

Oropharyngeal *Kingella kingae* carriage in children: characteristics and correlation with osteoarticular infections

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BACKGROUND: The aim of this study was to investigate changes in oropharyngeal *K. kingae* carriage during the first 4 y of life, including seasonal variation and comparison of asymptomatic carriage with cases of invasive osteoarticular infections (OAI).

METHODS: Oropharyngeal bacterial *K. kingae* carriage was screened in 744 healthy children aged 7–48 mo between January 2009 and December 2012. Oropharyngeal swabs were analyzed by rt-PCR targeting the DNA of *K. kingae* RTX toxin, epidemiological characteristics of asymptomatic carriers and OAI case patients were recorded.

RESULTS: The carriage prevalence showed no significant difference between age groups or seasons. Compared with asymptomatic carriers, OAI cases were more likely to be aged from 7 to 12 mo (OR = 2.5; 95% CI (1.2–5.0)) and 13–24 mo (OR = 2.2; 95% CI (1.2–3.9)), and less likely over 36 mo (OR = 0.2; 95% CI (0.1–0.7)). Fewer OAI cases were identified in spring compared to asymptomatic carriers (OR = 0.3; 95% CI (0.1–0.7)), while more were detected in autumn (OR = 2.5; 95% CI (1.4–4.4)).

CONCLUSION: Although oropharyngeal *K. kingae* colonization is a prerequisite for further invasive infection, this epidemiological study emphasizes that the carriage rate variations do not correlate with the variations of OAI incidence by gender, season, or age group.

Kingella kingae is considered the major bacterial cause of osteoarticular infections (OAI) in children less than 48 mo (1–3). This organism is a frequent component of the oropharyngeal flora of young children (4). There is evidence that *K. kingae* colonize the oropharynx before hematogenous spread to distant sites (5). Respiratory carriage of *K. kingae* appears to be a prerequisite for distant infection of the joint and bone (6–8).

Prior studies demonstrated that the prevalence of *K. kingae* colonization in young children ranged between 3.2 and 17.5%,

and was stable throughout the year (8–11). *K. kingae* is not usually isolated in infants under 6 mo of age and carriage is highest among 7–36 mo old children. Variation in *K. kingae* carriage rates, however, has not been shown to explain variations in incidence of invasive disease. Most studies on oropharyngeal carriage of *K. kingae* have been among children of southern Israel, so these results may represent a regional epidemiologic phenomenon (8–10). The aim of this epidemiologic study was to determine the respiratory carriage of *K. kingae* among a Swiss population of healthy children aged from 7 to 48 mo using our *K. kingae*-specific rt-PCR, which is more sensitive than seminested broad-range 16S rRNA gene PCR, and standard culture in detecting *K. kingae* (11,12). Secondly, we investigated the age and seasonal distributions of *K. kingae* OAI as well as the correlation with its respiratory carriage.

RESULTS

Demographic Characteristics

Overall, 744 throat swabs were obtained from asymptomatic children aged from 7 to 48 mo during the study period and subsequently analyzed. Demographic characteristics of screened children are shown in **Table 1**. We obtained a minimum of 144 and 135 oropharyngeal specimens for each age group and season respectively. Gender distribution for each age group and season are shown in **Table 1**; there was no significant difference in gender distribution within each group.

Respiratory Carriage Prevalence

The oropharyngeal prevalence's distribution of this microorganism by gender, age group, and season are shown in **Table 1**. Mean oropharyngeal carriage rate in children from 7 to 48 mo was 8.7% (65 of 744); ranging between 7.6 and 10.4% in 2010 and 2011 respectively. There was no significant difference of carriage rate in between the years of the study period, allowing us to merge the results for the further analysis. Carriage rate was significantly greater among male subjects (10.9%) than females (6.4%; RR: 1.69 (95% CI 1.04–2.76), $P < 0.05$; **Table 1**).

