The first report on epidemiology of oropharyngeal
Kingella kingae carriage in Scandinavian children:
K. kingae carriage is very common in children attending
day care facilities in Western Norway

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Kingella kingae colonizes the upper airways in children and has been recognized as the most common causative agent
of osteoarticular infections (OAI) in children below 4 years of age. This is the first Scandinavian study to investigate
oropharyngeal K. kingae carriage in healthy children. From June 2015 to August 2016, we recruited 198 healthy children
aged 11–14 months from routine consultations at health promotion centers in Hordaland County, Norway for a
cross-sectional study. After their parents had provided informed consent; demographic data were registered, and an
oropharyngeal swab was collected. The oropharyngeal swab was analyzed with a real-time PCR assay specific to
K. kingae targeting the RTX toxin locus. Results showed an asymptomatic carriage rate of 12.6%. A striking and
highly significant difference was observed between the children that had started attending day care facilities as com-
pared with children still being at home (33.33% vs 8.5%; p < 0.001). K. kingae is prevalent in young children in Nor-
way. This study emphasize that K. kingae should be considered an important etiological agent in OAI. Transmission
seems to be facilitated in day care facilities. The correlation between oropharyngeal carriage and OAI needs to be fur-
ther explored.

Key words: Kingella kingae; children; pediatric infectious disease; emerging infection.

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Throat colonization with the Gram-negative bacterium Kingella kingae is common in children under 4 years of age (1). K. kingae has only recently been acknowledged as the most common cause of osteoar-
ticular infections (OAI) such as osteomyelitis and septic arthritis in small children. Since the bacterium is difficult to culture in the laboratory, the incidence is probably underestimated (2). In synovial fluid samples, bone biopsies and throat samples the bacterium can be reliably detected with PCR (3).

Previous studies on K. kingae oropharyngeal colon-
zation or carriage have been performed in Israel, Switzerland, France, Australia, and New Zealand (2, 4–7). Using PCR, a group from New Zealand

found a 23% prevalence in the age group from 12
to 24 months, the highest reported prevalence to date (7). A Swiss study detected by similar tech-
niques K. kingae in the tonsils of 8.7% of asym-
 tomatic children (4). Israeli studies have found prevalence’s ranging from 5% to 12% with various
methodologies, 9%–11.4% in the same age group as our study (2, 8). An Australian study found
asymptomatic carriage at 0% during summertime in a small study, but this was culture based (6). Higher prevalence’s and outbreaks have been described in day care institutions among both children and employees in the United States, France, and Israel (9, 10). Day care center attendance has been found to be independently associated with
K. kingae colonization (11). The variations in
REPORTED CARRIER RATES ARE PROBABLY AT LEAST PARTLY DUE TO DIFFERENT METHODOLOGIES AND VARIATIONS IN POPULATION DELIMITATION.

ACCORDING TO THE LITERATURE, FEW CHILDREN BELOW 6 MONTHS ARE COLONIZED, BUT FROM 12 TO 24 MONTHS RATE OF COLONIZATION IS HIGH (12). SEASONAL DIFFERENCES HAVE BEEN EXPLORED IN AN ISRAELI STUDY (11). ALTHOUGH NO SIGNIFICANT DIFFERENCES WERE FOUND IN ISRAEL, THIS HAS NOT BEEN FULLY EXPLORED IN A COLDER CLIMATE WITH LARGER CLIMATIC DIFFERENCES BETWEEN SUMMER AND WINTER LIKE SCANDINAVIA.

K. KINGAE OAI IS ALWAYS PROCEEDED BY OROPHARYNGEAL COLONIZATION FROM WHERE THE BACTERIUM IS ASSUMED TO SPREAD HEMATOGENOUSLY TO BONE AND JOINT TISSUES. IN ORDER TO OPTIMIZE TREATMENT, THE CORRECT MICROBE SHOULD BE CONFIRMED RAPIDLY; SO CORRECT ANTIBIOTICS CAN BE GIVEN. IT IS OFTEN DIFFICULT TO PERFORM BIOPSY OR COLLECT JOINT FLUID FROM CHILDREN FOR DIAGNOSIS, AND PCR BASED DETECTION OF K. KINGAE RTX TOXINS IN THE OROPHARYNX HAS BEEN FOUND TO REPRESENT A VERY SENSITIVE SURROGATE MARKER OF K. KINGAE OSTEOARTICULAR INFECTION IN YOUNG CHILDREN. CONTRARY, A POSITIVE TEST DOES NOT NECESSARILY SUGGEST INVASIVE INFECTION BUT STUDIES DEMONSTRATE THAT IT CAN PROVIDE A strong INDICATION that K. KINGAE IS THE RESPONSIBLE MICROBE IN CHILDREN WITH CLINICAL AND RADIOLOGICAL EVIDENCE OF OAI (13, 14). IT IS IMPORTANT TO POSSESS KNOWLEDGE ABOUT EXPECTED COLONIZATION RATES IN YOUR POPULATION.

