of upper respiratory tract infection, stomatitis or diarrhea are found on admission in many children who later demonstrate invasive K. kingae infection. This suggests that the peculiar epidemiological features of invasive K. kingae infections result from the interplay of the respiratory carriage of the organism with viral infections and probably other still-undefined factors [27].

**Clinical presentation of K. kingae infections**

**Transient nonfocal bacteremia**

As mentioned previously, K. kingae is able to penetrate a mucosal layer previously damaged by a viral disease and to invade the bloodstream. Transient bacteremia is thus a prerequisite for an invasive infection, as it enables the bacterium to be seeded in distant organs. Therefore, concomitant infections of the skeletal, respiratory, vascular or central nervous systems may be present in most of these patients, even if a single focus is the rule in invasive K. kingae infections. Strains that show remarkable invasiveness for bone or joint tissues are usually rapidly cleared from the respiratory tract and the bloodstream, implying that persistence in blood may require a different biological specialization [20]. As clone N organisms (which are responsible for arthritis and osteomyelitis) seem to be unable to survive in the bloodstream for long, focal bacteremia due to this strain is therefore unusual.

K. kingae nonfocal bacteremia without evidence of invasive infection has been observed most frequently in children [18,56,58,60], and clone K organisms are probably the more frequent etiologic agents of these bacteremias [20]. A maculopapular rash resembling disseminated meningococcal or gonococcal infection may be present [61], the mean maximal fever is 39°C and the mean erythrocyte sedimentation rate reaches 50 mm/h [58]. Patients with K. kingae nonfocal bacteremia should be studied in order to exclude an invasive infection. Because of the risk for endocarditis, for example, evaluation of all patients with K. kingae bacteremia in order to exclude endocardial invasion has been strongly advocated [59]. Patients with K. kingae nonfocal bacteremia without endocarditis respond favorably to a short course of antibiotics [18,60].

**Bacterial endocarditis**

K. kingae is included in the HACEK group of organisms that are collectively responsible for up to 5% of cases of bacterial endocarditis [28]. Since the first description of K. kingae endocarditis by Christensen and Emmanouilides in 1967 [62], close to 50 cases have been reported involving native as well as prosthetic valves [63]. In contrast to other K. kingae infections, endocarditis is diagnosed primarily in older children, teenagers and adults [18,64]. In approximately 50% of patients, there is a predisposing cardiac malformation or rheumatic heart disease, and typically the left side of the heart is involved, usually the mitral valve [28]. Fever and acute-phase reactants seem to be more elevated in patients with endocarditis compared with those with uncomplicated bacteremia, even if there is currently no cutoff value that can distinguish accurately between the two conditions [28,56]. In many patients with endocarditis due to K. kingae, valve replacement is not always necessary, since the response to antimicrobial therapy is good if administered quickly [63]. However, serious life-threatening complications may happen, such as destruction of heart valves, cardiac failure, septic shock, mycotic aneurysms and cerebrovascular accidents, and the overall mortality rate remains high, at approximately 15% [18,28,56,58,63,65–69]. For these reasons, echocardiographic evaluation of patients from whom the organism is isolated from a normally sterile site, especially blood, has been recommended by a few authors [70]. Bacterial endocarditis is strongly associated with
clone P organisms, which are seldom carried asymptomatically and are rare in other infected sites [20,24].

**Osteoarticular infections**

Since the 1980s, the reported number of cases of *K. kingae* OAIs has markedly increased, mainly owing to improvements in culture techniques [16,71–76] and to the utilization of molecular methods [32,51–55,77–81]. Many studies have thus demonstrated that *K. kingae* has become the major bacterial cause of OAIs in children aged between 6 and 48 months; this microorganism is currently recognized to account for 30–93.8% of all culture-positive OAIs [18,32,51–54,77]. However, the presentation of *K. kingae* OAIs is often characterized by a mild-to-moderate clinical and biologic inflammatory responses to infection, with the consequence that these children present few, if any, criteria evocative of OAIs. In fact, only 10–33% of children with OAIs caused by *K. kingae* have a body temperature ≥38°C at admission [51–53,55,77], and most patients have normal or near normal white blood cell counts and C-reactive protein levels [51,52,58,77]. Erythrocyte sedimentation rate and platelet counts seem to be the most sensitive markers of inflammation when a *K. kingae* OAI is present [51,77]. A model to allow the differentiation of *K. kingae* OAIs from those due to typical pathogens, in children aged less than 4 years of age, has been described and consists of the following four parameters: temperature at admission <38°C; C-reactive protein <55 mg/l; white blood cell count <14,000 leukocytes/mm³; and band shift <150 forms/mm³ [77]. This model is a subject of controversy, but it highlights the need for prospective studies to better define the clinical presentation according to the children’s age and the causative organisms.

