Comparing the oropharyngeal colonization density of Kingella kingae between asymptomatic carriers and children with invasive osteoarticular infections

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Abstract

Colonization of the oropharynx by Kingella kingae is currently considered to be a prerequisite for later development of invasive infections. However, the oropharyngeal K. kingae DNA bacterial load in children with osteoarticular infections caused by this microorganism is not different than that of asymptomatic carriers.


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In the period from birth to last follow-up, SCN patients were most often affected with infections, requiring hospitalization in half of the episodes. The proportion was lower but not negligible in AN (40%), and IN (36% of episodes). These percentages are lower than what has been reported in other studies that showed “mild infections” in 80%–90% of AN. This may be due to a stricter infection definition applied in the present study.3,5

Skin and subcutaneous tissues were the most common sites regardless of the type of neutropenia, and accounted for greater than half the cases of infections. This is in agreement with the fact that skin/subcutaneous lesions are events often leading to diagnosis because they tend to recur and to be difficult to eradicate.1 The infection rate was significantly higher in SCN than in AN and IN, both before and after the diagnosis of neutropenia. This can be explained by the fact that in SCN deficiency of neutrophils is not only quantitative but also qualitative and is associated with functional defects in native immunity.10 Furthermore, in AN and sometimes in IN, unlike SCN, neutrophil count increases during infections due to enhanced marrow release and vessel demargination in response to infectious stimuli.

After diagnosis, the infection rate decreased in all 3 groups, although significantly only in SCN. This may be due to the start of G-CSF in SCN, whereas a more cautious attitude of the patient and the family might have played a role in all the 3 patients’ groups.3 Finally, using the infection rate, it is possible to compare the epidemiology of infections between children with different causes of neutropenia. In this sense, the rate observed in SCN before diagnosis results higher than that observed in aplastic anemia and in solid tumors, even aggressively treated,8,9 but lower than that of children aggressively treated for acute leukaemia/lymphoma.

REFERENCES
real-time polymerase chain reaction (rtPCR) assay targeting the toxin-encoding gene rtxB. Arthrocentesis or bone aspiration was performed under fluoroscopy guidance in the operating room, and the diagnosis of osteoarticular infections (OAI) caused by K. kingae was established by the same specific rtPCR assay, whenever it revealed positive either in blood, synovial fluid or bone aspirate samples. In parallel, healthy children between 6 and 48 months of age were screened for asymptomatic oropharyngeal carriage of K. kingae during the same period. Recruited children were among hospitalized for clean surgery, attending our orthopedic outpatient clinic or visiting the emergency department for minor problems; children of hospital staff were also screened. Exclusion criteria were the presence of invasive diseases and the administration of antimicrobial drugs during the 2 previous months. Oropharyngeal specimens were obtained by pediatric emergency physicians by rubbing a cotton swab on the tonsils and subsequently analyzed by rtPCR for K. kingae as described above. The study had received institutional review board approval (09-029R, Mat-Ped 09-008R) and was conducted in accordance with Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki.

Analytical Sensitivity

All biological materials (oropharyngeal swab, synovial fluid, bone biopsy specimen and peripheral blood) were analyzed with a qPCR assay targeting the toxin-encoding gene rtxB, specific to the K. kingae. TaqMan Universal PCR Master Mix with AmpErase Uracil N-glycosylase (Applied Biosystems, Foster City, CA) was used with 5 µL input DNA and nuclease-free water (Promega, Madison, WI). Each PCR analysis was performed in duplicate. Standard curves and minimal limit of detection were generated by plotting the cycle threshold values obtained on a dilution series of purified DNA from K. kingae cells (10^3–10^8 colonizing-formit unit [CFU]/mL) against the log input CFU/mL. K. kingae counts were determined using a viable cell plate count method, by plating serial 10-fold dilutions on Colombia agar plates. The plates were incubated at 37°C for 24 hours, and CFU were determined in duplicate. DNA was amplified with the primers rtxB. Results of the standard curve are shown in Figure 1. The lowest detectable concentration of the real-time PCR was 10 CFU/mL. Detection and quantification were linear over the studied range with a correlation coefficient of 0.99513.