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Table 1. *K. kingae* asymptomatic carriage depending on gender, age, and season

Results of oropharyngeal *K. kingae* rt-PCR screening among asymptomatic children

	Screened children (n) (male/female)	Oropharyngeal <i>K. Kingae</i> PCR + (n)	Carriage rate (%)	Relative risk	CI (95% CI)
Gender					
Male	386	42	10.9	1.69	1.04–2.76
Female	358	23	6.4	0.59	0.36–0.96
Age					
7–12 mo	144 (78/66)	9	6.3	0.67	0.34–1.31
13–24 mo	226 (114/112)	23	10.2	1.26	0.77–2.04
25–36 mo	194 (96/98)	19	9.8	1.17	0.72–1.98
37–48 mo	180 (98/82)	14	7.8	0.86	0.49–1.52
Season					
Winter	194 (94/100)	13	6.7	0.71	0.40–1.30
Spring	157 (82/75)	16	10.2	1.22	0.73–2.12
Summer	135 (71/64)	11	8.2	0.92	0.50–1.74
Autumn	258 (139/119)	25	9.7	1.17	0.72–1.88

Carriage expressed in % and n the number of positive oropharyngeal *K. kingae* carriers.

Respiratory carriage according to age: The median age of asymptomatic carriers was 25 mo and ranged between 8 and 48 mo. The prevalence of *K. kingae* carriage was highest among 13-to-24- and 25-to-36 mo-old children (10.2 and 9.8% respectively), however carriage rate did not vary significantly among different age groups ($P \geq 0.185$; **Table 1** and **Figure 1a**).

Respiratory carriage according to season: **Table 1** shows that prevalence of oropharyngeal *K. kingae* colonization showed no significant seasonal variation ($P > 0.245$; **Figure 1b**).

Osteoarticular *K. kingae* Infections

During the study period, 46 patients were diagnosed with *K. kingae* OAI; cases ranged from 9 to 14 per year. Similar to oropharyngeal colonization, *K. kingae* OAI showed no significant difference in incidence between study years. The case-control portion of the study, comparing OAI case patients with asymptomatic carrier controls, revealed the following:

OAI case patients by gender: Girls represented a higher percent of case-patients (21/46 = 45.7%) than of asymptomatic carrier controls (2176/5773=37.6%), but this difference was not significant (OR 1.38; 95% CI 0.8 to 2.5, **Table 2**).

OAI case-patients by age: The median age of children diagnosed with *K. kingae* OAI was 16.5 mo compared with 25 mo among asymptomatic carrier controls. Comparison between **Tables 1** and **2** reveals that fewer of the case-patients were over 36 mo of age (3/46 = 6.5%) compared with controls (1,513/5,996 = 25.2%; OR = 0.21; 95% CI = 0.1–0.7) and a higher number of case-patients were aged between 7 and 12 mo and 13 and 24 mo, 10/46 = 21.7% and 24/46 = 52.2% respectively, vs. 601/5,996 = 10.0% (OR = 2.47; 95% CI = 1.2–5.0) and 1,980/5,996 = 33.0% (OR = 2.20; 95% CI = 1.2–3.9) asymptomatic controls, respectively.

OAI case-patients by season: **Table 2** shows that a plurality of case-patients (22/45 = 48.9%) presented during autumn compared with only 1,575/5,825 = 27.1% of asymptomatic

carrier controls (OR = 2.45; 95% CI 1.4–4.4). There were fewer case-patients in spring (5/46 = 10.9%) than asymptomatic controls (1,727/5,825 = 29.6%; OR = 0.29; 95% CI = 0.1–0.7).

Correlation in Between Oropharyngeal *K. kingae* Carriage and OAI Incidence

No statistically significant correlation was observed in between the variations of asymptomatic carriage and OAI incidence when analyzed by age ($P = 0.750$) or season ($P = 0.917$).

Influence of URT Infection on *K. kingae* OAI Incidence

A parallel increase of URT infection and *K. kingae* OAI was noted over the months of August to November (**Figure 2**). Overall, there was no significant correlation between the incidence of URT infections and OAI cases in children aged from 7 to 48 mo ($P = 0.442$).

DISCUSSION

K. kingae has recently emerged as an important cause of OAI in young children (1–3,13). This organism belongs to the normal commensal flora of the posterior pharynx (14,15), but rarely penetrates the bloodstreams and invades distant organs (6,16). The *K. kingae* strains isolated from the pharynx of children with invasive infections are genotypically identical to those recovered from the blood (5). Colonization of the respiratory tract is, therefore, a prerequisite for later invasive infection; but most of those colonized will never be infected. The correlates of invasive disease among colonized children remain unknown.