*K. kingae* septic arthritis generally involves the large weight-bearing joints such as the hip, knee, ankle, shoulder or elbow [18,51–54,58,77]. In addition, atypical joints such as small metacarpophalangeal/metatarsophalangeal, sternoclavicular, acromioclavicular or tarsal joints are overrepresented in *K. kingae* arthritis compared with septic arthritis due to other pathogens [18,51–53,58,77,82]. The anatomical sites involved in patients with *K. kingae* osteomyelitis include long bones, such as the femur, tibia, humerus, radius and ulna [51–53,77,83–87]. Nevertheless, any bone rarely infected by other pathogens, such as the calcaneum, talus, sternum or clavicle, may also be affected by *K. kingae* osteomyelitis [18]. It is also interesting to highlight that the epiphysis or apophysis, which are almost never invaded by other organisms, may be commonly involved in *K. kingae* osteomyelitis [51,77,85,88,89]. Today, *K. kingae* is probably responsible for many cases of hematogenous spondylodiscitis in children younger than 4 years of age [90–93]. Patients usually present with limping, back pain, refusal to sit or walk, abdominal pain or, in rare instances, neurologic symptoms. Most of the time, infection involves the lumbar discs, and radiograph or MRI studies reveal narrowing of the intervertebral space.

Onset of *K. kingae* OAIs may be insidious, and the disease is frequently diagnosed with considerable delay (after more than 1 week in 70% of patients) [51,55,64,75]. As described previously, the clinical course is usually better for children with OAIs due to *K. kingae* than with other classical pathogens, as evidenced by shorter hospitalization and fewer adverse events. Some reports even suggest that transient involvement of the skeletal system during an episode of *K. kingae* bacteremia may occur, and that fever and skeletal complaints may resolve without antimicrobial therapy, thus supporting an abortive clinical course [15,82,94]. Evolution towards chronic osteomyelitis and orthopedic sequelae seems to be uncommon [18,75,95], but not impossible, as any case may develop serious epiphyseal lesions [LIHARRERBES B. UNPUBLISHED DATA]. Caution is therefore recommended, and adequate antibiotics should be administered to all patients for whom the organism is recovered from a normally sterile body fluid (Box 2) [28].

**Culture detection of *K. kingae***

The primary isolation of *K. kingae* from joint, bone or blood samples appears to be strongly dependent on the methodology used [87]. In fact, the recovery of *K. kingae* from purulent specimens seeded onto solid culture media is suboptimal and most of the time results in a frustrating proportion of negative cultures [18,32,51,77,87]. The yield of cultures has been significantly improved by inoculating clinical specimens into aerobic blood culture vials [73,76] from a variety of automated or manual blood culture systems such as BACTEC™ (Becton Dickinson, MD, USA) [18,87], BacT/Alert® (Organon Teknika Corp., NC, USA) [72–75], Isolator™ 1.5 Microbial Tube (Wampole Laboratories, NJ, USA) [16] or Hemoline™ DUO (bioMérieux, France) [71,96]. However, no controlled study has been performed to identify the best blood culture system for this purpose [18]. 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. The 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 the concentration of such inhibitory factors, thus improving the recovery of this fastidious organism [87]. Nevertheless, even when blood culture vials are used for culturing synovial fluid aspirates, *K. kingae* is isolated in only 37.5–67% of children with proven arthritis [52,54,55,83], which indicates that PCR detection of this organism should be routinely used to improve the bacteriological diagnosis.