AFTER WE ESTABLISHED A PCR FOR K. KINGAE IN OUR LABORATORY, WE HAVE DIAGNOSED SEVERAL CHILDREN WITH K. KINGAE OSTEOYIELTITIS. THE AIM OF THIS STUDY WAS TO TEST THE HYPOTHESIS THAT OROPHARYNGEAL CARRIAGE IS COMMON IN YOUNG CHILDREN IN NORWAY.

METHODS

Study population

This is a cross-sectional study based on screening of healthy children attending a universal 12-month checkup at a family health clinic. Participants were prospectively recruited from 4 selected peri-urban municipalities (Fjell, Sund, Os and Askøy), all situated in a coastal area within Hordaland County, approximately 1 h outside the city of Bergen. The area has a milder climate due to the Gulf Stream, which also gives this region a milder winter than other parts of Norway. The municipalities all have a similar temperate coastal climate with an average temperature of approximately 2 °C in winter, and 14–16 °C in the summer. Average annual rainfall varies from 1500 mm per year to above 2000 mm in the different municipalities. Norway is a welfare state, and the study area has a socioeconomically homogenous population with less than 10% immigrants.

The majority of children in Norway attend family health clinics with frequent intervals, one being the checkup at approximately twelve months of age (season/holiday-dependent). Prior to the study, information meetings were organized at each health promotion center to inform public health nurses and physicians. All children aged 11–14 months living in the district (based on data from the Population Registry) were invited to participate.

Each parent/care giver received a consent form per mail a few weeks before their child’s routine 12 months control appointment. Letters were sent to all families without discrimination. 615 invitation letters were sent out, the majority to the children living in the two largest municipalities Fjell and Askøy, followed by Os and Sund. The invitation letter included information about the purpose of the study and ensured confidentiality. Children were eligible if they: (i) lived in the study district; (ii) were 11–14 months at inclusion; (iii) did not have known previous or current bone/joint infection; (iv) their parent/care giver were able to give written consent; and (v) their physician returned a throat swab from the child.

Sample collection procedure and questionnaire

Throat swabs were collected in a standardized manner, similar to throat swabs for throat infection that all the participating physicians were familiar with.

A single oropharyngeal swabs was collected from each child by a municipality physician and transported in liquid transport media (Eswab, Copans Diagnostic Inc., US) to the laboratory for processing. The sampling was part of a clinical routine examination, but other findings from the routine examination were not registered for this study. We included a questionnaire (Q) for self-reporting with the invitation letter. This was returned to the physician by the parent/care giver when they attended their child’s routine appointment.

The oropharyngeal swabs were analyzed with a real-time PCR assay targeting the RTX-B toxin gene (15). For DNA extraction between 400 and 800 μL, sample material was added to a tube containing a mixture of glass and ceramic beads (SeptiFast Lysis kit; Roche, Mannheim, Germany), together with 400 μL Bacterial Lysis Buffer (Roche). The samples were run for 2 × 45 s in a MagnaLyser machine (Roche) at speed 6.5. After a short spin (16 060 g, 3 min), 400 μL supernatant was transferred to a MagNa Pure Compact automated extractor (Roche), and DNA was extracted and purified using the ‘Bacteria DNA v3.2’ programme and a selected elution volume of 50 μL. PCR was performed on a real-time SmartCycler apparatus (Cepheid, Sunnyvale, California, USA) with a final reaction volume of 25 μL. The PCR mixture consisted of 12.5 μL Premix ExTaq master mix (TaKaRa, Kusatsu, Japan), 0.4 μM F-primer (rtxB-F: 5’-CAACAATAGCCGCCAGTTGA-3’), 0.4 μM R-primer (rtxB-R: 5’-ACAATTAAGACCAATGGCCGTTGAG-3’) and 0.2 μM of probe (RtxB-P: [FAM]ATCCCAACGCGCGTCATTGTG[6FAM]) and 8 μL PCR-grade water, and 2 μL template. The PCR thermal profile included an initial polymerase activation step of 30 s at 95 °C followed by 45 cycles of 5 s at 95 °C and 30 s at 64 °C. A sample was considered positive if it reached the fluorescence threshold before cycle 40.

