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Kingella kingae: Carriage, Transmission, and Disease

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SUMMARY

Kingella kingae is a common etiology of pediatric bacteremia and the leading agent of osteomyelitis and septic arthritis in children aged 6 to 36 months. This Gram-negative bacterium is carried asymptomatically in the oropharynx and disseminates by close interpersonal contact. The colonized epithelium is the source of bloodstream invasion and dissemination to distant sites, and certain clones show significant association with bacteremia, osteoar-

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thritis, or endocarditis. *Kingella kingae* produces an RTX (repeat-in-toxin) toxin with broad-spectrum cytotoxicity that probably facilitates mucosal colonization and persistence of the organism in the bloodstream and deep body tissues. With the exception of patients with endocardial involvement, children with *K. kingae* diseases often show only mild symptoms and signs, necessitating clinical acumen. The isolation of *K. kingae* on routine solid media is suboptimal, and detection of the bacterium is significantly improved by inoculating exudates into blood culture bottles and the use of PCR-based assays. The organism is generally susceptible to antibiotics that are administered to young patients with joint and bone infections. β-Lactamase production is clonal, and the local prevalence of β-lactamase-producing strains is variable. If adequately and promptly treated, invasive *K. kingae* infections with no endocardial involvement usually run a benign clinical course.

INTRODUCTION

In the 1960s Elizabeth O. King, working at the U.S. Centers for Disease Control (CDC) in Atlanta, GA, described a novel bacterial species isolated from human respiratory secretions, blood, and bone and joint exudates (1). The organism, initially assigned to the genus *Moraxella* and designated *Moraxella kingii* in honor of King's seminal research, was later placed in a separate genus and renamed *Kingella kingae* (2).

The interest in *K. kingae* was initially limited (3–10), and only 55 reports on the organism were published in the medical literature until 1990. The number, however, has sharply increased in recent years, jumping to 105 in the following decade and 83 in the short period from January 2010 through August 2014 (determined by a PubMed search with "*Kingella kingae*" and "*Moraxella kingii*"), and has firmly established the status of *K. kingae* as a common agent of bacteremia with no focus (also called occult bacteremia) (11, 12) and the predominant etiology of joint and bone infections in 6- to 36-month-old children (13–16). This increasing detection of *K. kingae* does not indicate that the bacterium is really a novel human pathogen. It appears, rather, than this rapidly enlarging body of information is the result of improvement in detection methods, particularly the observation that inoculation of skeletal system exudates into blood culture vials (BCVs) enhances the isolation of the organism (17), and the development and increasing usage of sensitive nucleic acid amplification (NAA) assays (16).

The vast majority of publications on *K. kingae* infections have originated in countries in the developed world, particularly the United States, France, Switzerland, and Israel (18), whereas reports from the developing world are still scarce (19–25), probably reflecting the unavailability of expensive automated blood culture systems and NAA technology needed for detecting the bacterium in resource-poor countries.

Coinciding with the recognition of *K. kingae* as a major cause of pediatric disease, the growing attention of the scientific community to this pathogen has resulted in the rapid accumulation of knowledge in the fields of the epidemiology, pathogenesis, diagnosis, and treatment of invasive *K. kingae* infections (Fig. 1), which are the subject of this review.

BACTERIOLOGY

Taxonomy

The genus *Kingella* belongs to the *Neisseriaceae* family in the beta subclass of the *Proteobacteria* and comprises four recognized species: *K. denitrificans*, which has been implicated in cases of bacteremia, endocarditis, pleural empyema, pediatric vaginitis, chorioamnionitis, and granulomatous disease in AIDS patients (26–29); *K. oralis*, which is a commensal dweller of the human buccal cavity and is associated with dental plaque and periodontitis (30, 31); *K. potus*, a zoonotic organism recovered from an infected bite (32); and *K. kingae*. Although *K. kingae*'s taxonomic place remained uncertain for many years (1, 2), subsequent analysis of its biochemical profile and fatty acid composition (33–39) and genotypic studies (33, 40–44) have led to the conclusion that *K. kingae* constitutes a separate species, only distantly linked to other *Neisseriaceae* (44).

Identification

Kingella kingae appears as pairs or chains of 4 to 8 plump (0.6 to 1 μm by 1 to 3 μm) coccobacilli (Fig. 2A). *Kingella kingae* cells tend to resist decolorization, and thus the organism may be erroneously identified as Gram positive (45), but electron microscopic examination discloses a characteristic Gram-negative cell wall structure. The bacterium is beta-hemolytic, nonmotile, and nonspore forming, exhibits negative catalase, urease, and indol tests, and, with rare exceptions, has oxidase activity. *Kingella kingae* produces acid from glucose and usually from maltose (46, 47), hydrolyzes indoxyl phosphate and L-prolyl-β-naphthylamide, and exhibits positive alkaline and acid phosphatase reactions. The fatty acid content of the organism resembles that of *K. denitrificans* and comprises a high percentage of myristic acid and lesser concentrations of palmitic, lauric, palmitoleic, linoleic, oleic, 3-hydroxilauric, 3-hydroximyristic, and *cis*-vaccenic acids (35, 36).

The identification of *K. kingae* in the clinical microbiology laboratory can be accomplished with a wide array of commercial systems, such as the quadFERM+ kit (48), API NH card, Vitek 2 instrument (49, 50), matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) (50, 51), and 16S rRNA gene sequencing (52), but the organism is completely misidentified by the Remel RapID NH kit (50).

Culture

Kingella kingae is a facultative anaerobic bacterium that grows on conventional Trypticase-soy agar supplemented with 5% hemoglobin (blood agar medium), chocolate agar, Columbia-based blood agar (53), and GC-based media. Similar to the case for other *Neisseriaceae*, most *K. kingae* strains can be recovered on Thayer-Martin medium but do not develop on MacConkey or Krigler agar. Many isolates show poor growth on the cation-supplemented Mueller-Hinton medium used to determine the antibiotic susceptibility of the species. Similar to the case for other bacteria that inhabit the respiratory tract, growth is improved in a 5% CO_2 atmosphere, but only a minute proportion of isolates are strictly capnophilic (18).

Growth on solid media is characterized by marked pitting of the agar surface, which is best seen after removal of the colony (34). *Kingella kingae* strains produce three different colony types that are associated with the degree of pilus expression: a spreading-corroding morphology distinguished by a small central colony encircled by a wide fringe, a nonspreading/noncorroding type consisting of a flat colony surrounded by a narrow fringe, and a dome-shaped colony with no noticeable fringe. The first two morphologies are associated with the presence of long fimbriae, whereas strains growing as domed colonies are nonpiliated

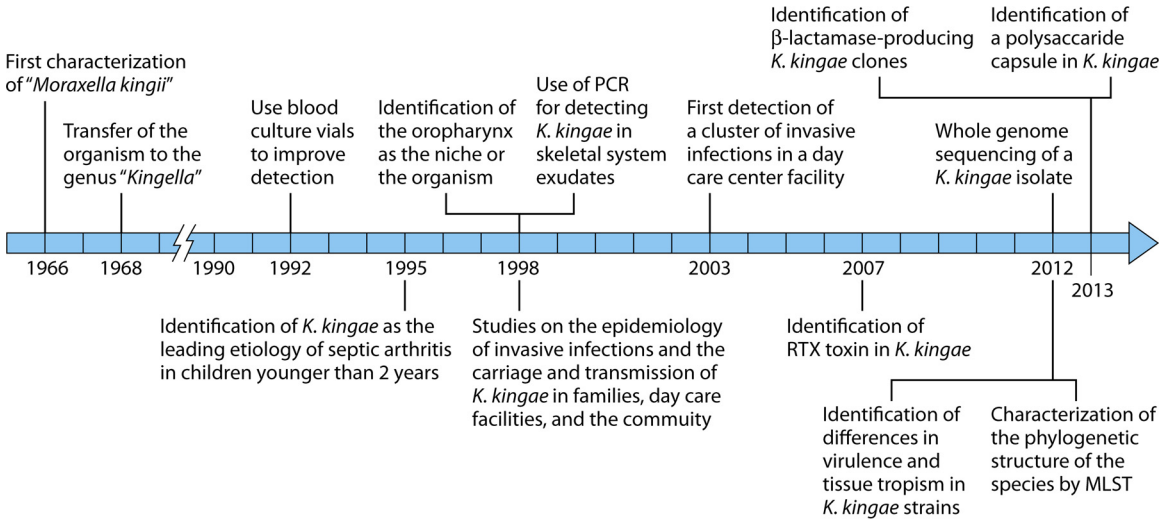

FIG 1 Timeline of milestone research studies on *K. kingae*.