Our results confirmed that *K. kingae* is frequently detected in the upper respiratory tract of young children. Previous studies conducted in Israel, using standard culture methods, found prevalences of oropharyngeal *K. kingae* colonization between 3.2 and 17.5% among children less than 4 y (8,14,16). Quantitative studies imply that the load of *K. kingae* among asymptomatic carriers changes little through the first 4 y of

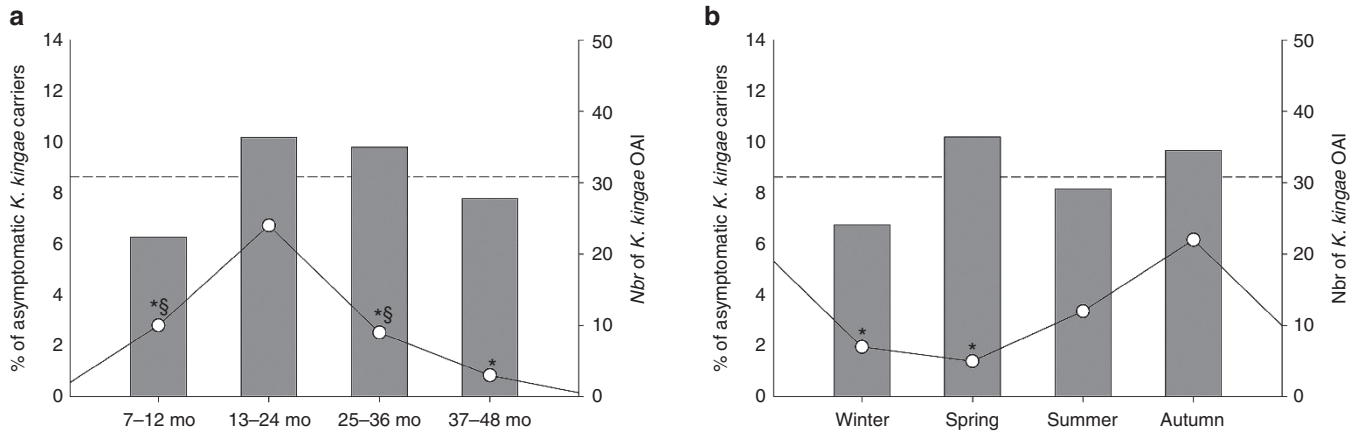


Figure 1. *K. kingae* asymptomatic carriage rate and OAI cases by age group (a) and season (b). Bars represent carriage rates, white circles correspond to the number of OAI cases diagnosed between January 2009 and December 2012, and dashed line marks the mean carriage rate. For Carriage rate data total $n = 744$ and for OAI cases total $n = 46$. (a) * $P < 0.001$ vs. 13-24-mo OAI cases, $^{\S}P < 0.05$ vs. 37-48-mo OAI cases; (b) * $P < 0.001$ vs. autumn OAI cases, Mann Whitney U-test.

Table 2. Distribution of *K. kingae* OAI cases by gender, age group, and season

Diagnosed <i>K. kingae</i> OAI cases between 2009 and 2012					
	Cases (n)	(%)	Asymptomatic carriers (n)	Odds ratio	CI (95% CI)
Gender					
Male	25	54.3	3,597	0.72	0.41-1.29
Female	21	45.7	2,176	1.38	0.78-2.47
Age					
7-12 mo	10	21.7	601	2.47	1.23-4.95
13-24 mo	24	52.2	1,980	2.20	1.24-3.91
25-36 mo	9	19.6	1,902	0.53	0.25-1.09
37-48 mo	3	6.5	1,513	0.21	0.06-0.67
Season					
Winter	7	15.2	1,142	0.74	0.33-1.64
Spring	5	10.9	1,727	0.29	0.12-0.74
Summer	12	26.1	1,381	1.13	0.59-2.19
Autumn	22	47.8	1,575	2.45	1.38-4.36

Total number of *K. kingae* cases diagnosed between 2009 and 2012. Number of asymptomatic carriers during the study period was estimated according to official Geneva Demographic data and the calculated carriage rates. Statistically significant results are highlighted with boldface text.

life and is similar to that of patients with invasive OAI (17,18). Our use of rt-PCR for detection did not increase the observed prevalence rate beyond the previously reported range. Taken together, these results imply that the bacterial load of colonized children is usually high enough to be detected with culture, and that colonization is a necessary but not sufficient condition for invasive disease.