**Detection of *K. kingae* by PCR assay**

In recent years, molecular diagnosis of *K. kingae* infections by novel nucleic acid amplification techniques has enabled the identification of *K. kingae* in invasive infections within 24 h [32,51,53,77,82,97]. There are currently two different nucleic acid amplification approaches. The broad-range 16S rRNA gene assay involves extracting DNA from clinical samples, incubating the DNA with broad-range oligonucleotide primers that anneal to constant regions of the 16S rRNA gene and amplification of the intervening sequence, which varies according to the bacterial species [97]. The resulting amplification products are either sequenced and compared with sequences in the GenBank database or hybridized with organism-specific probes [28]. Some authors have reported detection of *K. kingae* from culture-negative specimens by using broad-range PCR amplification [54,55,83,98]. The use of the broad-range 16S rRNA gene assay offers the tremendous advantage of not requiring any *a priori* knowledge of the causative bacteria. However, this method is hampered by an insufficient sensitivity to detect all agents directly from clinical samples, as the analytical sensitivity of the broad-range 16S rRNA gene PCR is only 300 CFU [80]. Currently, real-time PCR assays that amplify *K. kingae*-specific targets such as *cnp60* or RTX toxin genes have been developed and are associated with high reliability [32,51–53,77,80]. Real-time PCR assays that are specific to *K. kingae* targets, such as the RTX toxin, are tenfold more sensitive than the broad-range 16S rRNA gene PCR (30 vs 300 CFU) [80]. In addition, this assay does not show any cross-reactivity with several related species and common osteoarticular pathogens [80].

Ideally, three bacteriological investigations should be performed when a *K. kingae* invasive infection is suspected. First, the presence of *K. kingae* should be proven by a real-time PCR assay. Second, one should keep materials and perform a broad-range 16S rRNA gene PCR assay when the precedent test is negative in order to exclude infections caused by other fastidious microorganisms. Third, clinical specimens should be inoculated into aerobic blood culture vials for studying the organism’s antibiotic susceptibility profile (Box 3).

**Antibiotic susceptibility of *K. kingae***

The susceptibility of *K. kingae* to antimicrobial drugs that are commonly administered to children with confirmed or suspected invasive infection has been better recognized over recent years. *K. kingae* is considered highly susceptible to penicillin, ampicillin, second- and third-generation cephalosporins, macrolides, cotrimoxazole, ciprofloxacin, tetracycline and chloramphenicol [18,28]. Occasional *in vitro* resistance to erythromycin, cotrimoxazole and ciprofloxacin has been reported [17,64,99–101]. The organism exhibits decreased susceptibility to oxacillin and clindamycin [17,61], and is fully resistant to trimetoprim and vancomycin [17,64,99–102].

**Box 2. When to suspect a *Kingella kingae* invasive osteoarticular infection.**

- Children aged between 6 and 48 months
- Presence of concomitant viral infections (upper respiratory tract infection, stomatitis or diarrhea)
- Oropharyngeal carriage of *Kingella kingae*
- Osteoarticular infection with mild clinical presentation
- Osteoarticular infection with subnormal acute-phase reactants (only erythrocyte sedimentation rate and platelet counts may be affected)
- Involvement of atypical joints (e.g., sternoclavicular, acromioclavicular joints or spondylodiscitis)
- Involvement of epiphysis or apophysitis

**Box 3. Bacteriological investigations of suspected *Kingella kingae* invasive infections.**

- Take samples of normally sterile body fluid (joint fluid, bone aspirate or blood samples)
- Perform usual cultures in order to exclude classical pathogens
- Perform a specific PCR assay to confirm or infirm the presence of *Kingella kingae*
- Keep residual material to perform a broad-range 16S rRNA gene PCR when the precedent test is negative in order to exclude infections due to other fastidious microorganisms
- Inoculate a part of the sample into aerobic blood culture vials for studying the organism’s antibiotic susceptibility profile
Yagupsky et al. demonstrated that 38.5% of isolates from respiratory carriers and patients with invasive infection contained K. kingae strains that were resistant to clindamycin [17]. More worrying is the discovery of the production of β-lactamase found in an isolate recovered from an HIV-positive patient in the USA [103] and in three isolates from children in Iceland [104]. The culture detection of K. kingae, even when inoculating the samples from infected joint or bone directly into blood culture vials, remains suboptimal. In addition, as a result of the widespread use of nucleic acid amplification assays, the susceptibility of the organism to antimicrobial drugs that are administered to children with invasive infection due to K. kingae is being less frequently investigated. Therefore, in many clinical situations, clinicians do not have any information about the antibiotic susceptibility profile of the organism, with the potential risk of an antimicrobial-resistant organism infection being treated ineffectually. On this subject, a recent study demonstrated that the throat-isolated strain was responsible for arthritis, and thus highlighted the possibility of isolating the K. kingae strain responsible for arthritis using throat swabs with significant effectiveness [105]. Thus, there is a real interest performing throat swabs to isolate the bacteria and study its antimicrobial sensitivity.