(54–57). The ability to produce the spreading-corroding type of morphology can be irreversibly lost after repeated subculture (47).

Small-Colony Variants

In recent years, small-colony variants (SCVs) of *K. kingae*, characterized by growth as pinpoint colonies on blood agar plates and no growth on Thayer-Martin medium, have been isolated in approximately 15% of healthy pharyngeal carriers in southern Israel (58). These strains frequently fail to produce acid from maltose and tend to form atypical long chains of coccobacilli, indicating impaired cell separation (Fig. 2B). Although SCVs detected in other bacterial pathogens such as *Staphylococcus aureus* or *Pseudomonas aeruginosa* are commonly associated with chronic or difficult-to-eradicate infections, *K. kingae* SCVs have been only exceptionally isolated from patients with invasive disease, suggesting decreased virulence. Reversion to a rapidly growing phenotype, but without modification of the long-chain configuration, is obtained by seeding *K. kingae* SCVs on GC-based medium, but not on chocolate agar, implying a defect in electron transport, menadione metabolism, or thymidine uptake (59). *Kingella kingae* cultures in general, and SCVs in particular, should be subcultured frequently (every 2 to 3 days) to keep the organism viable.

The SCVs of *K. kingae* belong to several pulsed-field gel electrophoresis (PFGE) clones, of which clone T is the most commonly carried by the healthy pediatric population in southern Israel and is associated with β-lactamase production (60). Although clone T isolates are readily identified as *K. kingae* by MALDI-TOF technology and share alleles of the *rtxA* and *por* genes (encoding the RTX toxin and the porin protein, respectively) found in some other *K. kingae* clones, attempts to type clone T strains by multilocus sequence typing (MLST) failed, because even when diverse couples of primers were employed, amplification succeeded for only three housekeeping genes (*abcZ*, *adk*, and *cpn*60) (61). In addition, examination of the 16S rRNA gene sequence indicates that clone T isolates share only 96.9% nucleotide identity with the ATCC 23330 type strain (61). Taken together, these genomic findings suggest that clone T is only remotely linked to other *K. kingae* clones and probably represents a separate clade or quasispecies (61).

GENOMICS

Comparison of the genomes of 22 *K. kingae* isolates from asymptomatic carriers and 28 derived from patients with different infections and diverse geographic origins revealed that the genome size of the species ranges between 1,990,794 bp and 2,096,758 bp, com-

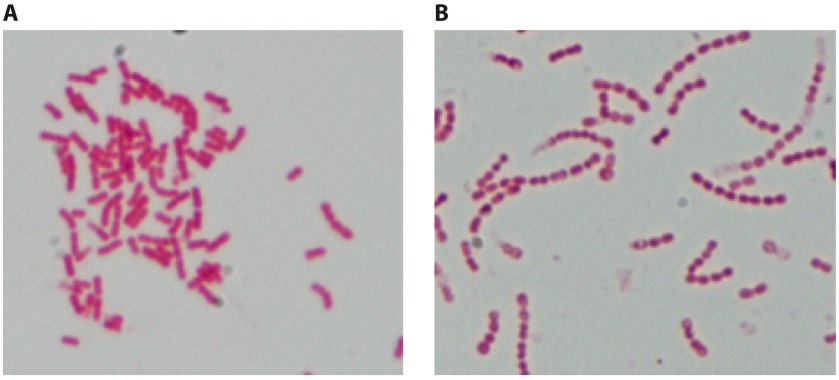

FIG 2 (A) Typical Gram stain of *K. kingae* organisms, depicting short Gram-negative coccobacilli with tapered ends arranged in pairs or short chains. (B) Gram stain of *K. kingae* small-colony variant, showing atypical long chains.

prises 1,981 to 2,300 protein-encoding genes and between 43 and 52 RNA genes, and has a GC content between 46.8% and 46.9% (62, 63; L. Rouli, unpublished data).

Genomic studies in which *K. kingae* isolates from different geographic origins were studied by MLST of 6 housekeeping genes (*abcZ*, *adk*, *aroE*, *cpn60*, *gdh*, and *recA*) demonstrated that the species displays notable genomic variability, and to date, 64 MLST sequence types (STs) comprising strains sharing identical alleles combinations, 74 PFGE clones, and 18 *rtxA* and 11 different *por* alleles have been identified in the species (61, 64, 65). Three unrelated MLST sequence type complexes (STCs) comprising strains that differ at ≤1 locus from at least one other member of the group, namely, STC-6, STC-14, and STC-23, have been isolated from healthy individuals and patients with *K. kingae* disease in North America, Iceland, continental Europe, Australia, and Israel for over 2 decades, whereas other STCs show a more restricted temporal and spatial distribution or appear to be country specific and sporadic (61, 64, 65).

Similarly to other members of the *Neisseriaceae* family, *K. kingae* organisms are naturally transformable, and this phenomenon represents a potent driving force of genetic diversity. The uptake of exogenous DNA in *Neisseriaceae* is finely regulated by DNA uptake sequences (DUS), which facilitate transformation by homologous DNA and discriminate against horizontal transfer of heterologous and possibly harmful genomic sequences (66). DUS are short (12-nucleotide) DNA sequences which are present in the genome in multiple copies, consisting of a conserved 5′-CTG-3′ core flanked by variable sequences, resulting in DUS variants named "dialects" (66). Integrity of the core is strictly required for transformation, whereas the grade of genetic resemblance between the dialects of the donor and recipient organisms correlates with the efficiency of the transformation process (66). The DUS are, therefore, efficient obstacles to between-species recombination, contributing to the genetic stability and sexual isolation of the species.

Examination of *K. kingae* strain ATCC 23330 revealed that a DUS named king3DUS with the sequence 5′-AAGCAGAGCCTG CA-3′ is present in 2,787 copies in the genome (66). An identical sequence was identified in 3,603 copies in the taxonomically close *K. denitrificans*, which differs from the DUS detected in *K. oralis* and in other *Neisseriaceae* (66). Given that the genome size of *K. kingae* is approximately 2 Mb (62, 63), DUS should be randomly repeated, on average, every 500 to 1,000 bp. Because the number of DUS and their chromosomal location vary between strains, a novel PCR assay targeting this highly ubiquitous sequence was developed to investigate the genomic polymorphism of the species (65). The test amplifies sequences located between two consecutive DUS, resulting in multiple DNA bands that are unique and differ in length and thus can be separated in an electrophoretic gel. In an innovative study, DUS typing results showed excellent correlation with those obtained with the more costly and labor-intensive MLST method (65).