In our population, the colonization rate was greatest among children aged between 12 and 36 mo. After 36 mo, the carriage rate declined, probably due to the maturation of the children's immune system and/or an acquired humeral immunity to *K. kingae* carriage and invasive infection as a result of previous expositions (15-19). Our results show marked similarities to

a recent study carried out by Amit *et al.* (20) among a large cohort of healthy Israeli children; and previous data (13,16). A recent study by Amit *et al.* (19) confirmed the age of 6-29 mo to be a significant risk factor for *K. kingae* colonization in two different ethnic groups of Israel. In general, age-specific colonization rates show remarkable consistency between patients of varying geographic and ethnic characteristics.

K. kingae oropharyngeal carriage rates and OAI cases in our study both showed a marked increase between the 7-12-mo and 13-24-mo age groups, while the decreased incidence of OAI was sharper than the decrease in prevalence of colonization after 24 mo. Vanishing maternally derived immunity (16), and increased social contacts, especially day-care attendance (21), may result in an increasing acquisition of *K. kingae* within the two first year of life. The available data suggest that by 24 mo most children who will be infected by *K. kingae* already have been infected resulting in acquired immunity (15,22). Furthermore, recent investigation demonstrated the presence of a carbohydrate capsule in *K. kingae* (23). Since immunity to polysaccharides in humans matures between the ages of 2-4 y (24), this may explain the increased risk of clinical apparent invasive infection in colonized children below the age of 24 mo.

Seasonal prevalence of *K. kingae* colonization in our study showed minimal variations, with a modest increase in the fall and a subsequent decline in winter. Yagupsky *et al.* hypothesize that prevalence of *K. kingae* could be reduced by exposure to antibiotics during the winter months, since *K. kingae* is highly sensitive to antibiotics commonly used for respiratory infections (13,25). In our study of asymptomatic carriage, throat swabs were collected only from children without clinical findings of OAI who did not receive any antibiotic treatment in the previous 2 mo. Although these exclusion criteria weaken the argument that antibiotic exposure is the primary cause of lower *K. kingae* prevalence in winter, this theory cannot be entirely discounted. Choosing a longer antibiotic free interval as exclusion criteria may also introduce selection bias, since most children undergo one

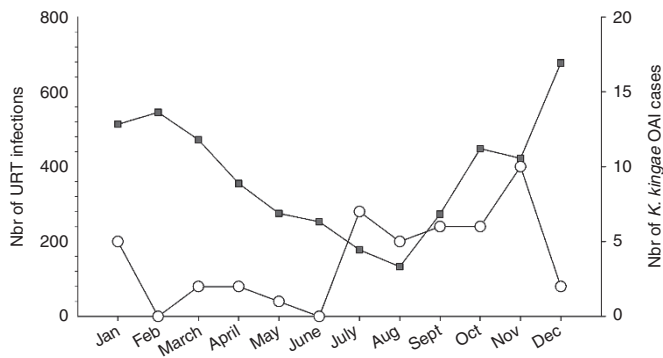


Figure 2. Monthly distribution of upper respiratory tract (URT) infections and *K. kingae* OAI cases in our hospital. Gray squares represent the monthly number of URT infections seen in our emergency room in 2011–2012 and white circles correspond to the number of OAI cases diagnosed every month in between January 2009 and December 2012.

to two antibiotic courses annually during the first 4 y of life (26–28).

In our series, more than two-thirds of OAI cases were diagnosed between July and November, as previously described (8,10). Our results showed no correlation between prevalence of *K. kingae* carriage and OAI incidence. We previously demonstrated a constant bacterial load among asymptomatic carriers throughout the first 4 y of life (17), although the OAI incidence greatly changes; and the absolute risk for asymptomatic carriers to sustain an invasive OAI is below 1% (29). Like most infectious diseases, OAI represents a confluence of agent, host and environment, and although *K. kingae* carriage is clearly involved in pathogenesis, disease only occurs in the presence of other factors not yet identified (8).