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<th>Treatment</th>
<th>General considerations about antibiotic treatment</th>
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<td>Today, there are still too few controlled studies that permit the formulation of evidence-based recommendations on the preferable antibiotic, on the necessity or not to research the bacterium’s production of β-lactamase and on the optimal length of therapy for K. kingae disease [28]. However, the antimicrobial drug resistance profile of the organism is quite consistent and predictable [17,64,99–102] and, with the exception of cases of endocarditis, infections promptly respond to antibiotics that are empirically administered to young febrile children or those with presumptive skeletal system infections. Nevertheless, the treatment of invasive K. kingae infections faces three unexpected problems. First, most of the infections due to K. kingae are currently detected by novel nucleic acid amplification techniques, and clinicians often do not have any information about the antibiotic susceptibility profile of the organism. Second, the clinical presentation of K. kingae disease is subtle and may be associated with normal levels of acute-phase reactants, and therefore, these parameters can often not be used to guide switching to oral antibiotics and defining the duration of treatment. Third, some reports even suggest that invasive K. kingae infections may resolve without treatment, and question the necessity of antimicrobial therapy [15,82,94]. However, this statement has to be considered to be controversial, since K. kingae organisms may be seeded elsewhere more easily, causing more precarious complications. Thus, patients from whom the organism is recovered from a normally sterile body site should be treated. Many patients with skeletal system involvement respond promptly to conservative treatment with appropriate antibiotics, but we currently continue to perform surgical procedures (especially taking samples for bacteriology) in these cases only to confirm that there is no pyogenic infection.</td>
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**Recommended antibiotic regimes**

Drug therapy for OAIs usually consists of intravenous administration of nafcillin or a second- or third-generation cephalosporin while pending culture results [51,53,60], whereas some authors still recommend oxacillin despite K. kingae demonstrating decreased susceptibility to this antibiotic [53]. In areas in which community-associated methicillin-resistant Staphylococcus aureus is prevalent and when the clinical presentation of OAIs is acute, a combination of a β-lactam antibiotic and vancomycin may be suggested [106]. Clinical response and acute-phase reactants (when abnormal) are then used to guide switching to oral antibiotics (usually after 3 days) and defining the duration of therapy. Antibiostatic treatment generally varies from 2 to 3 weeks for K. kingae arthritis, from 3 to 6 weeks for osteomyelitis and from 3 to 12 weeks for spondylodiscitis [18]. However, in the future, we can expect that OAIs due to K. kingae, characterized by subacute course and low bacterial concentrations, could be treated by shorter oral antibiotic therapy. Patients with K. kingae bacteremia are treated with an intravenous β-lactam antibiotic, which is subsequently switched for an oral β-lactam once the clinical situation has improved. Usually, the total duration of treatment ranges from 1 to 2 weeks [18,60]. Patients with K. kingae endocarditis must be treated longer with an intravenous β-lactam alone or in combination with an aminoglycoside for 6–7 weeks. In rare cases, early surgical procedures may be mandatory for life-threatening complications that are unresponsive to medical therapy [18,28,56,58,65–69].
Prophylactic treatment in cases of outbreaks of invasive infections among child DCC attendees

Young children in DCCs have an excess of infectious morbidity, especially of that caused by respiratory organisms [23]. Enhanced transmission of bacterial pathogens in this setting is related to a complex inter-relation between increased exposure to these microorganisms and the immunologic status of this particular population [23]. Thus, dissemination of K. kingae in a young susceptible population may result in ‘veritable’ outbreaks of invasive disease [23,68,107]. Although no treatment is currently recommended to eliminate the carriage state, clusters of K. kingae invasive infections in children attending DCC facilities raise questions about the management of contacts in this setting, and thus represent a public health consideration. Even if solid data are still lacking, many authors consider it more prudent to offer empiric antimicrobial therapy to close contacts of infected children in case of outbreaks of invasive infections due to K. kingae in DCCs [23,68,107]. Similar to N. meningitidis, prompt administration of antibiotic prophylaxis is therefore advised when a case of invasive disease is detected in the daycare setting [23,68,107]. Most of the time, contacts of infected children receive prophylaxis with rifampicin (10 mg/kg) orally twice daily for 2 days [68,107], but one must keep in mind that prophylactic rifampicin is only moderately successful (67%) in eliminating K. kingae colonization [107].

