Person-to-Person Transmission of *Kingella kingae* among Day Care Center Attendees

Ariela Slonim, Elaine S. Walker, Esther Mishori, Nurith Porat, Ron Dagan, and Pablo Yagupsky

Clinical Microbiology Laboratory and Pediatric Infectious Disease Unit, Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel; Department of Internal Medicine, James H. Quillen Veterans Affairs Medical Center, East Tennessee State University, Johnson City, Tennessee

Fifty *Kingella kingae* organisms, isolated from tonsillar cultures of day care center attendees during an 11-month period, and 60 isolates derived from epidemiologically unrelated individuals, including 19 isolates from respiratory carriers and 41 isolates from patients with invasive infections, were typed by immunoblotting, pulsed-field gel electrophoresis, and ribotyping. One strain, defined by unique immunoblotting, pulsed-field gel electrophoresis, and ribotyping patterns, represented 14 day care isolates (28%) and was frequently isolated during the first half of the follow-up period; a second strain represented 23 (46%) isolates and prevailed during the last 5 months. Children frequently carried the same strain continuously or intermittently for weeks or months, when it was replaced by a new strain. Epidemiologically unrelated organisms showed greater variability, and no strain represented >5% of isolates. The present results support person-to-person transmission of *K. kingae* among young children in the day care setting.

Because of better awareness of the organism and improved culturing techniques, *Kingella kingae* is being recognized as a common cause of bacteremia and skeletal infections in young children [1–4]. Despite this increasing interest in the organism, the epidemiology of *K. kingae* remains incompletely understood. In recent years, the prevalence of *K. kingae* in the respiratory tract was studied in a population of children attending a day care center (DCC) in southern Israel [5]. To investigate the possibility of person-to-person transmission of *K. kingae,* organisms isolated from the DCC attendees were studied by three different typing methods and compared with a collection of epidemiologically unrelated organisms.

Materials and Methods

Background. Tonsillar cultures were obtained every 2 weeks during the 11-month period of September 1993 through July 1994 from 2 cohorts of DCC attendees [5]. Inoculated swabs were plated onto blood agar and onto a selective vancomycin-containing medium especially designed to enhance the recovery of *K. kingae* [6]. Overall, *K. kingae* was recovered from 109 (18%) of 624 cultures, and 34 (71%) of 48 children carried *K. kingae* at least once. Sporadic, continuous, and intermittent patterns of carriage were observed. None of the DCC attendees had an invasive *K. kingae* infection during the study period.

Bacterial isolates. A single *K. kingae* colony recovered in the primary pharyngeal culture was subcultured on blood-agar medium and kept frozen at −70°C for further testing. Three years after the original isolation, 50 (47%) of 109 DCC isolates could be retrieved.

In addition, a collection of 60 *K. kingae* organisms isolated from individuals who were not epidemiologically related to the day care facility was also studied. This group comprised 18 isolates from asymptomatic respiratory carrier residents of southern Israel, 17 organisms isolated from blood since 1992 in different areas of the country, and 22 organisms isolated from children with septic arthritis or osteomyelitis since the late 1980s, mostly from southern Israel. Three American Type Culture Collection (ATCC) strains of *K. kingae* (respiratory isolate ATCC 23330 and blood isolates ATCC 23331 and ATCC 23332) were also studied.

Immunoblotting. A suspension of 3 formalin-killed *K. kingae* isolates was mixed with complete Freund's adjuvant and injected intracutaneously to rabbits. Animals were reinoculated after 4 and 8 weeks. Blood samples were taken from rabbits before the first inoculation and 2 weeks after each inoculation. Serum from the last blood sampling was used for the immunoblotting procedure.

A whole cell lysate of *K. kingae* organisms was prepared as described [7], and the protein concentration of the suspension was determined. Proteins present in the lysate were resolved by SDS-PAGE, electrophoretically transferred to a cellulose nitrate membrane, and stained with Ponseau S, and the unreacted sites on the nitrocellulose membrane were blocked. The nitrocellulose membrane was incubated with the rabbit antiserum diluted 1:10,000. The blot was then incubated with goat anti–rabbit IgG conjugated to horseradish peroxidase. Reactive bands were visualized using H_2O_2 and 4-chloro-1-naphthol.

Pulsed-field gel electrophoresis (PFGE). DNA of *K. kingae*

Received 26 January 1998; revised 22 July 1998.

The ethics committees of the Soroka Medical Center and the Ministry of Health approved the performance of the study. Informed consent was obtained from parents of the children studied.