Possibly, concurrent infections causing damage to the upper respiratory mucosa facilitate local invasion by *K. kingae* residing in the pharynx (30,31). If so, OAI could result from the interaction of *K. kingae* colonization and acute URT infection (8–14). Recent studies have evidenced associations between viral respiratory diseases (32), such as primary herpetic gingiva-stomatitis (30), hand-foot-mouth disease/herpangina (33) and invasive *K. kingae* infections. Interestingly, in our study only the seasonal OAI prevalence increase occurring between September and November corresponds to an increase in respiratory tract infections among our pediatric population. Although evidence underlines an association between a viral infection caused breach of the oropharyngeal mucosal lining and invasive infections, this study suggests that other factors may influence this direct link.

Previous evidence suggested differences in virulence, colonization capacity and affinity for osteoarticular tissue among different *K. kingae* strains (23,34). This raises the question of whether age, gender, or season influences the susceptibility of an organism to be colonized with a more or less virulent clone. Unfortunately, the rt-PCR method we used does not allow further analysis of this variable.

Another limitation of this study concerns the recruitment of the healthy controls. The majority was recruited upon arrival

to our Children's Hospital, which may not be representative of the healthy 7- to 48-mo-old Geneva population. However in order to offset this potential bias we did not include children with invasive disease or having sustained an antimicrobial treatment in the preceding 2 mo. This, as well as the screening of hospital staff children, allowed us to reduce the hospital setting induced selection bias of chronically ill children which could affect prevalence of *K. kingae*. Finally the sample size of screened children remains small for an epidemiologic study. We collected 744 oropharyngeal samples over 4 y. Considering that the number of children aged from 7 to 48-mo living in Geneva and surroundings is 17,000 children (according to official Geneva Demographic data), our sample size represents 1.1% of the local 7–48-mo-old population during the study period. However, despite these limitations, we were able to show an absence of correlation between *K. kingae* carriage and invasive OAI incidence.

Conclusion

Epidemiological features of asymptomatic carriage and invasive *K. kingae* infections appear similar in at least two different geographic locations (Israel and Switzerland), but more epidemiological studies should be conducted in order to generalize these results. Although oropharyngeal colonization with *K. kingae* is a prerequisite for further invasive infection, variations in OAI incidence are not explained fully by variations in *K. kingae* colonization. Age related immunity, antibiotic exposure, coinfection with other agents, and intrinsic virulence of specific *K. kingae* clones each merit further investigation as possible disease cofactors.

METHODS

In this prospective, consecutive patient study, controls were children without symptoms or signs of OAI aged from 7 to 48 mo. They were screened for asymptomatic oropharyngeal *K. kingae* carriage between 1 January 2009 and 31 December 2012. Controls were either hospitalized for clean surgery, attending our orthopedic outpatient clinic, or visiting the emergency department for minor problems; children of hospital staff were also screened in order to reduce the hospital setting induced selection bias of chronically ill children. Exclusion criteria were the presence of invasive diseases and the administration of antimicrobial drugs during the 2 previous months. Oropharyngeal specimens were obtained by rubbing a cotton swab on the tonsils, which were subsequently analyzed by rt-PCR specific for *K. kingae*.

In parallel, we collected data for all children aged 7–48 mo admitted to our emergency department from 1 January 2009 until 31 December 2012 in whom an OAI due to *K. kingae* was confirmed. All patients admitted for suspected OAI were evaluated clinically, underwent blood analysis and oropharyngeal screening for *K. kingae*. Further on, MRI investigations and diagnostic osteoarticular puncture were performed if the first investigations were suspicious for OAI. The diagnosis of *K. kingae* OAI was established as a positive culture or PCR assay for this microorganism in blood, synovial fluid or bone aspiration samples. Cases with presumed osteoarticular *K. kingae* infections (patients with conclusive signs of infection on MRI but for which joint-fluid or bone sample was not obtained for diagnostic PCR assay) were not taken into consideration.

Analyzed data included gender and age of the subjects as well as the year and season in which oropharyngeal screening tests were performed. Age limits were chosen accordingly to previous studies, which showed that the vast majority of invasive infections caused by *K. kingae* occurred from 7 to 48 mo of age (1,8,35). Age intervals

were defined as follows: 7–12, 13–24, 25–36, and 37–48 mo. Seasons were defined considering local weather changes: winter included the months of December through February, spring the months of March through May, summer, and autumn the months of June–August and September–November respectively.

