Kingella kingae a Potentially Emerging Pathogen: a Comprehensive Review

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Abstract Kingella kingae are a group of fastidious Gram negative bacilli that were first described way back in 1960’s as CDC group M-1. Initially placed in moraxella family, Kingella spp. have later been classified separately under Neisseriaceae family. Kingella kingae is the most common species responsible for human infections and is characterized along with other fastidious bacteria named as HACEK group. Kingella kingae, K. denitrificans, K. indologenes and K. oralis are few species of Kingella. Kingella kingae has gradually evolved from a bacterium that normally colonizes oral cavity, upper respiratory tract and genital tract in to a potential pathogen in children and debilitated patients. Recent trends of Kingella kingae infections among adult population should be considered as an alarming signal. The spectrum of infections, indifferent cultural and biochemical characters, antimicrobial susceptibility pattern, complex pathogenicity and genetic polymorphism has attracted a lot of interested among paediatricians, orthopedicians and clinical microbiologists.

Keywords Kingella kingae; Pathogen; Colonization

Introduction

Kingella kingae are a group of gram negative bacteria appearing as coci, diplococci and short coccobacillary forms, placed previously under Moraxella family (Snell et al., 1976). Due to the presence of coccoid forms and production of oxidase enzyme, Kingella kingae are placed in the family Neisseriaceae way back in 1976 by Henriksen and Bovre (1976). Kingellae are now included in a separate group called as HACEK (Haemophilus aphrophilus, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens and Kingella kingae) which share similar phenotypic and biochemical characters in being fastidious and catalase negative (Winn et al., 2006; Von Graevenitz et al., 2003). Kingella kingae, K. denitrificans, K. indologenes (now named as Suttonella indologenes under Cardiobacteriaceae based on G+C contents of its DNA) and K. oralis are few species of Kingella (Dewhirst et al., 1990). Being present as a normal flora of oral cavity, upper respiratory tract and genitourinary tract, Kingellae can become invasive and are associated with serious infections, mostly in children (Winn et al., 2006; Manuselis et al., 2000).

Pathogenicity, Virulence and Clinical features

The possible mode of invasion is by abrasion of the mucus membrane. Kingella kingae, the common species responsible for occasional human infections has been first described in 1960’s as CDC group M-1 (Winn et al., 2006). Kingella kingae was initially thought to be a rare causative of infections in patients with endocarditis and has evolved in to a potential pathogen especially in paediatric age patients causing bacteremia and osteoarticular infections (septic arthritis, osteomyelitis, diskitis, tenosynovitis and dactylitis). Other infections associated with K. kingae include meningitis, hematogenous endophthalmitis, soft-tissue infection and corneal ulcers and abscess (Yagupsky et al., 1997; Mollee et al., 1992). Pneumonia, epiglottitis and tracheobronchitis are some clinical conditions where K. kingae has been isolated (Kennedy et al., 1988). It has been reported that more than 70% children below 5 years are colonized with Kingella kingae in their upper respiratory tract and oropharynx (Henriksson et al., 1976).
Children usually show symptoms including fever, viral upper respiratory tract infections, stomatitis and swollen joints, show decreased mobility and their blood cultures remain negative (Dodman et al., 2000). Predisposing factors for invasive *Kingella kingae* infection include acute lymphocytic leukaemia, sickle cell anaemia, presence of prosthetic devices and congenital heart disease. Other predisposing factors for *K. kingae* infection include poor oral hygiene, pharyngitis, or mucosal ulceration due to cancer chemotherapy. *Kingella kingae* has also been associated with cardiovascular complications in children with mitral valve perforation resulting in infective endocarditis in children (Holmes et al., 2011). Predisposing factors in adults include history of major cardiac surgery, old age, chronic kidney diseases, diabetes mellitus, cancer patients and presence of orthopaedic and other prosthetic devices (Henrikssen et al., 1976). Acquired immunodeficiency due to infection with HIV or immunocompromised due to immunosuppressive therapies (solid organ transplants), autoimmune conditions like systemic lupus erythematosus (SLE) and haematological malignancies may also predispose to invasive *Kingella kingae* infections (Wolak et al., 2000) (Table 1). A recent study has reported the genome sequence of *Kingella kingae* (strain-PYKK081) that has been isolated from joint fluid of an 8-month old child who was suffering from septic arthritis in 1991. The study revealed that the genome sequence was unique and not matching with other *Kingella kingae* (nasal isolate ATCC 23330/gene bank No: AFHS0100000) and members of *Neisseria* warranting a separate status. The study also mapped several protein coding genes including some genes responsible for resistance to antibiotics and some coding for invasive activity (Jeffrey et al., 2012). It has been revealed that 27.4 out of every 100 000 children suffer from *Kingella kingae* invasive infection annually in Israel (Yagupsky and Dagan, 1997).

