

## MOLECULAR DETECTION OF OROPHARYNGEAL KINGELLA KINGAE IN CHILDREN AND ITS CORRELATION WITH OSTEOARTICULAR INFECTIONS AND ONCOLOGY DISEASES IN BASRAH, IRAQ

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**Abstract:** *Kingella Kingae* is gram negative bacterium. It can be a serious etiology of invasive infections detected by molecular technology. The aim of this study was to detect the prevalence of oropharyngeal *Kingella Kingae* carriage during the first 5 years of life, association of asymptomatic carriage with cases of osteoarticular infections (OAI), and to compare the prevalence of *Kingella Kingae* in normal and immune compromised children. Children were divided into: 61 normal, 18 OAI, and 32 Oncology patients. Throat swab was taken from 111 children that was included in the study, and 18 joint fluid samples from OAI children. All samples submitted to culture and real time PCR targeting *rtxA* and *rtxB* gene. Prevalence of *Kingella Kingae* was 2.7% in all children, and according to disease group: 1/61 (1.6%) were detected in the normal group, while 2/32 (6.3%) was the prevalence in the oncology group, and none were detected in the OAI group. In conclusion, *Kingella kingae* was detected in Iraqi children population, and that was the first report in this area regarding this microorganism. *Kingella kingae* was best detected by real-time PCR targeting RTX toxin gene (A and B).

**Keywords:** *Kingella Kingae*, OAI, real time PCR, RTX toxin gene

### 1. Introduction

Due to expansions that occur in the molecular technology, there's increase in identification of novel microorganisms that are not found previously or aid in the detection of microorganisms that previously diagnosed at a low level, *Kingella Kingae* is one of such microorganism (Yagupsky et al., 2011). *Kingella Kingae* is part of Neisseriaceae family,  $\beta$ -hemolytic, gram negative coccobacilli, aerobic and facultatively anaerobe. posterior pharynx of young children below four years of age is the usual environment of this bacterium (Yagupsky et al., 2002; Yagupsky et al., 2011). While habitually it is a normal flora in the pharynx of children, *Kingella Kingae* can be a serious etiology of invasive infections, like bacteremia, osteoarticular infections, infective endocarditis, meningitis, lower respiratory tract infection, central nervous system infection, and eye infection (Yagupsky et al., 2011). Majority of research on *Kingella Kingae* disease have been occurred in advanced countries (Yagupsky, 2004). Whereas, the developing countries reports are still lacking (Urs et al., 1994; Elyès et al., 2006; Isolda-Budnik et al., 2011; Mardaneh et al., 2011), which might be reflecting the inaccessibility of computerized blood culture systems and the requirement of molecular technology for identifying the bacterium in developing countries. Concurring with the identification of *Kingella Kingae* as the main cause of pediatric infection, the emergent awareness of the medical community toward this bacterium has resulted in the prompt development of information about this microorganism (Yagupsky, 2015). Diagnosis of *Kingella Kingae* by culture is suboptimal and outcomes is a no growth in various instances (Yagupsky et al., 1992; Yagupsky, 2004).

Recently, PCR tests that augment *Kingella Kingae* specific target like *cpn 60* or RTX toxin genes emerged and have been related to a high reliability (Chometon et al., 2007; Cherkaoui et al., 2009a; Ilharreborde et al., 2009). PCR assays can diagnose the causative agent even after antibiotic administration (Bonacorsi et al., 2006; Maas et al., 2007). In conclusion, a real-time PCR assay that is specific to the *Kingella Kingae* RTX toxin is highly sensitive than a semi-nested broad-range 16S rRNA gene PCR, thus providing improved analytic performance when applied in the routine clinical microbiology laboratory. We expect that the application of this technique will

improve information of *Kingella Kingae* infections, by considerably increasing the number of identified cases (Cherkaoui et al., 2009b). Therefore, this study was conducted to investigate the prevalence of oropharyngeal *K. kingae* carriage in the children less than 6 y of age at Basrah province, southern of Iraq as well as investigation of the carriage status with cases of invasive OAI infections and compare it with normal and compromised children.