Future perspective

The use of specific PCR assays has completely revolutionized the diagnosis of K. kingae and changed the microbiological ecology for OAI s in young children. Since the early 1990s, there has been an explosion of reports of K. kingae disease, deriving mostly from European and Israeli sources, whereas limited reports have been described in the USA. This discrepancy is probably attributable to the fact that specific PCR assays are not in broad use in US hospitals. However, we can expect that future studies focusing on children aged between 6 and 48 months and performing specific PCR assays will demonstrate that OAI s due to K. kingae are a worldwide phenomenon among young children.

Improving recognition of infections due to K. kingae is the next problem to resolve, since the clinical presentation of K. kingae disease is often subtle and may be associated with normal acute-phase reactants. There is thus a need for new diagnostic tests in order to improve the diagnosis of these infections. The next years will probably see the emergence of noninvasive methods for diagnosing invasive K. kingae infections. As mentioned previously, a recent study demonstrated that the throat-isolated strain was responsible for arthritis and thus highlighted the possibility of isolating the K. kingae strain responsible for the arthritis using throat swabs with significant effectiveness [105]. In another forthcoming study, the authors demonstrate that a simple technique of detecting K. kingae DNA in the oropharynx can provide strong evidence that this microorganism is responsible for the OAI s, or even stronger evidence that it is not [108]. 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 s 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 might become an early decision-making tool for treating K. kingae OAI s in young children in a near future. By using a combination of oropharyngeal swab PCR tests, the predictive score for K. kingae and radiologic investigations [89], when appropriate, it should become possible to achieve a higher accuracy of invasive infection diagnosis at lower cost, in less time and with less danger and distress for the patient [108].

Last but not least, one field of investigation has recently focused on the differences of pathogenicity of K. kingae strains. In fact, it is currently thought that not all K. kingae strains have the same capacity to cause human disease, and questions are being asked as to whether certain clones are associated with particular clinical syndromes. On this subject, a recent study demonstrated that five clones of K. kingae (B, H, K, N and P) were responsible for the majority of the invasive infections, exhibiting genetic stability, long-term persistence and wide geographic dispersal [20]. Recently, an animal model has been developed and may contribute to the discrimination between high- and low-virulence clones in the future, and would point to clones to be further investigated in order to identify genetic determinants of virulence [49].
Detecting Henriksen and Bøvre

Drug therapy consists of the administration of β-lactam or a second- or third-generation cephalosporin.

Five of these strains (B, H, K, N and P) are responsible for most invasive infections.

K. kingae

Pathogenesis is related to the production of a potent cytotoxin, RTX.

Improvement of the yield of cultures can be obtained by inoculating clinical specimens into aerobic blood culture vials.

All patients with bacteremia have to be evaluated in order to exclude endocardial invasion.

Detection of K. kingae

Improvement of the yield of cultures can be obtained by inoculating clinical specimens into aerobic blood culture vials.

PCR assays specific to K. kingae targets are tenfold more sensitive than broad-range 16S rRNA gene PCR.

Detecting K. kingae DNA in the oropharynx can provide strong evidence that this microorganism is responsible for invasive infection.

Treatment

Drug therapy consists of the administration of β-lactam or a second- or third-generation cephalosporin.

Antibiotic treatment duration varies from 2 to 4 weeks for osteoarticular infections, 1 to 2 weeks for nonfocal bacteremia and 6 to 8 weeks for endocarditis.

No treatment is currently recommended to eliminate the carriage state.

References

Papers of special note have been highlighted as:


** Important study regarding genotyping different Kingella kingae strains and the investigation of their associations with specific clinical syndromes.**


** Investigation of the respiratory carriage of K. kingae among healthy children.**


** Epidemiological study of invasive K. kingae infections according to the respiratory carriage of the organism.**


** Description of the type IV pili, which are essential for mediating adherence to respiratory epithelial and synovial cells.**


** Study regarding the production of the RTX cytotoxin, which is responsible of the pathogenesis of K. kingae.**


Ilharreborde B, Bide P, Lorrot M et al. New real-time PCR-based method for Kingella


**Study regarding the mild-to-moderate clinical and biologic inflammatory responses to *K. kingae* infections.**


New concept for diagnosing *K. kingae* osteoarticular infections by oropharyngeal swab PCR.