Table 1 Spectrum of infections and possible predisposing factors caused by *Kingella kingae* in children and adults

<table>
<thead>
<tr>
<th>Children</th>
<th>Predisposing factors</th>
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<tbody>
<tr>
<td>Osteomyelitis</td>
<td>Children between 6months-24months</td>
</tr>
<tr>
<td>Osteoarticular infections (Septic arthritis, diskitis, tenosynovitis and dactylitis)</td>
<td>Congenital heart disease (Valvular pathology) or other anomalies</td>
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<tr>
<td>Endocarditis</td>
<td>Sickle cell anaemia</td>
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<tr>
<td>Hematogenous spondylodiscitis</td>
<td>Acute lymphocytic leukaemia</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>Presence of prosthetic devices</td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>Poor oral hygiene</td>
</tr>
<tr>
<td>Meningitis</td>
<td>Chronic Pharyngitis</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>Mucosal ulceration due to cancer/cancer chemotherapy</td>
</tr>
<tr>
<td>Cellulitis</td>
<td>Old age</td>
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<tr>
<td>Ocular infections</td>
<td>History of major cardiac surgery</td>
</tr>
<tr>
<td>Haematogenous endophthalmitis</td>
<td>Chronic kidney diseases</td>
</tr>
<tr>
<td>Soft tissue infections</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Cancer patients with solid tumors/ Haematological malignancies</td>
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<tr>
<td>Adult</td>
<td>Presence of orthopaedic and other prosthetic devices</td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td>Autoimmune conditions like systemic lupus erythematosus (SLE)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>Solid organ transplants</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>Liver cirrhosis</td>
</tr>
<tr>
<td>Intervertebral diskitis</td>
<td>Cardiac vascular pathology</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>HIV infection</td>
</tr>
<tr>
<td>Spondylodiscitis</td>
<td>Herpetic gingivostomatitis</td>
</tr>
<tr>
<td>Sacroillitis, Pericarditis</td>
<td>Oral Varicella blisters</td>
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<td>Post menopause abnormal bleeding</td>
<td>Aphthous ulcers</td>
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<tr>
<td>Lower respiratory tract infections</td>
<td>Other viral respiratory tract infections</td>
</tr>
<tr>
<td>Arthritis, Epiglottitis and Tracheobronchitis</td>
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</table>
From being almost an established pathogen among children, *Kingella kingae* has recently been reported to cause invasive infections in adults. A case of osteomyelitis pubis has been reported in an adult patient aged 66 years, with underlying end stage kidney disease and breast carcinoma (Wilmes et al., 2012).

**Prevalence and Colonization of *Kingella kingae***

Considering the fact that there has been an increase in the incidences of invasive *Kingella kingae* infections both in children and in adults, a recent study carried out to assess the respiratory tract colonization and revealed that 97% of the pharyngeal samples were positive for *Kingella kingae* (Yagupsky, 2013). Sheep Blood agar with 2 mg/mL vancomycin (BAV) was used for the culture to increase the isolation rate which acts as a selective medium by inhibiting the growth of other Commensal gram positive bacteria (Yagupsky et al., 1995; Basmaci et al., 2012). Previous studies have confirmed that children below 6 months are not colonized with *Kingella kingae* and that the colonization rates vary among 6 months to 4 years (10%) children and school going children (4~14 years) (Goutzmanis et al., 1991). Yagupsky et al. (1995) in their study have noted that as the age increases the rate of colonization decreases with 3.2%, 1.5% and 0.8% colonization of *Kingella kingae* in respiratory secretions in children < 4 years old, 4~14 years and adults respectively (Yagupsky, 2013). A study from Switzerland, by Ceroni et al. (2012) which included 431 young asymptomatic young children used real-time PCR for the detection of *Kingella kingae* from pharyngeal secretions and revealed a colonization rate of 8.1% (Ceroni et al., 2012). Previous studies have also confirmed the colonization of *Kingella kingae* in respiratory secretion among patients with microbiologically confirmed osteo-articular infections (Chometon et al., 2007).