Reprints or correspondence: Dr. Pablo Yagupsky, Clinical Microbiology Laboratory, Soroka Medical Center, Beer-Sheva 84101, Israel.

The Journal of Infectious Diseases 1998; 178:1843–6
© 1998 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/98/7806-0047$02.00

Downloaded from https://academic.oup.com/jid/article/178/6/1843/850121 by guest on 24 April 2024

isolates was purified by the method of Maslow et al. [8]. Restriction digestion with *Bgl*II, *Sma*I, and *Spe*I was performed according to the manufacturer's guidelines (Promega, Madison, WI), and restriction fragments were separated in a CHEF DRII PFGE system (Bio-Rad Laboratories, Hercules, CA). Electrophoretic conditions used were 200 V, 14°C, ramp 5–50 s, and 22 h. Restriction patterns were visualized by ethidium-bromide fluorescence. To estimate relatedness among strains, DNA fragments were scored as present or absent for *Bgl*II and *Sma*I restriction digests. Cluster analysis was performed with MEGA software [9] based on the number of fragment differences and the neighbor-joining method, with the results displayed in dendrogram form.

Ribotyping. Genomic DNA of *K. kingae* isolates was extracted by the cetyltrimethylammonium-bromide method. The restriction enzymes *Bsu*15I and *Mlu*I were used to digest the DNA samples. The resulting restriction fragments were separated by electrophoresis, denatured, and transferred to Gene-Screen plus membranes (DuPont, Boston). The membranes were incubated in a prehybridization solution and then hybridized with a probe consisting of a 2.5-kb *Eco*RI-*Hin*dIII restriction fragment of plasmid pKK_{3535}, encoding the 16S rRNA and part of the 23S rRNA of *Escherichia coli* [10]. The probe was radiolabeled by the Rediprime DNA Labeling System (Amersham International, Buckinghamshire, UK) with [α-^{32}P]dCTP (3000 ci/mmol). Membranes were washed with 2× SSC and 1% SDS and exposed to radiographic film.

Results

Western blot results. No reactive bands were visualized with the rabbit preimmune serum, but when the postimmunization serum was used, distinct patterns could be clearly visualized. Overall, six different immunoblotting patterns (hereafter named I, II, III, IV, V, and VI) were observed among the 50 DCC isolates that could be retrieved. Pattern V accounted for 28 (56%) isolates and pattern IV for an additional 15 (30%). The 60 isolates of *K. kingae* from DCC-unrelated sources showed remarkable variability, and 14 distinct immunoblotting patterns were identified, including 4 that were also found among the DCC isolates (II, IV, V, and VI). Overall, 8 different patterns could be distinguished among the 19 respiratory isolates derived from DCC-unrelated carriers, 7 among the 19 blood isolates, and 10 among 22 organisms isolated from skeletal system infections.

PFGE results. Restriction digestion of individual isolates with *Bgl*II and *Sma*I produced 2–6 and 4–6 restriction fragments, respectively. The number of fragments of unique size among all isolates tested was 13 for *Bgl*II and 15 for *Sma*I. *Spe*I produced more complex patterns of 15–25 fragments, but individual fragments were not sufficiently separated to score presence or absence with certainty. Each unique pattern produced by a single restriction enzyme is designated by a capital letter, and the combined results of the three restriction enzymes are listed by three letters, where the first letter refers to *Bgl*II, the second to *Sma*I, and the third to *Spe*I results. Five different patterns were present among the DCC isolates, with combinations GBA and ACB accounting for 28 and 13 isolates, respectively. Thirty-two distinct patterns were noted among the 60 isolates from individuals who did not attend the DCC, 13 among 19 respiratory carriers, 14 among the 19 blood isolates, and 14 among 22 organisms isolated from patients with skeletal infections. Bootstrap analysis of dendrograms supported relatedness for 5 groups: those with *Bgl*II patterns B, F, G, E, and D, with no evidence for relatedness across the groups (figure 1).

Ribotyping results. Restriction of DNA isolates from the DCC with *Bsu*15I revealed 5 different patterns, and restriction with *Mlu*I resulted in 4 patterns and a total of 5 combinations of both results among organisms isolated from DCC attendees. Each unique pattern produced by a single restriction enzyme is designated by a lowercase letter, and the combined results of the two restriction enzymes are listed by two letters, where the first letter refers to *Bsu*15I and the second to *Mlu*I results. Ribotyping of the 60 isolates from DCC-unrelated sources showed 12 patterns with *Bsu*15I, 13 patterns with *Mlu*I, and 17 different combinations of both ribotyping results. Ten combinations were found among isolates recovered from respiratory carriers, and 9 combinations each among blood and skeletal system isolates.