Despite the transformation competency of *K. kingae*, almost perfect correlation has been found between the different genotyping methods, disregarding the isolates' clinical, temporal, or geographic source, and strains are usually characterized by a unique combination of PFGE profiles and consistent allele content of housekeeping, *rtxA*, and *por* genes (61, 64, 65). For instance, all PFGE clone A isolates studied so far belong to the MLST STC-34 and share *rtxA* allele 3 or 13 and the *por* 4 allele, PFGE clone ψ organisms belong to MLST STC-29 and harbor the *rtxA* 4 and *por* 12 alleles, and PFGE clone K isolates belong to MLST STC-6 and possess the closely related *rtxA* 8 or 9 allele and the *por* 1 allele (61, 64, 65). It should be noted that these different genotyping methods investigate different sections of the bacterial genomic content: the PFGE approach randomly probes the entire genome, MLST explores a tiny fraction of the core genome that encodes critical metabolic enzymes and, therefore, evolves at a slow pace, and *rtxA* and *por* sequencing analyzes putative *K. kingae* virulence factors which are exposed to the host's immune response and undergo frequent genetic changes (67). The remarkable linkage disequilibrium disclosed by these diverse methods thus is striking and implies that *K. kingae* strains comprise idiosyncratic and seemingly quite homogeneous bacterial populations. It is postulated that circulating *K. kingae* strains are subjected to positive selection and resist the disrupting effect of recombination because they harbor favorable genetic traits and experience clonal expansion, explaining their extensive geographic dissemination and genetic stability over time (68).

MECHANISMS OF COLONIZATION AND VIRULENCE FACTORS

Pili

To colonize the human mucosae, bacteria first have to anchor to epithelial surfaces and tissues, and many pathogens express adhesive proteinaceous appendages to avoid being washed out. The presence of fimbriae was detected in *K. kingae* as early as 1978 (55), and in 1992, Weir and Marrs, using electron microscopy, DNA transformation patterns, and immunoblotting, convincingly demonstrated that these fibers are type 4 pili (56). In a series of elegant studies performed in the last decade, St. Geme and coworkers showed that *K. kingae*'s pili are essential for the adherence of the bacterium to the respiratory epithelium and synovial layer (69). The investigators disclosed a chromosomal gene cluster homologous to that found in other Gram-negative organisms, consisting of a *pilA1* gene that encodes the major pilin subunit and *pilA2* and *fimB* genes of unknown function that appear not to be necessary for pilus expression or attachment (69). As is the case for other surface-exposed virulence factors, the PilA subunit exhibits significant between-strain variation in sequence and antibody reactivity, suggesting that it is subjected to selective pressure by the immune system (70). The expression of pili in *K. kingae* appears to be finely regulated by three genes (the σ^{54} gene, *pilS*, and *pilR*) (70), and the majority of colonizing strains, as well as those isolated from individuals with bacteremia, express piliation, whereas those derived from patients with bone, joint, or endocardial infections are nonpiliated (57). This observation suggests that piliation offers a selective advantage in the colonization process and at the early mucosal and bloodstream stages of the disease but is detrimental to the bacterium for invading deep body tissues.

Additional work by the same research group identified two other genes, named *pilC1* and *pilC2*, in physically separated chromosomal locations, that encode homologs of the *Neisseria* PilC proteins, which play an essential role in the adherence and piliation processes (69, 71). The *Kingella kingae* PilC1 and PilC2 proteins have only partial similarity to each other and contain calcium-binding sites, and at least one PilC protein is essential for pilus expression (69, 71). While the PilC1 site is required for twitching motility and adherence, the PilC2 site exerts only a minor effect on

motility and has no functional role in adherence (71). Additional research indicated that a trimeric autotransporter protein called Knh is crucial for firm adherence of *K. kingae* organisms to the epithelium (72). The Knh protein, however, is covered by the bacterial carbohydrate capsule, which renders it inaccessible for attachment to the host cell. Based on the available data, it has been proposed that the adherence process is initiated by attachment of the long pili to their specific membrane receptor on the mucosal surface. This appears to be followed by a strong retraction of the pilus fibers that, by displacing the capsule, enables close contact between the bacterium and the host cell membrane, unmasking the Knh element, which can then anchor to the host's respiratory epithelium (72).

Polysaccharide Capsule

Synthesis of polysaccharide capsules is a convergent evolutionary strategy shared by many important human pathogens that colonize the upper respiratory tract. Capsules are lipid-anchored, outer-membrane-associated, and surface-exposed structures that confer protection from phagocytosis and complement-mediated killing. Capsules are, then, crucial virulence factors that enable bacterial survival on the mucosal surfaces by thwarting the host's defensive response. These tools, originally developed and selected to allow colonization of the respiratory epithelium, also enable survival of encapsulated organisms in the bloodstream and deep body tissues. Bacterial capsules exhibit chemical and antigenic heterogeneity within members of the same species as the result of the highly selective pressure exerted by the immune system, and this diversity is the basis for serotyping strains of organisms such as pneumococci, *Haemophilus influenzae*, and *Neisseria meningitidis*.

It has been recently demonstrated that *K. kingae* is also coated with a polysaccharide capsule (72). A search of the draft genome of the organism detected a locus that showed remarkable similarity to the ABC-type capsule export operon *ctrABCD*, as well as separate gene homologs to the *ctrE/lipA* and *ctrF/lipB* genes of the taxonomically closely related *N. meningitidis* species (43, 72). Wild-type *K. kingae* organisms stained with cationic ferritin exhibited an electron-thick perimeter on the bacterial surface, consisting of an anionic bacterial capsule, when examined by thin-section transmission electron microscopy. When the *ctrA* gene was insertionally inactivated, nonmucoid colonies were visualized and no capsule could be demonstrated (72). Glycoyl analysis of bacterial surface extracts of the invasive *K. kingae* strain 269-492 by gas chromatography under acidic conditions, mass spectrometry, and nuclear magnetic resonance (NMR) revealed that the polysaccharide capsule contains *N*-acetylgalactosamine (GalNAc) and 3-deoxy-D-manno-oct-3-ulosonic acid (Kdo) with a →3)-β-Gal*p*NAc-(1→6)-β-Kdo*p*-(2→ structure, chemically identical to the capsule synthesized by *Actinobacillus pleuropneumoniae* serotype 5 (the causative agent of a contagious lower respiratory tract infection in swine) (73). A second capsular polysaccharide, extracted from a different invasive *K. kingae* strain (PYKK181), exhibited the chemical structure →6)-α-D-GlcNAc*p*-(1→5)-β-D-OclA*p*-(2→ (74), indicating that the *K. kingae* capsule shows chemical and, most probably, also antigenic heterogeneity, similar to the case with other bacterial pathogens. Preliminary results indicate that all *K. kingae* isolates produce a polysaccharide capsule, regardless of their clinical source (asymptomatic carriage or a variety of invasive diseases) (J. W. St. Geme, personal communication). These important findings may shed light on the peculiar age-dependent epidemiological curves of *K. kingae* carriage and disease, because immunity to polysaccharides in humans matures between the ages of 2 and 4 years (75), explaining the increased susceptibility of young children to mucosal colonization and invasive diseases caused by encapsulated organisms in general and *K. kingae* in particular and the reduction in the colonization rate and incidence of invasive infections in immunologically mature older children and adults.