Statistical Analysis

Variables are reported as mean ± standard deviation. Comparison between groups was performed using unpaired t tests. P values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS (version 20.0 for Windows, Chicago, IL).

RESULTS

Cases

Between January 2008 and September 2012, 35 patients had K. kingae OAI identified by a PCR assay performed on bone/joint aspirates (33) or blood. The mean age for children with OAI due to K. kingae was 18.8 ± 8 months. All children with confirmed OAI due to K. kingae had a positive rtPCR in oropharyngeal specimens. During the same period, 47 oropharyngeal asymptomatic carriers were discovered by screening 620 healthy children, using rtPCR assay (7.6%). The mean age for asymptomatic oropharyngeal carriers was 25.5 ± 11 months.

Quantitative Colonization Density

Oropharyngeal colonization densities were similar in young children with OAI due to K. kingae and in asymptomatic carriers (P = 0.370): children with OAI due to K. kingae (mean number of cycles to detection; 32.1 ± 4.9 cycles) and asymptomatic

FIGURE 1. Quantification of Kingella kingae using quantitative real-time PCR. Ct indicates cycle threshold; SD, standard deviation.
oropharyngeal carriers (mean number of cycles to detection; 33 ± 3.9 cycles).

DISCUSSION

The normal flora of the oropharynx contains a large number of bacterial inhabitants. The most important group of microorganisms routinely cultured from this body niche is constituted by the α-hemolytic Streptococci. Additionally, cultures from this region usually show also large numbers of Moraxella (formerly Branhamella) catarrhalis, Neisseria spp and HACEK organisms such as Kingella.

Invasive infections in young children are frequently caused by organisms carried asymptptomatically in the respiratory tract.\(^6,11,12\) Colonization of the respiratory tracts 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 to present with invasive diseases.\(^6,7,11,12\) For any of these microorganisms, such as \textit{S. pneumoniae} or \textit{H. influenzae}, there are some evidences that higher nasopharynx colonization densities play major roles in the development of invasive infections.\(^13-18\)

However, less is known about the relationship between asymptomatic respiratory carriage and the incidence of invasive \textit{K. kingae} infections.\(^1,8,15\) Epidemiological investigations demonstrated that the asymptomatic respiratory carriage rate in young children ranged between 3.2\% and 17.5\%.\(^11,20-22\) and remained stable throughout the year.\(^7\) A few studies focused in elucidating the relationship between oropharyngeal colonization with \textit{K. kingae} and the subsequent risk to develop invasive infections. These studies found no correlation between the pattern of carriage of \textit{K. kingae} and the incidence of invasive disease.\(^6,19\)

Our study also attests for the first time that the \textit{K. kingae} colonization density in young children is not a risk factor for invasive OAI. These results suggest therefore that the intrinsic virulence of the colonizing strain is probably more important than the colonization density in the development of the invasive infections. In fact, many strains that colonize the oropharynx are not necessarily involved in clinical infections because invasion of the bloodstream and infection of deep tissues requires specific biological specialization, especially in their invasiveness capacities. Recent reports demonstrated, on this subject, that only a limited number of \textit{K. kingae} clones cause the majority of invasive infections\(^23,24\); clone K was significantly associated with bacteremia and skeletal system infections, clone N with osteoarticular infections and clone P with endocarditis.\(^24\)

In conclusion, and in contrary to our underlying assumption, there is no difference between children with OAI and asymptomatic carriers in density of oropharyngeal colonization with \textit{K. kingae}. However, there are many different \textit{K. kingae} clones in healthy oropharyngeal carriers, and children colonized by more virulent strains are more likely to develop serious systemic infections.

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REFERENCES