Combined typing results. Although, in general terms, PFGE was the most discriminative method, immunoblotting was more sensitive for typing DCC isolates and distinguished 3 different patterns (I, II, and III) among organisms exhibiting identical BDD profiles by PFGE. On the other hand, ribotyp-

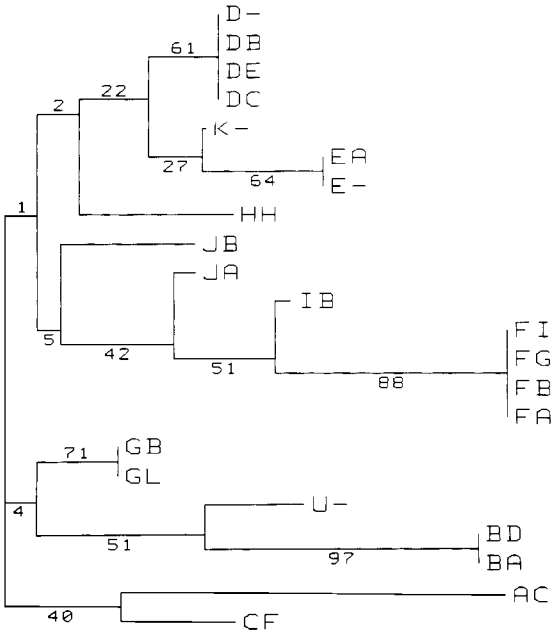

Figure 1. Dendrogram formed by cluster analysis with neighbor-joining method and subjected to bootstrap analysis. Nos. indicate percentage of 500 replicated dendrograms that support each cluster. Distance matrix is based on no. of *Bgl*II and *Sma*I DNA fragment differences.

Downloaded from https://academic.oup.com/jid/article/178/6/1843/850121 by guest on 24 April 2024

ing distinguished two profiles (dd and ed) among organisms with the IV and GBA configurations by immunoblotting and PFGE, respectively.

When isolates showing identical immunoblotting, PFGE, and ribotyping profiles were considered to represent the same strain, 8 different strains (I/BDD/aa, II/BDD/aa, III/BDD/aa, IV/ACB/cc, IV/D-E/cc, V/GBA/dd, V/GBA/ed, and VI/JAC/bb) could be identified among DCC isolates, and 2 strains combined (IV/ACB/cc and V/GBA/dd) represented 37 (74%) of 50 isolates studied. The 60 non-DCC isolates showed much variability, and 43 different strains could be recognized, including a few strains indistinguishable from those isolated in the DCC. Overall, 17 strains were identified among 19 respiratory carriers, 15 strains among 19 isolates from patients with bacteremia, and 17 among the 22 isolates from patients with skeletal infections. The patterns observed among ATCC strains of *K. kingae* were as follows: ATCC 23330, XVI/GBX/ih; ATCC 23331, VII/DBY/rr; and ATCC 23332, V/JBZ/ee.

It should be pointed out that 2 isolates recovered on the same day from pharyngeal cultures of 2 young siblings were identical by the three typing methods and exhibited the VIII/K-O/gg combination. No particular strain was overrepresented among organisms derived from the same geographic area or isolated during the same period, nor from patients with similar clinical conditions.

Strains of *K. kingae* isolated from the DCC population showed a clear temporal clustering (figure 2). Strains I/BDD/aa and II/BDD/aa were isolated at the end of 1993 and beginning of 1994; strain IV/ACB/cc was first isolated in December 1993 and spread through cohort A until March 1994, when it was almost entirely replaced by strain V/GBA/dd. This strain disseminated throughout the facility and was the prevailing organism in both cohorts until the end of the study period. A close strain, V/GBA/ed, was also isolated at the end of the follow-up period from 2 children. Strain VI/JAC/bb was isolated from 2 different attendees, and strains III/BDD/aa and IV/D-E/cc were isolated only once.

Individual children acquired and carried a *K. kingae* strain for weeks or months, when it was replaced by a new one. In a few children, best exemplified by attendee number 17, multiple strain shifts occurred in the course of the year.