Studies have also revealed that *Kingella kingae* colonization was observed more in the oropharynx than in the nasopharynx confirming the fact that *Kingella kingae* occupies a rather narrow niche in the upper respiratory tract (Yagupsky et al., 2002). Reports have also indicated that there is an increased colonization and infection rates among day-care attendees, suggestive of overcrowding as a predisposing factor for person-to-person spread (Robinson, 2001).

Studies on molecular typing with PFGE have confirmed that spread of *Kingella kingae* is associated with close mingling as seen among family members, playmates and community gatherings which should be considered as a predisposing factor for exposure (Yagupsky et al., 2009).

**Determinants of Colonization and Invasive Properties**

Microbial entry in to the human is very common but for it to cause infection/disease, the microbe should be able to adhere, adapt and invade. Pili are the surface projections present on the bacterial cell wall that help the organism to adhere. Studies have confirmed that *Kingella kingae* possesses type IV pili which play a key role in attachment to the respiratory epithelial cells and synovial cells (Kehl-Fie et al., 2008). Studies have also revealed certain genes (*pilA1, pilA2, fimB, pilS, pilR, pilC1, pilC2*), that are coding for the expression of pili in *Kingella kingae* and have noted that strains isolated from colonized persons had pili and those isolated from invasive infections were non-piliated indicating that the process of piliation is self regulating and influenced by immune response (Kehl-Fie et al., 2010). Another protein, a trimeric autotransporter protein called Knh also helps *Kingella kingae* to firmly bind to the colonization sites (Porsch et al., 2012). *Kingella kingae*, has the property to form biofilms (ability of microorganisms to produce a polymeric matrix like substance surrounding them to evade immune response and antibiotics entry), that helps in colonization and periodic dispersion of bacteria to other parts of the host and enabling the bacteria to evade immune detection, dessication and antimicrobial action; it has also been noted that *Kingella kingae* has an anti-biofilm activity that prevents other bacterial colonization and thereby establishing itself in the respiratory mucosa (Bendaoud et al., 2011). A toxin named RTX was identified as an exotoxin that is coded on the *Kingella*
Kingella genome that plays an important role in initiation of inflammation and thereby increases the chances of invasion (Kehl-Fie et al., 2007). Kingella kingae has been reported to produce a polysaccharide capsule, early during an infection mostly among strains colonizing in the respiratory mucosa of very young children (< 3 years) which indicates that the immune system plays an important role in the colonization and invasion of Kingella kingae, where ineffective immune responses of children are not sufficient to resist colonization and later invasion (Porsch et al., 2012, Yagupsky et al., 2011) immunological responses to invasive Kingella kingae infections has not been completely understood, but studies from the past have reported the presence of circulating IgG antibodies acquired from mother would help in resisting colonization and infection in children aged below 6 months and that as age increases till 24 months the susceptibility to invasive infection also rises (Slonim et al., 2003).