Sample size calculations and statistics

Based on previous studies from other countries the prevalence of K. kingae oropharyngeal carriage in children...
below 4 years of age range from 8% to 12% (2, 16). We hypothesized the prevalence in West-Norwegian children in the age 12–24 months to be 12%. With a 5% point level for precision and standard levels for 95% confidence interval, we then needed at least 163 participants. Due to the uncertainties in prevalence level estimations, we planned to recruit approximately 200 children. Data were analyzed with SPSS.

Ethics
The survey was approved by the Regional Committee for Ethics in Medical Research, REK Western Norway (Ref. number: 2015/322). Written informed consent was obtained from parents/care-givers of all subjects.

RESULTS
Data collection was continued until we had a sufficient number of samples. From June 2015 to August 2016, 198 healthy children (97 boys and 101 girls) were recruited giving a participation rate of 32% (198/615). The median age was 12 months (mean 11.96). Due to differences in data collection intensity, the number of responses each calendar month ranged from \(N = 5\) (March) to \(N = 41\) (June). The number of samples collected per yearly month is provided in Table 1. All 198 swabs were analyzed successfully.

The results are summarized in Table 2. Overall, \(K. \)kingae was detected in 12.6% of the samples (25/198). The bacteria was detected in 14.43% of the boys (14/97) and 10.89% of the girls (11/101). This gender difference was not significant.

At the time of inclusion, only 16.67% (33/198) of the children were attending day care facilities. However, among these children, 33.33% (11/33) had \(K. \)kingae DNA detectable in their throat, while only 8.48% (14/165) of the children not attending day nursery institutions had a positive throat swab. This was significantly different (\(p < 0.001\)).

Table 1. Annual distribution

| Month | Number of samples | Positive samples, n (%) |
|-------|-------------------|------------------------|
| January | 12 | 1 (8.3) |
| February | 25 | 2 (8.0) |
| March | 11 | 3 (27.3) |
| April | 27 | 3 (11.1) |
| May | 14 | 0 (0.0) |
| June | 41 | 3 (7.1) |
| July | 13 | 1 (7.7) |
| August | 7 | 2 (28.6) |
| September | 12 | 1 (8.3) |
| October | 7 | 3 (42.9) |
| November | 19 | 4 (21.1) |
| December | 9 | 2 (22.2) |
| Sum | 198 | 25 (12.6) |

68.69% (136/198) of the children reported to have older siblings. The prevalence of \(K. \)kingae in children without older siblings was 12.9% (8/62), while prevalence in children with siblings was almost identical at 12.5% (17/136).

Thirty-eight % (75/198) of the throat samples were collected during the summer months May, June, July, and August, while the remaining 62% (123/198) were collected in the other months of the year. In the summer, 6.66% (5/75) of the samples collected were positive for \(K. \)kingae DNA, while 16.26% (20/123) of the samples collected in the colder months were positive for \(K. \)kingae DNA. There was a weak association between season and carrier rate when comparing summer swabs with swabs from the rest of the year (\(p = 0.049\)), but the summer period also coincide with a period when fewer children are attending day care facilities. If we split the year into 6-month periods (6 coldest months vs 6 warmest months), no significant difference was found.

DISCUSSION

In this study, we examined oropharyngeal carriage of \(K. \)kingae in asymptomatic 1-year old children and found an average prevalence of 12.6%. Asymptomatic carriage rates in Norway have not previously been explored, and this study confirms that \(K. \)kingae is likely to represent an important etiological agent of OAI also here.

Some of the children in our study population had already started in day care. These children had an exceptionally high carriage rate of 33% giving reason to believe that day care facilities is an important risk factor for colonization also in Norway. Although the number of children in this subpopulation was small and the calculated carrier rate has a large confidence interval, the difference was nevertheless large enough to conclude that there was a highly significant difference.

This is of importance in Norway were most parents re-enter the workforce 12–18 months after birth, and day care attendance reaches 91% among 1–5 year olds (17). Previous investigations from Israel, France, and the USA have described that \(K. \)kingae disease is common in day care institutions. Outbreaks have been described in day care facilities causing clusters of serious infections such as osteomyelitis, septic arthritis, endocarditis, and meningitis (9, 10, 18–21).

Evidence of seasonal variation has been conflicting (1, 2, 22). We found a tendency of lower colonization rate in the summer months, but the association was weak. Numbers are also very small, and the results must be interpreted with caution.
We did not ask for nor excluded children who had recently been on antibiotic treatment. Antibiotic prescriptions to children in Norway for common respiratory infections in the winter months is generally conservative and low (23). However, we cannot totally exclude that this has affected our results.

A potential limitation of our study was that the participants were not representative for the Norwegian population according to geographic area since the whole study was conducted in the western, coastal, mild part of Norway.