## 2. Materials and Methods

### 2.1. Population and study design:

This is a descriptive prospective cross-sectional study conducted in the period between 20th of September 2016 till 15th of June 2017, the sampled population were children from Basrah General Hospital, Basrah Specialist Children's Hospital, and primary health care centers aged between 6 months-6years. One hundred and eleven (111) samples were collected and distributed as following; 61 normal children, 18 child with osteoarticular infection and 32 had oncology diseases. Throat swabs were taken from all children; from each child, two throat swabs were taken one for culture study and the second one for molecular study. The synovial fluid and fluid aspirated samples from bone were taken by an orthopedic specialist doctor from children with diagnosed septic arthritis and osteomyelitis.

### 2.2. Microbiological detection

Sample inoculated into blood culture vials (spectrum) for 4 days and then sub cultured on blood agar containing vancomycin (2µg/ml) for throat swab, while synovial fluid subculture on blood agar without vancomycin then positive culture submitted to catalase and oxidase tests.

### 2.3. Molecular detection

DNA extraction was done using QIAamp® DNA Mini kit (50) (QIAGEN, GERMANY), samples stored at 20 °C till real time PCR detection. Real time PCR was done using taq-man probe targeting RTX A and RTX B gene with flourogenic probe (Korea, bioneer) primers and probes were established from published studies (Cherkaoui et al., 2009a) (Table 1). Reaction PCR volume was 20 µL, real time PCR mixture contained 10µl of GoTaq® Probe qPCR Master Mix (promega USA) 1µl of each primer (RTX A and RTX B) 1 µl of each probe and 5 µl of DNA in a final volume of 20 µl. Amplification was done in a Rotor-GeneQ (QIAGEN, GERMANY) with an initial step of 2 minute at 95 °C followed by 45 cycles of 15 seconds at 95°C 60 sec at 60 °C anneal and 15 seconds at 72°C / extension.

**Table 1: The primers and probe sequence were manufactured by bioneer company (Korea).**

| Gene        | Primer/probe | Primer sequence (5»3)              | LENGTH | Accession no. |
|-------------|--------------|------------------------------------|--------|---------------|
| <i>rtxA</i> | rtxA-F       | TGCCAAAGTAAAACC - AGCTGAA          | 22     | EF067866      |
|             | rtxA-R       | AACTTACCTAATTTTG<br>GCAAAGCAA      | - 25   | EF067866      |
|             | rtxA- P*     | TGACAACAAACCGCTA<br>ATCATTCTAAGGCC | - 31   | EF067866      |
| <i>rtxB</i> | rtxB-F       | CAACATAAGCCGCCAG - TTGA            | 20     | EF067866      |

|         |                             |    |          |
|---------|-----------------------------|----|----------|
| rtxB-R  | ACAATTAAAGCAATGG - CAGTTGAG | 25 | EF067866 |
| rtxB-P* | ATCCCAACGGCGCGT - CATTTGT   | 22 | EF067866 |

- ☐ Fam dye is at reporter side of the probe.
- ☐ Tamra dye at quencher side of the probe.

### 3. Results

One hundred and eleven children were included in this study, throat swab, synovial fluid and fluid aspirate (14m/4f), and Oncology 32 (20m/12f), the number of cases per gender age range, mean, and the SD for each study groups where shown in (Table 2).

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**Table 2: Demographic table**

| Group      | Normal | OAI    | Oncology | Total  |
|------------|--------|--------|----------|--------|
| Count      | 61     | 18     | 32       | 111    |
| Percentage | 55%    | 16.2%  | 28.80%   | 100%   |
| Male**     | 33     | 14     | 20       | 67     |
| Female**   | 28     | 4      | 12       | 44     |
| Age range* | 6-60m  | 6-60m  | 9-64m    | 6-64m  |
| Mean age*  | 30.38  | 29.28  | 37.06    | 32.13  |
| SD         | 15.992 | 16.799 | 13.796   | 15.712 |

\* Age was not statistically different (P value = 0.104).

\*\* Sex does not seem to be statistically different (P value = 0.172).

Out of 111 children, *Kingella kingae* were detected in 3 (2.7%) children (Table 3).