Discussion

Colonization of the upper respiratory tract by pathogenic bacteria such as *Streptococcus pneumoniae* or *Haemophilus in-*

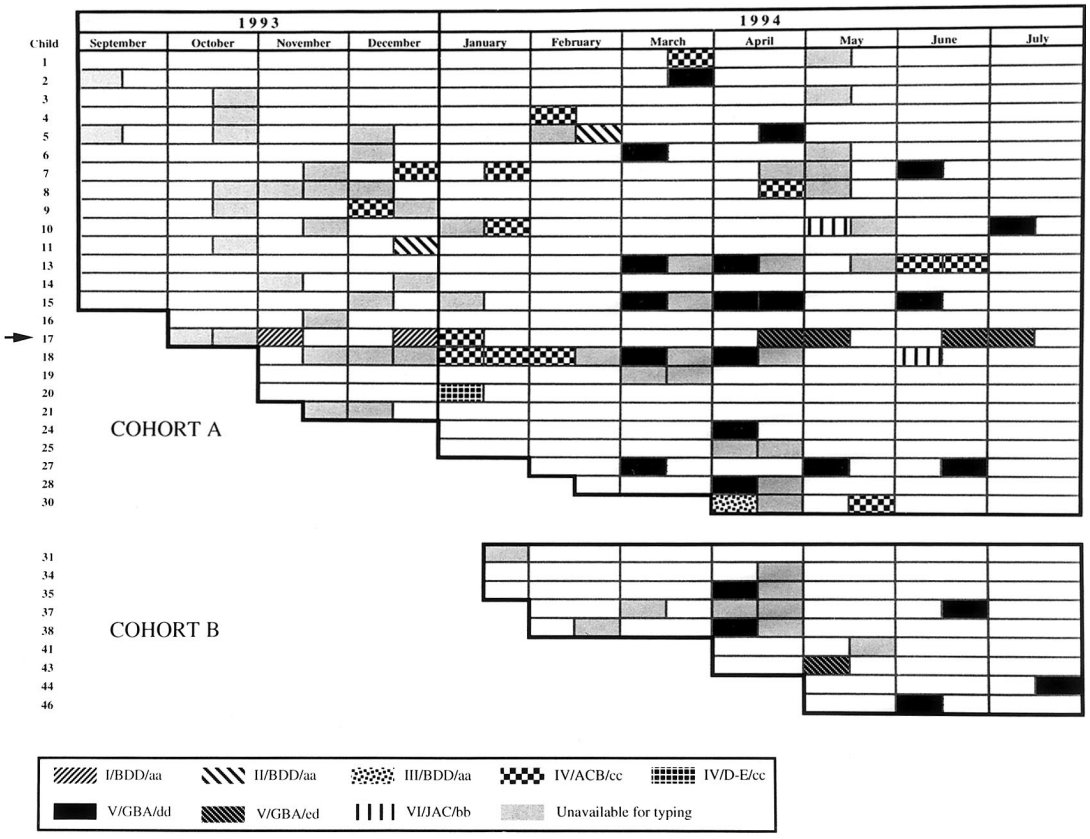

Figure 2. Longitudinal isolation of *K. kingae* strains in day care facility during study period. Arrow points to attendee no. 17, from whom 3 different strains were sequentially identified during study period.

Downloaded from https://academic.oup.com/jid/article/178/6/1843/850121 by guest on 24 April 2024

fluenzae type b usually precedes invasion of the blood and seeding to remote sites [11]. Prolonged carriage of these organisms in the pharynx without symptoms of disease may also occur, and the respiratory mucosal surface constitutes the reservoir from which these pathogens can be transmitted to susceptible hosts [12]. Although *K. kingae* is known to be a component of the normal respiratory flora, the person-to-person transmission of the organism has not been previously studied. The rather limited biochemical repertoire of the species and the uniform antibiotic susceptibility pattern exhibited by *K. kingae* organisms do not allow differentiation between strains on the basis of simple phenotypic markers [5, 13].

The results of the present study show that *K. kingae* organisms can be adequately typed by immunoblotting, PFGE, and ribotyping techniques. The three methods proved to be reproducible and showed excellent correlation, enabling recognition of several distinct strains. Epidemiologically unrelated *K. kingae* organisms, collected over a prolonged period of time from patients and healthy carriers living in different locations, showed remarkable heterogeneity. No particular strains were clearly associated with either respiratory carriage outside the DCC or with specific clinical conditions such as bacteremia, osteomyelitis, or septic arthritis. In contrast, isolates from a large fraction of DCC attendees and the 2 isolates from members of the same family were identical, supporting person-to-person transmission of *K. kingae* among young children.

Overall, a limited number of strains circulated in the day care facility during the 11-month study. Strains IV/AC/cc and V/GBA/dd were particularly successful colonizers and accounted for the vast majority of typed organisms, whereas, in general, other strains did not persist in individual hosts over time and were not able to disseminate and become endemic in the facility. In addition, organisms that differed slightly from the prevalent strains were also recovered, suggesting that subtle genomic changes occur over time, resulting in gradual divergence of *K. kingae* strains.