We neither collect socioeconomic data nor perform regression analysis to control for confounders. However, inclusion was universal via family health clinics. Since participation rate was low, it is possible that augmented participation from parts of the population (e.g., higher socioeconomic status) can contribute to an overestimation or underestimation of prevalence and hence a selection bias. However, it is more likely to believe that the busy time schedule of parents and nurses/physicians led to a universal lack of throat sampling from some children regardless of socioeconomic status. We believe responders and non-responders share similar characteristics. Norway has a population with a high mean income and good housing quality overall with no crowding, so we argue that this difference is negligible.

Seeding of K. kingae to bone or joint occurs via hematogenous spread from oropharyngeal colonization (2). A K. kingae DNA negative throat swab has a high negative predictive value and can guide pediatricians to choose a treatment that does not necessarily include empiric coverage for K. kingae OIA. A positive throat swab is predictive of K. kingae in children with clinical and radiological findings of OIA, and should be used as an argument to broaden empiric treatment to include antimicrobial coverage of K. kingae. Due to the high carriage rate, a positive throat swab alone should not be used as an argument to narrow empiric treatment. Nor should it impact decisions regarding collection of proper sample material from bone or joint. These results underscore the need for continuous awareness of K. kingae as a cause of osteoarticular infection in children below 4 years of age. European guidelines recommend that empirical therapy should include an antibiotic with effect against Staphylococcus aureus and for young children also coverage for K. kingae in relevant areas (24). However, in Norway where S. aureus is normally methicillin sensitive, Oxacillin is often the drug of choice for empiric treatment according to national guidelines (25). Oxacillin is not the drug of choice for K. kingae, and high in-vitro MIC values indicate a poorer effect than other beta-lactam antibiotics (26). The high prevalence of K. kingae in throat swabs might indicate a need of revision of treatment guidelines in Norway.

### CONCLUSIONS

This study describes an overall 12.6% prevalence; hence, there are high rates of K. kingae carriage in tonsils in young, healthy children in western Norway. Given Norway’s homogenous population, it seems reasonable to assume that these findings apply to children in Norway in general. Transmission seems to be highly facilitated in day care facilities with an almost 4-fold increase in carrier rate associated with day care attendance. This epidemiological study emphasizes that K. kingae should be considered an important etiological agent in osteoarticular infection (OAI) also in Norway. A positive throat swab should be used as an argument to broaden empiric treatment to include antimicrobial coverage of K. kingae. Numbers are small, and further studies are warranted. Correlation with oropharyngeal carriage and OAI in Scandinavia needs to be further explored in a hospital study, preferably a multicenter trial, since OAI is relatively rare.

|                          | K. kingae positive, n (%) | 95% CI for prevalence | p-value |
|--------------------------|---------------------------|------------------------|---------|
| Boys                     | 14/97 (14.43%)            | 7–22                   | 0.453   |
| Girls                    | 11/101 (10.89%)           | 5–17                   |         |
| Day care attendance      | 11/33 (33.33%)            | 16–50                  | <0.001  |
| No day care attendance   | 14/165 (8.48%)            | 4–13                   |         |
| Older siblings           | 17/136 (12.5%)            | 7–18                   | 0.937   |
| No older siblings        | 5/62 (12.9%)              | 4–21                   |         |
| Throat sample in summer  | 5/75 (6.66%)              | 10–23                  | 0.049   |
| Throat sample colder     | 20/123 (16.26%)           | 1–12                   |         |

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We are grateful to all children and parents in Fjell, Sund, Askøy, and Os municipalities who contributed to this study. We also thank the public health nurses who helped and facilitated in recruitment of volunteers. A special thanks to Astrid Hermansen Paulsen (Fjell kommune), Britt Roti (Askøy kommune), Svanhild Forland (Sund kommune), and Dagmar Hilde (Os kommune). Thanks to all the general physicians who contributed in collecting the swabs, and especially the Municipality Senior Doctors Stein-Inge Stigen (Sund and Fjell Municipalities), Arild Iversen and Christian Redisch (Askøy Municipality), and Jan Rune Stangeland (Os Municipality) who approved the study locally. We thank the medical doctors and the laboratory technicians at the laboratory for help with the analyses and professor and head of the department Elling Ulvestad for supporting the study. We also thank pediatrician Dr. Franciscus Bosse, Haukeland university hospital, for kindly providing his advice.

FUNDING

This work was supported by internal funding of the Department of Medical Microbiology, Haukeland university hospital.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

KL and OK conceived and designed the study; KL, RIB, JVA, and OK performed the experiments; KL and OK analyzed the data; KL, OK, JVA, and RIB contributed reagents/materials/analysis tools KL, RIB, and OK drafted the manuscript; KL, OK, JVA, and RB involved in revision of manuscript. All authors have read and accepted the final manuscript.

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