**Microbiological, Laboratory Identification, Confirmation and Antimicrobial Susceptibility Testing**

*Kingella kingae* are facultatively anaerobic gram negative bacilli, which on primary isolation appear as cocci (resembling *Neisseria* spp.) and coccobacilli (resembling *Moraxella* spp.) later on showing bacillary forms (Ramana and Mohanty, 2009) (Figure 1). Though not strictly fastidious, Kingela kingae takes up to 48 hours for growth from clinical specimens and on trypticase soy agar with added blood (sheep blood agar), produces 1~3 mm pin point to small β-haemolytic colonies, which are observed sometimes to pit, spread or corrode the medium (Kehl-Fie et al., 2009) (Figure 2). Kingella kingae are catalase negative (differing with *Moraxella* which are catalase positive), oxidase positive and non motile. *K. kingae* are indole, urease negative and ferment glucose and maltose only with production of acid and no gas. *K. kingae* can be differentiated from *Neisseria* spp by using penicillin-G disc test, where Kingella kingae form elongated bacillary forms in the presence of penicillin G disc (Yagupsky, 2004). Improved isolation is achieved in case of strong clinical suspicion, the clinical samples are incubated for at least 48 hours and incubation in 5%~10% CO₂ chamber can improve the growth (Yagupsky, 2004). On isolation, the regular biochemical reactions will be sufficient to identify Kingella kingae. Primary isolation from specimens can be improved by using automated blood culture system (BACTEC (BD-Becton Dickinson, Cockeysville, MD), Bac T Alert systems (Yagupsky, 2004). Further confirmation will be facilitated by API 20 system and Microscan (Dade Behring, Germany) automated identification and antimicrobial sensitivity systems depending on their availability. Conventional PCR and real-time PCR (RT-PCR) are the molecular methods that target specific areas of DNA (cpn 60 and RTX genes) can be used for confirmation and reducing the time for diagnosis (Baticle et al., 2008; Ilharreborde et al., 2009). Other methods including Multi-locus sequence typing (MALT), SYBR green and TaqMan assays have been used to sequence rtxA gene for identification of *Kingella kingae* from various clinical specimens using controls (*Kingella kingae* ATCC 23330) (Basmaci et al., 2012; Philippe et al., 2011).
Studies have demonstrated that *Kingella kingae* is susceptible to various groups of antibiotics including the aminoglycosides, fluoroquinolones, cephalosporins, macrolides and others. Resistance was observed against trimethoprim-sulphamethoxazole, glycopeptides and clindamycin (Yagupsky et al., 2001).

**Future Implications**

In view of increasing reports of invasive infections with *Kingella kingae* both in paediatrics age and in adults, it becomes necessary for, paediatricians, orthopaeditians and clinical microbiologists to consider this organism as a potential pathogen. More than fifty years since its first description, *Kingella* have evolved from being a normal commensal in children and adults to an established pathogen in paediatrics age group and a potential pathogen in adults. In most of the cases it is clearly imperative that a precise clinical suspicion is necessary and that recovery of these bacteria depends on appropriate laboratory methods/culture techniques used by clinical microbiologists (Dubnov-Raz et al., 2008; Gen’e et al., 2004; Yagupsky et al., 1992). Bacteriological identification is only possible when laboratory personnel are aware of the unusual cultural, morphological and biochemical characters of *Kingella kingae* group. Further, as these bacteria are reported worldwide, studies on the carriage rates among children of different ages and in adults, possible predisposing factors in different geographical regions is an area of much interest that should be explored (Yagupsky and Dagan, 2000; Yagupsky et al., 2002;). Molecular epidemiology of colonizing and invasive *Kingella kingae* infections is the need of the hour as indicated by recent studies that have revealed a remarkable genetic variability among various clinical isolates of *Kingella kingae*. Studies have confirmed that strains isolated from colonized individuals were genetically significantly different from those isolated from strains responsible for invasive infections (Basmaci et al., 2012; Amit et al., 2012).

**Conclusion**

Existing literature about the *Kingella kingae* bacterium suggests that this bacterium though is present as a normal flora, has the potential to cause serious invasive infections. Use of automated blood culture systems for primary isolations will improve bacteriological diagnosis which otherwise are culture negative by conventional methods and novel PCR based nucleic acid amplification assays aid in confirmation and study on virulence characters. Studies further should be concentrated on the antibiotic susceptibility profile of the clinical isolates of *Kingella kingae*. Finally prompt clinical suspicion and rapid laboratory confirmation would certainly help in reducing the morbidity and mortality due to invasive infections caused by *Kingella kingae* especially in children and debilitated adults.

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