Exopolysaccharides and Biofilm Production

Growth as biofilms is the usual lifestyle for bacteria in most environments and particularly on human body surfaces. Many colonizing species build up biofilms consisting of large quantities of crammed bacteria contained in a polysaccharide "slime" that firmly attaches to the mucosal epithelium. Life in such enclosed and crowded conditions protects the organism from the deleterious effects of the immune response, desiccation, and antimicrobial drugs. Consequently, biofilms play an important role in bacterial adherence and establishment of colonization, as well as in the pathogenesis of persisting and difficult-to-cure infections, such as chronic osteomyelitis or lung disease in cystic fibrosis patients (74, 76). The sequence of biofilm establishment, growth, and architectural remodeling is a precisely regulated and highly dynamic process. Periodic inhibition of biofilm formation is crucial for many pathogens because it makes possible the release of trapped bacterial cells, enabling dispersion and colonization of new body niches and hosts. This complex cycle is usually modulated by secretion of exopolysaccharides that do not remain attached to the bacteria. Bendaoud et al. have shown that *K. kingae* strain PYKK181 synthesizes a linear galactan homopolymer with the structure →3)-β-(1→6)-Galf-(1→ that exerts potent antibiofilm activity upon other bacterial species (74), and Starr et al. found a different exopolysaccharide in strain 269-492 that contains only galactose and has the structure →5)-β-Galf-(1→ (73). Targeted mutagenesis demonstrated that production of *K. kingae*'s capsule and exopolysaccharide involves separate genetic loci for surface location (73).

It is speculated that *K. kingae* exopolysaccharides facilitate colonization of the pharyngeal epithelium by inhibiting biofilm production by other organisms competing for the same niche. It is also plausible that these exopolysaccharides play a role in the regulation of the periodic release of *K. kingae* cells from the biofilm matrix, enabling dissemination of the bacterium by droplet transmission.

RTX Toxin

In a study carried out by Kehl-Fie and St. Geme, it was demonstrated that *K. kingae* organisms exert a potent and wide-spectrum cytotoxic effect, being especially harmful to macrophages, leukocytes from many animal species, synoviocytes, and, to a lesser degree, respiratory epithelial cells (77). Using mariner mutagenesis followed by screening of mutants for loss of cytotoxic activity, a locus encoding an RTX toxin system was detected in the organism's genome (77). The locus consists of 5 genes named *rtxA*, *rtxB*, *rtxC*, *rtxD*, and *tolC*, located in a single cluster in the chromosome, required for the manufacture and secretion of the toxin. The locus is flanked by insertion sequences and has a decreased G+C content compared to the entire *K. kingae* genome, suggesting that, despite the DUS barriers that limit horizontal gene trans-

fer, it has been acquired from a donor bacterial species (77). This possibility is further supported by finding that the *rtxA*, *rtxB*, and *rtxC* genes encode proteins that share >70% identity with their homologs in *Moraxella bovis*, whereas the *rtxD* and *tolC* genes encode proteins that exhibit 81% and 64% identity, respectively, with their *N. meningitidis* counterparts (77). Disruption of the RTX toxin gene *rtxA* by transposon mutagenesis caused loss of cytotoxic activity to cultured cell lines (77). Like the RTX toxins of other bacteria, *K. kingae*'s RTX toxin contains an amino-terminal hydrophobic domain, potential lipidation sites, and a calcium-binding motif (77). *Kingella kingae* RTX toxin is a 100-kDa protein that appears to be secreted in the extracellular environment in a soluble form, as well as a component of outer membrane vesicles (OMVs) that are internalized by host's cells, suggesting that *K. kingae* utilizes multiple mechanisms for toxin release (78).

All carried and invasive *K. kingae* isolates studied so far produce RTX toxin, whereas it is conspicuously absent in the less virulent *K. denitrificans* and *K. oralis*, suggesting that this bacterial constituent is universally conserved in *K. kingae* because it improves colonization fitness by disrupting the oropharyngeal epithelium. Incidentally, the RTX toxin may also enable survival of *K. kingae* in the bloodstream and invasion of the skeletal system tissues (77) and therefore have a disease-promoting effect.

The potential role of *K. kingae* RTX toxin has been further investigated using an animal model of infection in a recent study (79). Intraperitoneal inoculation of 7-day-old Sprague-Dawley rats with 8×10^6 CFU of a virulent *K. kingae* strain isolated from a child with septic arthritis (strain PYKK081) resulted in a marked reduction in the circulating white blood cells (WBCs) compared with the leukocyte count in animals injected with a toxin-deficient mutant of the same wild-type strain (designated KKNB100), suggesting that the depressed acute immune response induced by the RTX toxin may represent a strategy aimed to guarantee *K. kingae*'s subsistence in the host's bloodstream, skeletal tissues, and endocardium (79). Animals inoculated with the wild PYKK081 strain developed a rapidly fatal illness characterized by weight loss, bacteremia, abdominal wall necrotic lesions, and damage to the pups' thymuses, spleens, liver, lungs, and bone marrow, whereas inoculation of the animals with the KKNB100 mutant did not result in clinical disease or demonstrable bacteremia, corroborating the importance of the RTX toxin as a virulence factor and its role in the pathogenesis of disease (79). Remarkably, intraperitoneal or intra-articular inoculation of 21-day-old rats with the wild-type strain did not result in observable disease, bacteremia, or septic arthritis, indicating that the age-dependent susceptibility to invasive infections observed in humans can be closely reproduced in the animal model (79).

Outer Membrane Vesicles

Gram-negative bacteria interact with the surroundings by releasing toxins and other proteins that exert distal effects without the need to expend energy in moving themselves. In addition, secreted material may reach sections of the environment that are inaccessible to the whole bacterium. Frequently, secretion of these bacterial products to the extracellular space is accomplished through formation of small (20- to 250-nm) spherical elements named OMVs. These structures consist of small portions of the outer membrane enclosing and entrapping periplasm proteins that bulge away from the cell, pinch off, and are then released (80) (Fig. 3). Maldonado et al. described blebbing OMVs in clinical *K. kingae* isolates that contain several major proteins, including the RTX toxin and the PilC2 pilus adhesin (78). These OMVs are hemolytic and leukotoxic and are internalized by human osteoblasts and synoviocytes in an *in vitro* model. Upon OMV acquisition, the host cells synthesize large amounts of human granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 6 (IL-6), suggesting that these cytokines may be components of the human inflammatory response to *K. kingae* joint and bone infections (78).

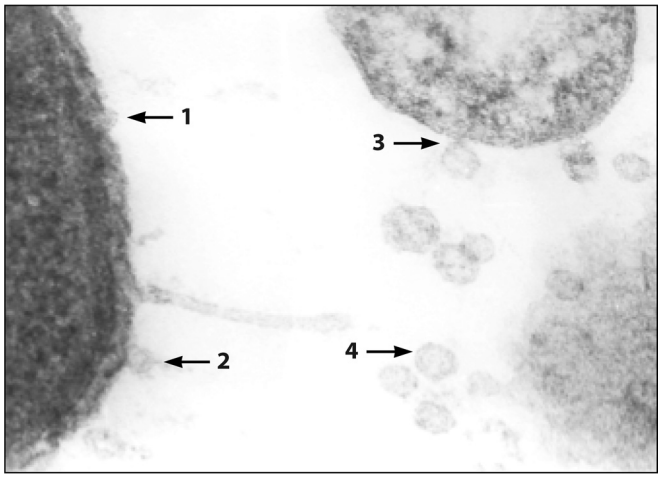

FIG 3 Electron microscope picture of *K. kingae* organisms, showing sequential formation of outer membrane vesicles.