**Table 3: Total prevalence of *K. kingae* in the study.**

| Category | Result | Percentage | Total |
|----------|--------|------------|-------|
| Negative | 108    | 97.30%     | 111   |
| Positive | 3      | 2.70%      | 111   |

According to disease group: 1/61 (1.6%) were detected in the normal group, while 2/32 (6.3%) was the prevalence in the oncology group, and none were detected in the OAI group (Table 4).

**Table 4: Prevalence of *K. kingae* in normal, oncology, and OAI groups.**

| Disease Group | Positive | %    | Total |
|---------------|----------|------|-------|
| Normal        | 1        | 1.6% | 61    |

|                 |   |      |    |
|-----------------|---|------|----|
| <b>Oncology</b> | 2 | 6.3% | 32 |
| <b>OAI</b>      | 0 | 0%   | 18 |

According to age 2/30 (6.7%) was the prevalence in 25-36 months of age, and the prevalence in 49-72 months of age was 1/13(7.6%) (Table 5).

**Table 5: Prevalence of *K. kingae* in different age groups.**

| <b>Age group</b>    | <b>Positive</b> | <b>%</b> | <b>Total</b> |
|---------------------|-----------------|----------|--------------|
| <b>6-12 months</b>  | 0               | 0%       | 15           |
| <b>13-24 months</b> | 0               | 0%       | 29           |
| <b>25-36 months</b> | 2               | 6.70%    | 30           |
| <b>6-36 months</b>  | 2               | 2.70%    | 74           |
| <b>37-48 months</b> | 0               | 0%       | 24           |
| <b>49-72 months</b> | 1               | 7.60%    | 13           |

All positive cases were female, and no case detected in male, and the prevalence in female were 3/44(6.8%.) (Table 6).

**Table 6: Prevalence of *K. kingae* in both genders.**

| <b>Gender</b> | <b>Positive</b> | <b>%</b> | <b>Total</b> |
|---------------|-----------------|----------|--------------|
| <b>Male</b>   | 0               | 0%       | 67           |
| <b>Female</b> | 3               | 6.80%    | 44           |

#### 4. Discussion

This study was performed for first time in Basrah to detect *Kingella kingae* prevalence in children below 6 years and to estimate the association of carriage rate in OAI and oncology patients. The infections that caused by *Kingella kingae* necessitate a rapid and sensitive diagnosis thus allowing correct management directed against the contributing pathogen (Ferroni, 2007). Recently, polymerase chain reaction has made *Kingella kingae* detection in 24 hours (Baticle et al., 2008; Fenollar et al., 2008).

The PCR technique used in this study targets the RTX toxin genes (*rtxA* and *rtxB*) and has been confirmed to be 100% specific for *K. kingae* (Lehours et al., 2011) with a sensitivity ten times higher than targeting other genes like broad-range 16S rRNA gene and *cpn60* gene PCR (Chometon et al., 2007) as well, PCR detect genetic materials up to 6 days after the starting of the antibiotic therapy (Ilharreborde et al., 2009).

Detection of *Kingella kingae* by culture was found to be difficult, so PCR targeting the specific gene sequence of *K. kingae* directly from the specimen have become a common approach for identifying *K. kingae* from specimens of affected patient (Yagupsky, 2015). However, in this study *Kingella kingae* was not detected by culture method and instead real-time PCR was the reliable practical method used to detect possible infections with this organism.

In current study prevalence of *Kingella kingae* in children 6 months - 6 years was 2.7% ( 3 cases out of 111 cases) and 2/98 (2%) in children 6-48 months, while in other studies the carriage rate ranged between 3–12 % in children aged 6-48 months and 1.5% for older children (4-14) years (Yagupsky et al., 2002; Amit et al., 2013; Amit et al., 2014; Anderson et al., 2015) this discrepancy may attribute to the duration of study and sample size in this study which was shorter duration and smaller sample size than that for the mentioned studies.

The carriage rate in normal children population was 1.6% (1/ 61) which found at a lower rate than the carriage rate in related studies (Yagupsky et al., 2002; Amit et al., 2013; Amit et al., 2014; Anderson et al., 2015) this may be attributed to the different study environments which may affect the prevalence rate (Yagupsky et al., 2001; Yagupsky et al., 2011; Anderson et al., 2015) and geographic distribution (Basmaci et al., 2014). In the current study, the normal population case was detected at 36 months of age which is similar to the age of *Kingella kingae* carriage as shown by (Ceroni et al., 2012; Amit et al., 2014; Anderson et al., 2015; Yagupsky, 2015). While (Anderson et al., 2015) stated that the carriage rate is 8.7% in 7-48 months of age in the Swiss healthy children population.