For comparison purposes, the numbers of upper respiratory tract (URT) infections diagnosed in our emergency department in children aged from 7 to 48 mo were recorded in 2011 and 2012.

The study received institutional review board approval (09-029R, Mat-Ped 09-008R, Commission cantonale d'éthique de la recherche; CCER, Geneva University Hospitals) and was conducted in accordance with Good Clinical Practice guidelines and the provisions of the Helsinki Declaration. Parents or guardians provided written informed consent on behalf of each participant.

PCR Assays

All biological samples (oropharyngeal swabs, osteoarticular puncture specimen, peripheral blood) were analyzed with a rt-PCR assay targeting the toxin-encoding genes *rtxA* and *rtxB*, specific to *K. kingae* (11). DNA was extracted with a MagNAPure LC instrument using the MagNAPure LC DNA isolation kit II (Roche Molecular Biochemicals, Basel, Switzerland) according to manufacturer's instructions. TaqMan Universal PCR Master Mix with AmpErase UNG (Applied Biosystems, Zug, Switzerland) was used with 0.5 μmol/l of each primer, 0.25 μmol/l of the probe, 5 μl input DNA, and nuclease-free water (Promega, Basel, Switzerland). For a standard quantitative real-time PCR reaction, the conditions below are the recommended default conditions on all Applied Biosystems Real-Time PCR instruments: a 50 °C UNG activation step, a 95 °C AmpliTaq Gold enzyme activation, and 40 cycles of 95 °C denaturation and 60 °C anneal/extension. C_t detection cutoff was of 30 c.f.u. Each PCR analysis was performed in duplicate.

Statistical Methods

Differences among groups were analyzed using Mann–Whitney *U*-tests and considered statistically significant for $P < 0.05$. Relative risks (RRs) and their confidence intervals (CIs) were calculated to compare prevalence rates among the different risk groups. A case-control analysis (OAI case patients vs. asymptomatic carrier controls) was performed calculating Odds ratios (ORs) and the respective CIs for each gender-, age-, and season group. Statistical significance was considered at 95% CI. The numbers of asymptomatic carriers during the study period (January 2009 to December 2012 = 4 y) were determined for each risk group using the official local demographic database and the calculated carriage rates. According to official Geneva Demographic data, 17,000 children aged from 7 to 48 mo live in Geneva and surroundings. This number has not varied significantly over the study period, therefore a cumulative total of $17,000 \times 4 = 68,000$ children in between 7 and 48 mo lived in Geneva and surroundings during the study period. An equal distribution of male and female children (50% boys and 50% girls) as well as an equal distribution among age groups (14.2% of children aged 7–12 mo and 28.6% of children in every other age group) was considered. Since the total number of screened children was not equal in between genders, age groups, and seasons, the total of derived asymptomatic carriers for each case-control analysis varied slightly (5,773; 5,996; and 5,835 for gender, age, and season analysis, respectively). The Spearman rank correlation test was used to analyze correlations. Statistical analyses were performed using the IBM SPSS 20.0 (SPSS, Armonk, NY).

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R.A.de la.L. collaborated in the patient recruitment, performed the data analysis, statistical measurements, drafted the initial manuscript, and approved the final manuscript as submitted. She had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. V.D.-F. and S.M. participated in study design, patient recruitment, reviewed the manuscript, and approved the final manuscript as submitted. A.M. performed statistical analysis, revised the manuscript, and approved the final manuscript as submitted. A.C., J.H., and J.S. designed the *K. kingae* specific rt-PCR technique, supervised and coordinated the PCR analysis, critically revised the manuscript, and approved the final manuscript

as submitted. G.R. performed all the PCR analysis, revised the manuscript, and approved the final manuscript as submitted and D.C. conceptualized and designed the study, critically revised and reviewed the manuscript, and approved the final manuscript as submitted. The authors thank the children and their families for their willing cooperation to participate in this study. We acknowledge the staff of the paediatric orthopaedics service, the Pediatric Emergency Division, and above all Florence Hugon for their assistance.

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