KINGELLA KINGAE CARRIAGE

Colonization Site

The human upper respiratory tract can be colonized by many potential bacterial pathogens, such as the pneumococci, *Moraxella catarrhalis*, or meningococci, that establish a foothold on the body's mucosal surfaces, persist, and spread from person to person. The colonized epithelial surfaces are also the gateway through which virulent bacteria enter the bloodstream, an event that may be followed by dissemination of circulating organisms to remote normally sterile body sites, such as the skeletal or the central nervous system, causing secondary focal infections.

For many years it was suspected that *K. kingae* could also be a member of the residing respiratory tract microbiota, and this assumption was founded on the anecdotal isolation of *K. kingae* from respiratory cultures of healthy individuals (1, 34, 81) and from the blood of patients with pneumonia (18, 82, 83) and the fact that many members of the *Neisseriaceae* family are upper respiratory tract commensals. In a pioneering study, biweekly nasopharyngeal and oropharyngeal cultures were obtained from young attendees of a child care facility over an 11-month period. A total of 109 of 624 (17.5%) oropharyngeal specimens grew *K. kingae*, whereas the organism was not recovered from the nasopharynx (84). These results have been confirmed in a more recent study in which 4,472 oro- and nasopharyngeal specimens were prospectively obtained from a cohort of 716 healthy children. A total of 388 (8.7%) oropharyngeal cultures but only a single nasopharyngeal culture grew *K. kingae*, indicating that the organism occupies a restricted upper respiratory tract niche (85).

Culture Detection of *K. kingae* in the Pharynx

Due to the extraordinary complexity and high concentration of the colonizing bacterial flora, culture detection of *K. kingae* in throat specimens poses obvious difficulties. A differential and selective medium combining blood agar with 2 μg/ml of added vancomycin (BAV medium) has been designed to facilitate identification of the organism in respiratory cultures (86). The rationale behind this formulation is to ease the identification of beta-hemolytic *K. kingae* colonies by inhibiting the Gram-positive flora. In a blinded study, BAV plates recovered *K. kingae* in 43 of 44 (97.7%) oropharyngeal cultures, compared to only 10 (22.7%) specimens seeded onto petri dishes containing plain blood agar ($P < 0.001$). The original BAV medium and similar media (87) have been successfully employed in epidemiological studies aimed to investigate the respiratory carriage of the organism (18, 58, 84, 85). If not plated immediately, inoculated swabs should be kept at room temperature in Amies or similar transport medium and promptly sent to the laboratory for further processing (84).

Detection by Molecular Methods

In recent years, novel molecular detection assays have enabled diagnosis of *K. kingae* infections in patients for whom cultures of joint exudates on routine media and in BCVs did not reveal the presence of the bacterium (88). This PCR-based strategy has been also implemented in investigations of *K. kingae*'s carriage and the relationship between respiratory colonization and clinical disease.

Because the RTX toxin is produced by all *K. kingae* strains examined so far, the encoding RTX locus genes appear to be appropriate targets for detecting the organism in the blood, synovial fluid, and solid tissues, as well as in upper respiratory tract specimens (87–90). It has been shown that NAA assays targeting conserved segments of the RTX toxin-encoding genes (*rtxA* and/or *rtxB*) are able to detect as few as 30 CFU of the organism and therefore are more sensitive than PCR tests that amplify the 16S rRNA gene (88, 90, 91) or the *cpn60* gene (90). The NAA assays targeting the RTX toxin genes are highly specific, can be applied to a variety of clinical specimens, and allow detection of strains exhibiting *rtx* locus polymorphisms (87–90, 92).

Employing a real-time PCR assay that amplifies portions of the *rtxA* and *rtxB* genes, Ceroni et al. detected *K. kingae* DNA sequences in the oropharynges of 8.1% of 431 young asymptomatic Swiss children and in all 27 patients with *K. kingae* osteoarticular infections confirmed by an NAA of the bone or joint exudates (93). More recently, the same research group found specific *rtxA* gene sequences in the oropharynges of all 10 children aged 6 to 48 months with a roentgenologically confirmed diagnosis of spondylodiscitis, suggesting that *K. kingae* was the causative agent (94). In a study comprising 123 patients younger than 4 years with skeletal system complaints, the DNA detection assay performed on an oropharyngeal specimen was positive in all 30 children with *K. kingae* osteoarthritis as proven by culture and/or NAA of the synovial fluid (95). In the same study, 8 oropharyngeal samples, derived from 84 patients with microbiologically unconfirmed joint or bone infections or with skeletal infections caused by other bacteria, were also positive for *K. kingae* DNA sequences (95), reflecting the background respiratory carriage of the organism in the young pediatric population (85). The practical implications of these results are that the sensitive NAA tests have a high negative predictive value and that failure to detect *K. kingae*-specific genomic sequences in a pharyngeal specimen may practically exclude the organism as the etiology of an osteoarticular infection. However, because *K. kingae* is carried on the pharynx by approximately 10% to 12% of children in the relevant age group (85) and by >25% of those in day care (84), the predictive value of a positive pharyngeal NAA for diagnosing an invasive infection is limited.

In a refined study, Basmaci et al. grew *K. kingae* in the pharyngeal cultures of 8 of 12 NAA-positive/culture-negative synovial fluid specimens of young children with arthritis (87). The researchers succeed in extracting and sequencing the *rtxA* gene amplicons from 6 PCR-positive synovial fluid samples and compared them with the *rtxA* sequences of the pharyngeal isolates. The 6 paired pharyngeal and synovial fluid amplicons were found to contain identical sequences, establishing a firm link between *K. kingae* organisms colonizing the pharynx and those invading the skeletal tissues (87).

More recently, a real-time PCR test that amplifies the toxin-encoding *rtxB* gene was employed to assess the bacterial load in asymptomatic pharyngeal carriers and children in whom the diagnosis of *K. kingae* joint or bone diseases was confirmed by a positive PCR test performed in either blood or skeletal system exudate (96). The number of amplification cycles required to obtain a positive result was used as a surrogate for colonization density. Contrary to what has been observed in infections induced by *Streptococcus pneumoniae* or *H. influenzae* type b, no quantitative differences in bacterial density between healthy carriers and sick children were found, suggesting that other factors are more important for the development of an invasive *K. kingae* infection (96). Using the same approach, it was shown that the bacterial density remains remarkably stable in colonized children aged 8 months to 4 years, despite substantial differences in the risk to develop a *K. kingae* infection during this wide age interval (97).

The sensitivities of cultures and NAA assays for detecting *K. kingae* colonization have been compared in a single study in which the yields of both approaches were assessed during the investigation of a large cluster of invasive diseases caused by the organism in a Paris day care center (98). Overall, 12 of 18 pharyngeal specimens were positive by real-time PCR, compared to 6 of 18 positive by culture on modified BAV medium ($P < 0.01$), suggesting that NAA assays are more sensitive than cultures to determine the true carriage rate. It should be noted, however, that when multiple strains are found to be circulating in the population, the culture approach has the advantage of enabling a comprehensive genomic comparison of recovered isolates. In addition, cultures detect living organisms, while the viability of *K. kingae* cells in clinical samples positive by NAA assays but negative by culture is uncertain (98). This aspect could be important when assessing the effectiveness of antibiotic prophylaxis to reduce pharyngeal *K. kingae* carriage.