All positive cases in this study were female and the prevalence in the female in this study was 3/44(6.81%). On the other hand, no case was detected in the male population although the male to female ratio in the present study was 1.5:1. In several studies, there was a variability in oropharyngeal carriage according to sex; Anderson de la Llana had been reported that the female could be less likely to be a carrier than the male (Anderson et al., 2015) whereas, (Dubnov-Raz et al., 2010) showed 1.3:1 male-to-female ratio among cases studied and equal carriage rate in an another study had been reported. Equal carriage rate between male and females 1.14 male per 1 female had been also noticed by (Al-Qwbani et al., 2016). This discrepancy of the prevalence of the carriage rate according to sex might be due to the differences in the geographic distribution of the organism (Basmaci et al., 2014).

Regarding oncology patients, two cases (6.25%) were detected, and that was in agreement with other study; like the study of (Wolff et al., 1987) which showed that the immune-compromised patients were at risk to be infected with *Kingella kingae*, and (Elyès et al., 2006) study which also described *Kingella kingae* as an opportunistic infection existing entirely in an immune deficient patient. The oncology cases in the current study were detected in 25-72 months age group this increased rate of carriage may be due to impairment in their immune system which is vital for protection against pharyngeal colonization and consequent invasive infection of *Kingella kingae* (Slonim et al., 2003; Yagupsky, 2004).

This study was unable to isolate *K. kingae* from the patients that were suffering from OAI. (4 cases of septic arthritis and 14 cases of osteomyelitis) of OAI in this study, while (Al-Qwbani et al., 2016) found that the *K. kingae* is the cause of OAI in majority of cases (septic arthritis 73.1%, osteomyelitis 15.7%, spondylodiscitis 5.4%). A study by Ferroni showed 76.1% of OAI due to *K. kingae*, while 52.9% of osteomyelitis cases were due to *K. kingae* in children less than 4 years (Ferroni et al., 2013), this might be attributed to that the patient with *Kingella kingae* septic arthritis have mild presentation and fewer complication and may not reach to hospital (Basmaci et al., 2011). In addition, septic arthritis cases in this study were few (three) and majority of cases were osteomyelitis (73% of OAI cause by *Kingella kingae* are septic arthritis) (Al-Qwbani et al., 2016) Another point, child with skeletal system infection in this study were sampled in winter months while *K. kingae* infection mostly in autumn. Yagupsky and his co-authors, postulated decrease prevalence of *K. kingae* in winter due to antibiotics usage meanwhile *K. kingae* is very sensitive to antibiotics generally used for treatment of respiratory infections (Yagupsky et al., 2001; Yagupsky et al., 2011). Studies showed that *Kingella kingae* culturing is difficult due to demanding nature of *K. kingae* (Yagupsky, 2015). Despite optimization of culture technique (Yagupsky, 2004) isolation of *Kingella kingae* hardly succeeds (Yagupsky et al., 2011).

However, development in culture methods by using blood culture vials; the cultural diagnosis persisting difficult and the type of solid culture or broth media for *K. kingae* detection remain questionable (Yagupsky, 2008). In conclusion, *Kingella Kingae* present in our population in children and best detected by real-time PCR using TaqMan probe targeting RTX toxin gene (A & B) besides that *Kingella Kingae* detected at increase rate among oncology patient. Further studies on *Kingella Kingae* by participating a larger population of children to clarify its prevalence in normal children and its association with OAI and another disease like bacteremia and endocarditis are essential in this geographical area.

### Acknowledgment

The authors would like to thank Dr. Mona Mohammed from Al-Tafeel Specialized Hospital for her kindly assist in sampling and culturing and Ms. Hanan Salman for assistant with molecular diagnosis. We would also like to express our gratitude to Dr. Salah-Aldeen Mahdi from Al-Basrah General Hospital for his valuable advices on statistical analysis.

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