Longitudinal follow-up indicated that resident organisms, simultaneously carried by multiple DCC attendees, were usually replaced by new strains after weeks or months, whereas reacquisition of a previously carried strain did not occur. Because less than half of the DCC organisms isolated in the original study were still viable after 3 years, and only a single *K. kingae* colony from each positive pharyngeal culture was selected for cloning and storage, neither unrecognized persistence of "old" strains nor colonization of individual children by multiple strains can be definitely excluded. The time clustering of strains observed rather than the random distribution of profiles, however, suggests eradication or at least quantitative reduction of carriage of individual strains over time.

The close contact between young DCC attendees who lack appropriate hygienic behavior and who are immunologically naive has been shown to facilitate enhanced person-to-person transmission of members of the respiratory flora [12, 14]. In addition, pneumococci or *H. influenzae* type b isolates carried simultaneously by DCC attendees are frequently identical, and the prevailing organisms are replaced by new strains after variable periods of time [12, 15]. The results of the present study indicate that *K. kingae,* once considered an exceptional human pathogen, is frequently carried and transmitted from child-to-child in the day care setting. This carriage is a dynamic process with frequent turnover of strains, similar to that observed among other pathogens of respiratory origin.

References

1. Claesson B, Falsen E, Kjellman B. *Kingella kingae* infections: a review and a presentation of data from 10 Swedish cases. Scand J Infect Dis **1985**; 17:233–43.
2. deGroot R, Glover D, Clausen C, Smith AL, Wilson CB. Bone and joint infections caused by *Kingella kingae:* six cases and review of the literature. Rev Infect Dis **1988**; 10:998–1004.
3. Goutzmanis JJ, Gonis G, Gilbert GL. *Kingella kingae* infection in children: ten cases and review of the literature. Pediatr Infect Dis **1991**; 10:677–83.
4. Yagupsky P, Dagan R, Howard CB, Einhorn M, Kassis I, Simu A. Clinical features and epidemiology of invasive *Kingella kingae* infections in southern Israel. Pediatrics **1993**; 92:800–4.
5. Yagupsky P, Dagan R, Prajgrod F, Merires M. Respiratory carriage of *Kingella kingae* among healthy children. Pediatr Infect Dis J **1995**; 14: 673–8.
6. Yagupsky P, Merires M, Bahar J, Dagan R. Evaluation of a novel vancomycin-containing medium for primary isolation of *Kingella kingae* from upper respiratory tract specimens. J Clin Microbiol **1995**; 31:1426–7.
7. Sambrook J, Fritsch EF, Maniatis F, eds. Molecular cloning. A laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, **1989**:18.47–.75.
8. Maslow JN, Slutsky AM, Arbeit RD. Application of pulsed-field gel electrophoresis to molecular epidemiology. In: Persing DH, Smith TF, White TJ, eds. Diagnostic molecular microbiology principles and practices. Washington, DC: American Society for Microbiology, **1993**:563–72.
9. Sudhir K, Tamura K, Nei M. MEGA: molecular genetic evolutionary analysis. Version 1.01. University Park, PA: The Pennsylvania State University, **1993**.
10. Brosius I, Ullrich A, Raker MA, et al. Construction and fine mapping of recombinant plasmids containing the rnB ribosomal RNA operon of *Escherichia coli.* Plasmid **1981**; 6:112–8.
11. Aniansson G, Alm B, Andersson B, et al. Nasopharyngeal colonization during the first year of life. J Infect Dis **1992**; 165(suppl 1):S38–42.
12. Arnold KE, Leggiandro RJ, Breiman RF, et al. Risk factors for carriage of drug-resistant *Streptococcus pneumoniae* among children in Memphis, Tennessee. J Pediatr **1996**; 128:757–64.
13. Holmes B, Pickett MJ, Hollis DG. Unusual gram-negative bacteria, including *Capnocytophaga, Eikenella, Pasteurella,* and *Streptobacillus.* In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology. Washington, DC: American Society for Microbiology, **1995**:499–508.
14. Holmes SJ, Morrow AL, Pickering LK. Child-care practices: effects of social change on the epidemiology of infectious diseases and antibiotic resistance. Epidemiol Rev **1996**; 18:10–28.
15. Harabuchi Y, Faden H, Yamanaka N, et al. Nasopharyngeal colonization with nontypeable *Haemophilus influenzae* and recurrent otitis media. J Infect Dis **1994**; 170:862–6.

Downloaded from https://academic.oup.com/jid/article/178/6/1843/850121 by guest on 24 April 2024