Epidemiology of *K. kingae* Carriage

Because bacterial colonization of the mucosal surfaces is established at the interface between humans and their surroundings, the forces that shape the composition, acquisition, and elimination of residing organisms reflect a complex array of intermingling host, microbial, environmental, and socioeconomic determinants. Factors such as sampling season, the population's age and health status, crowding of living quarters, number of young children at home, day care attendance, antibiotic consumption, and

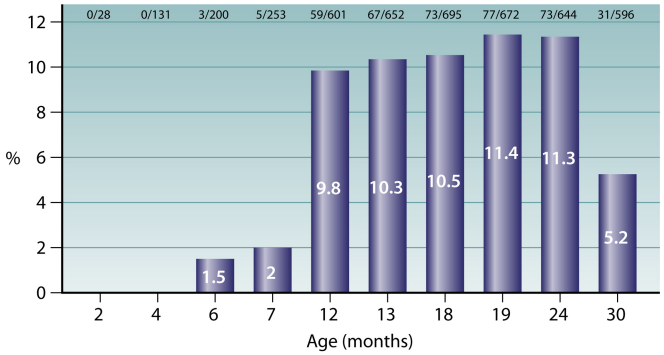

FIG 4 Prevalence of pharyngeal *K. kingae* colonization among children aged 0 to 36 months.

smoke exposure may profoundly influence the results of prevalence studies (99). Monitoring the presence of individual components of the flora is also strongly dependent on technical and methodological aspects, such as sampling site, specimen collection technique, quality of swabs, time for transport to the laboratory, use of selective media, and number of bacterial colonies examined (99). Not surprisingly, results of epidemiological investigations on *K. kingae* carriage have found discrepancies in the prevalence rate, although the overall picture indicates that the pharyngeal colonization of the bacterium is strongly age dependent (85). In an early study carried out in southern Israel, *K. kingae* was not recovered in oropharyngeal cultures obtained from healthy infants aged <6 months, the prevalence of the organism was 10.0% in children aged 6 months to 4 years, and the prevalence decreased to 6.0% in older children (84). In a later study, oropharyngeal specimens submitted to a clinical microbiology laboratory for isolation of *Streptococcus pyogenes* were also plated onto BAV medium (100). The prevalence of *K. kingae* decreased significantly with increasing age: the organism was detected in 22 of 694 (3.2%) samples obtained from children younger than 4 years, in 10 of 679 (1.5%) of those derived from patients aged 4 to 17 years, and in 5 of 671 (0.8%) cultures from adults ($P < 0.001$ for trend) (100). In a longitudinal study in which the younger age group was targeted, 716 healthy children living in southern Israel were sequentially cultured between the ages of 2 and 30 months (85). *Kingella kingae* was not isolated before the age of 6 months, and the colonization rate was low at 6 months, increased in 12-month-old children, remained relatively stable between 12 and 24 months of age, and decreased significantly at 30 months ($P < 0.001$) (85) (Fig. 4). Remarkably, very similar carriage rates were found in a study in which 431 children aged 6 to 48 months living in Geneva, Switzerland, were screened for *K. kingae* colonization by employing a NAA test, showing that the epidemiological features of *K. kingae* colonization are consistent among diverse pediatric populations and can be generalized (93). In a recent investigation carried out to identify risk factors for *K. kingae* carriage, multivariate analysis demonstrated that being 6 to 29 months old carries a strong and independent statistical association with oropharyngeal colonization (101), encompassing the age interval with the highest incidence of culture-proven infections (18, 102).

Living conditions are known to affect the transmission of respiratory pathogens and associated morbidity. Populations in the developing world, as well as economically disadvantaged individuals living in Western countries, have high rates of colonization with respiratory organisms such as pneumococci and suffer from an increased burden of clinical disease (103). Similar to populations in the developing world, the indigent Bedouin residents of southern Israel, who live in crowded households and have large families, exhibit early acquisition and high respiratory pathogen colonization rates, as well as increased morbidity and hospitalization rates for infectious diseases (104). In a recently completed study in which the effect of living conditions on the acquisition of *K. kingae* was prospectively investigated, Bedouin children demonstrated significantly earlier carriage of the organisms than Jewish children who enjoy better socioeconomic status, and by the age of 13 months, 71 of 316 (22.5%) Bedouin children but only 47 of 363 (13.0%) Jewish children had already experienced colonization by *K. kingae* organisms ($P = 0.002$) (P. Yagupsky, unpublished data).

In a study aimed to determine the temporal pattern of *K. kingae* carriage, throat cultures were obtained between February and May, to represent the time of the year when invasive *K. kingae* infections are less common, and from October to December, coinciding with the peak attack rate for clinical disease (100). Overall, 21 of 1,020 (2.1%) specimens cultured between February and May and 16 of 1,024 (1.6%) of those studied from October to December grew the organism ($P > 0.4$). The lack of a seasonal pattern in *K. kingae* carriage was confirmed in an investigation of potential risk factors for pharyngeal colonization, which showed no significant association between month of the year and prevalence rate (101). It appears, then, that in addition to pharyngeal carriage, other, still-unidentified cofactors, perhaps seasonal viral respiratory infections, are important in the pathogenesis of invasive disease.

Carriage Dynamics and Turnover of Colonizing Strains

The dynamics of carriage of respiratory bacteria can be studied in longitudinal surveys in which the target population is repeatedly sampled over a prolonged period of time and isolates are analyzed using highly discriminative typing methods. In a cohort study, pharyngeal swabs were obtained from attendees in a day care facility in southern Israel over an 11-month period and cultured on BAV medium (84). *Kingella kingae* isolates thus detected were genotyped by employing a combination of PFGE and ribotyping with multiple restriction enzymes and immunoblotting with rabbit immune serum (105). To conclusively demonstrate strain identity and consequently person-to-person transmission of individual strains, a stringent criterion (complete identity by all three methods) was used for the data analysis. Overall, sporadic, intermittent, or continuous patterns of strain carriage were observed (105). Two distinct strains, characterized by a distinct combination of typing profiles, represented 28.0% and 46.0% of the studied isolates and, once they took root in the day care center, disseminated effectively, displacing a variety of older strains (105). A few children were found to carry a strain whose PFGE fingerprint differed from the prevalent profile by a single DNA band, suggesting gradual genetic divergence and microevolution of *K. kingae* in the course of prolonged carriage and repeated transmission events (105).

In a prospective investigation in which 716 healthy children were serially cultured between 2 and 30 months of age, the genomic similarity of recovered isolates was assessed by PFGE in the cohort members in whom *K. kingae* organisms were isolated on >1 occasion (85). *Kingella kingae* was detected in 283 (39.5%)

children, of whom 64 had two positive cultures for the organism, 13 had three, and 3 had four. The study showed that the genotypic similarity of isolates was lost over time, and sequential colonization by organisms belonging to as many as four different PFGE genotypes was observed. Based on these data, carriage of *K. kingae* appears to be a dynamic process characterized by repeated acquisition and carriage of antigenically diverse strains, whereas simultaneous carriage of multiple strains is uncommon. After having been carried continuously or intermittently for a few weeks up to 8 months, colonizing strains are replaced by newly acquired strains, similar to the strain turnover noted in other bacterial pathogens that inhabit the respiratory mucosae (85). It should be pointed out, however, that in these two studies (85, 105) a single colony per positive culture was analyzed and, therefore, colonization by >1 *K. kingae* clone and/or persistence of previously carried strains at a reduced level, instead of strain extinction and substitution, cannot be ruled out. Recurrent detection of a strain that was isolated earlier but lost was infrequent (85, 105). This observation is consistent with eradication or at least a quantitative decrease in the presence of individual strains after a prolonged colonization period. It is suggested that sustained carriage of a given *K. kingae* strain induces a specific immune response that eliminates the colonizing organism but does not prevent acquisition of a novel strain characterized by a different array of antigenic determinants (106). This concept is backed by the strain-to-strain diversity of immunogenic and surface-exposed bacterial components that contribute to the colonization of the oropharyngeal mucosa, such as outer membrane proteins (OMPs) (107), the PilA1 pilus subunit (57), the RTX toxin (62, 90), and the *K. kingae* capsule (J. W. St. Geme, personal communication).

Immunity to *K. kingae* Carriage and Disease

The dynamics of *K. kingae* carriage and infection constitute a sequential phenomenon, characterized by almost complete lack of colonization and invasive infections in early life, followed by high colonization rates and increased morbidity in the 6- to 36-month age interval and a sharp decline of both carriage and invasive disease rates in children over 4 years of age and adults. This observation, coupled with the increased susceptibility of immunocompromised individuals to invasive *K. kingae* infections, indicates that an acquired immune response is required to shield from pharyngeal colonization and prevent clinical disease (18, 107). It should be noted, however, that whereas the rate of carriage of *K. kingae* is highest and remains stable between 12 and 24 months of age (Fig. 4), the epidemiological curve of children with clinical disease is markedly skewed to the left, and over 75% of patients are younger than 18 months (Fig. 5), indicating that acquisition of resistance to infection precedes the development of effective mucosal immunity to respiratory colonization.

Because the OMPs of many bacterial species play important functions in the establishment of mucosal colonization and, as such, elicit production of protective antibodies against mucosal and invasive infections, it was postulated that the antibody response to these antigens could serve as a tool to study the immune response to *K. kingae* carriage and disease. In a cohort investigation, serum samples were obtained in the acute and convalescent periods from children with culture-proven *K. kingae* endocarditis, occult bacteremia, and skeletal system infections and studied with an enzyme-linked immunosorbent assay (ELISA) employing the organism's OMPs as the coating antigen (106). IgG concentrations significantly increased in the convalescent period, whereas serum IgA antibodies decreased between the acute and convalescent phases, suggesting that the IgA immune response is short lived (106). When OMPs of the infecting isolate and those of heterologous strains were used as coating antigens, higher IgG antibody levels against the infecting strain were measured, indicating a more specific and probably a more effective immune response (106). When sera from pediatric patients with different *K. kingae* infections were reacted with OMP extracts of invasive strains, antigens bands of 108, 43, and 41 kDa were usually recognized, whereas reaction with other proteins showed variability depending on the strains and patient serum source (106). These experiments suggest that some immunogenic *K. kingae* OMPs are highly conserved, while others are inconstant and/or the exposed epitopes are polymorphic, translating to marked differences in the affinity of the antibody response.

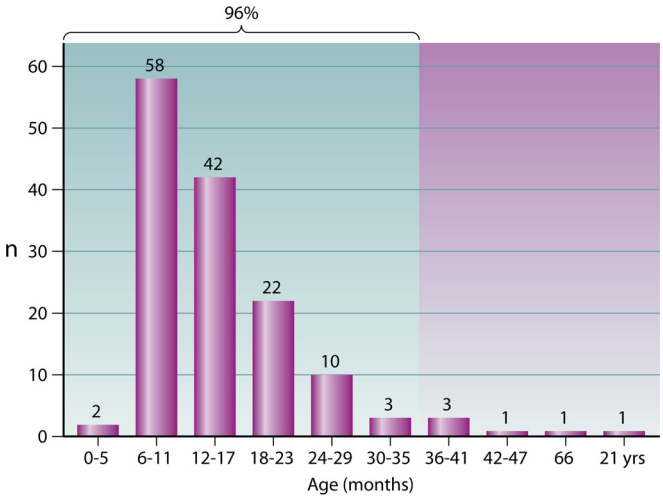

FIG 5 Age distribution of 143 patients with culture-proven *K. kingae* infections detected in southern Israel between 1988 and 2013.

In a longitudinal investigation in which the prevalence and fluctuations of levels of antibodies to *K. kingae* OMPs in a healthy population were studied, levels of IgG and IgA antibodies were measured in the sera of children at different ages with no previous clinical disease caused by the organism (107). Because IgA does not traverse the placenta, IgA- and IgG-type antibody levels were measured and compared to establish whether antibodies found in early childhood denote actual exposure to *K. kingae* antigens or represent maternally derived immunity. The mean IgG level was high at the age of 2 months, slowly declined thereafter (reaching an all-time nadir at 6 to 7 months), persisted at a low level until the age of 18 months, and peaked in children older than 24 months. Serum IgA was almost undetectable at 2 months, slowly increased between 4 and 7 months of age, and exhibited a further increment in children over 24 months of age (107). The scarcity of colonization and disease in early life, combined with absent serum IgA and high IgG levels, suggests that defense against mucosal carriage and infection is provided by vertically acquired immunity, because on the one hand, social contacts and potential sources of contagion multiply and maternal antibodies wane, and on the other, children older than 6 months experience increased rates of *K. kingae* carriage and disease. Low prevalence of respiratory colonization rates coupled with decreasing incidence of disease and rising an-

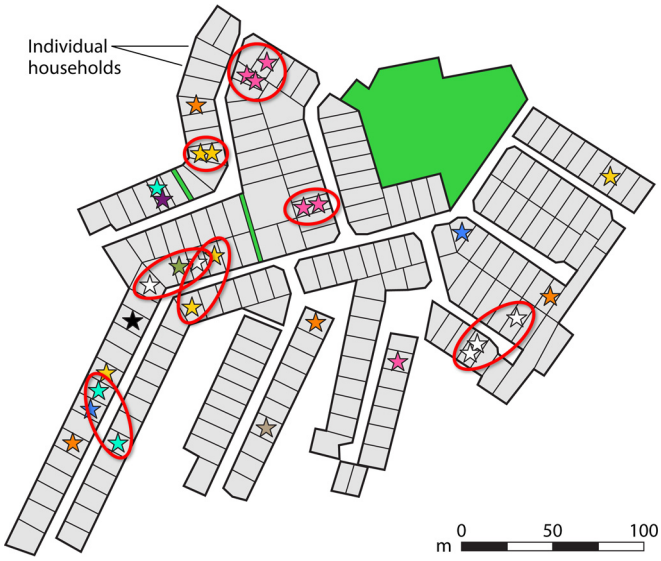

FIG 6 Spatial distribution of *K. kingae* clones carried in a Bedouin neighborhood as determined by PFGE with restriction enzyme EagI. Each star represents a positive pharyngeal culture, while the different colors represent distinct clones. Red ovals depict geographic clusters of identical strains.

tibody levels in older children most likely denote acquisition of immunological maturity and development of a protective immune response through reiterated exposure to the organism (107).

Transmission

The persistence and dissemination of respiratory organisms in the human milieu are dependent on the successful establishment of endless chains of person-to-person transmission. In the last few decades, the development of sensitive molecular typing methods made it possible for the first time to discriminate between genetically different strains, enabling deep understanding of the dynamics of transmission of individual bacterial lineages in the human population across time, space, and social networks. The propagation of *K. kingae* was prospectively investigated in a large cohort of asymptomatic Israeli Jewish and Bedouin children living in separate cities and towns (58). All *K. kingae* isolates recovered from pharyngeal cultures during a 12-month period were analyzed by PFGE, employing the restriction enzyme EagI (58). Organisms derived from Bedouin children tended to differ from those isolated from Jewish children, corroborating the geographic isolation and relative lack of personal interaction between the two ethnic groups (58). Significant spatial clustering of clones was detected in Bedouin towns, quarters, and households, indicating transmission between family members and other close contacts (58) (Fig. 6). Because the traditionally nomadic Bedouin population has recently settled in towns keeping the ancestral tribal divisions, household location closely follows family ties and social mingling. Bedouin children do not usually receive out-of-home care services, and most of their social intercourse takes place within their extended families and clans, explaining the genomic similarity of *K. kingae* isolates detected in neighboring households. No association between place of residency and distribution of individual *K. kingae* clones, however, was noted among Jewish carriers, who live in Westernized conditions and attend day care centers since an early age. It is postulated that Jewish children are connected by multiple and complex social networks through which respiratory bacteria circulate, obscuring the connection between house location and spatial scattering of *K. kingae* strains (58).

Using a sensitive NAA assay, Kampouroglu et al. studied the intrafamilial transmission of the organism in a Swiss pediatric population. The research found that 55.0% of children with a PCR-documented invasive *K. kingae* infection and 40.0% of asymptomatic carriers had siblings with a positive pharyngeal PCR assay, suggesting dissemination of the organism within susceptible family members (108).

PATHOGENESIS OF *K. KINGAE* DISEASE

From Colonization to Clinical Disease

The link between *K. kingae* colonization of the oropharyngeal mucosa and the development of an invasive infection was convincingly demonstrated in three bacteremic children in whom a genomically identical organism was also recovered from a pharyngeal culture (109). This finding was later confirmed by the results of two studies employing NAA technology. In a survey comprising 27 young children with PCR-proven *K. kingae* osteoarthritis, a PCR-based assay performed on the pharyngeal swab was positive for *rtxB* gene sequences in all cases (95). In a second and more conclusive study, young children with arthritis and culture-negative/PCR-positive synovial fluid samples had *K. kingae* isolated from the pharynx. The *rtxA* gene sequences of the synovial exudate amplicons and those of the pharyngeal isolate were indistinguishable, suggesting that the oropharynx is probably the gateway through which invasive *K. kingae* organisms enter into the bloodstream, disperse, and invade the skeletal system and endocardium, for which the bacterium shows a striking affinity (87).

Strain Virulence and Tissue Tropism

Similar to observations made with other bacterial species, *K. kingae*'s genomic heterogeneity is especially noticeable among carried organisms, whereas a large fraction of invasive diseases are induced by a few distinct strains, suggesting that *K. kingae* isolates differ in their virulence and some strains that colonize the pharyngeal epithelium have diminished invasive capability (64). Using an animal model, Basmaci et al. have recently demonstrated wide strain-to-strain variation in terms of virulence among *K. kingae* isolates (64). When 5-day-old albino Sprague-Dawley rats were inoculated intraperitoneally with 10^7 CFU of *K. kingae*, the ATCC type strain 23330 (a respiratory isolate) proved to be nonvirulent, whereas two invasive strains, derived from children with bacteremia and osteoarthritis, caused disease but showed significant differences in terms of animal survival (64).

In an investigation to characterize the *K. kingae* strains causing invasive infections in Israel in the period from 1991 through 2012 and the possible association, at the population level, of certain genotypes with specific clinical diseases, 181 invasive isolates from patients with bacteremia, arthritis, osteomyelitis, or endocarditis were typed by PFGE (110). A total of 32 genotypically distinct clones could be recognized, of which five (B, H, K, N, and P) were responsible for 72.9% of all cases and were detected countrywide for over 2 decades. Clone K showed a significant positive association with occult bacteremia, clone N with pediatric arthritis or osteomyelitis, and clone P with endocarditis. Other clones, which

are frequently isolated from asymptomatic children in Israel (58), are rarely detected in patients with invasive diseases (110). This discrepancy suggests a compromise between transmissibility and virulence, because diseased persons are isolated from other individuals, administered antibiotic drugs, and may even have died from the disease, interrupting the chain of interpersonal transmission and causing the death of the pathogen. Clone N, which was a frequent cause of clinical disease among Israeli patients in the decade of the 1990s and exceptionally colonizes the pharynges of healthy children (58), was found to be significantly linked to skeletal system infections (110). These results imply that strains associated with deep tissue invasiveness may be rapidly eliminated from the pharyngeal mucosal surface and removed from the bloodstream, indicating that bacterial survival in different body sites requires a specific biological specialization. Interestingly, clone K organisms appear to present an ideal equilibrium between transmissibility and virulence. Organisms belonging to this clone predominated among attendees at a day care center in southern Israel as early as 1993, persisting in the oropharynx for up to 4 months (105), and this was the second most common pharyngeal clone in healthy Jewish children between 2006 and 2007 (58). On the other hand, clone K organisms exhibit enhanced invasive capabilities, representing 41.7% of all southern Israel isolates from patients over the last 2 decades, and were accountable for the excess of *K. kingae* infections detected among children in the region (104). Remarkably, all clone K isolates harbor multiple copies of a 33-bp fragment of the *rtxA* gene, which is rarely found in other *K. kingae* organisms (64). The possibility that the repetition of the toxin-encoding gene adds to the invasiveness and epidemic success of clone K is intriguing but remains, as yet, unproven.

The PFGE results indicating association of Israeli clones of *K. kingae* with particular infections have been recently corroborated in an international study in which 324 strains derived from unrelated individuals were typed by MLST and results were correlated with the patient's clinical syndrome (65). ST-35, ST-14, and ST-25 (which correspond to PFGE clones N, H, and Sp, respectively) were significantly associated with pediatric arthritis or osteomyelitis, and ST-24 (PFGE clone P) was associated with endocarditis (65).

In two recent reports, *K. kingae* organisms belonging to ST-25 were found to be strongly associated with childhood osteomyelitis, causing a cluster of 4 bone infections among 5 affected day care attendees (98) and later an additional sporadic case (111), and an ST-6 strain caused a cluster of 3 cases of osteomyelitis in an Israeli day care center (112). These observations imply that carriage of particular strains entails increased risk for clinical disease and invasion of specific body tissues.

Viral Infections as Cofactors in *K. kingae* Disease

Observations made at the time of patients' presentation to the hospital and before *K. kingae* disease is microbiologically confirmed frequently reveal symptoms and signs consistent with a concurrent nonspecific upper respiratory infection, hand-foot-mouth disease (113), or varicella virus (114, 115)-, or herpes simplex virus (18, 114, 116)-induced buccal ulcers. In a study aimed to assess the effect of acyclovir administration on the duration of symptoms and viral shedding in children with culture-proven herpetic gingivostomatitis, 29 affected patients also underwent bacteriological blood cultures. Four (13.8%) of these children were bacteremic, and *K. kingae* was isolated in all cases (114). Because the peak incidence of infections with primary herpes simplex virus and many respiratory viruses coincides with the age of *K. kingae* carriage, it seems plausible that damage to the mucosal layer caused by a previous or concomitant viral disease facilitates the entry of colonizing *K. kingae* organisms into the bloodstream and dissemination to distant sites (18, 100, 109). In a recent report, Basmaci et al. strengthened the link between invasive *K. kingae* disease and viral upper respiratory tract comorbidity by detecting rhinoviruses in two children with concomitant PCR-proven *K. kingae* infections of bone and soft tissues (111).

EPIDEMIOLOGY OF INVASIVE *K. KINGAE* DISEASE

As occurs with other bacteria that colonize the upper respiratory tract mucosal surfaces, at any given time, the number of asymptomatic children that carry *K. kingae* is far in excess of those with clinical disease. For instance, the calculated risk of young Swiss carriers to develop a *K. kingae* infection of the skeletal system was found to be <1% per year (93). Because the entire pediatric population of southern Israel receives health services in a single hospital (the Soroka University Medical Center [SUMC]) in which the BCV technique has been regularly employed for over 2 decades, the morbidity caused by culture-proven *K. kingae* infections could be accurately computed in this captive population. Despite a carriage rate of 5% to 12% in young children living in the region (85), the annual incidence of invasive *K. kingae* disease was only 9.4 per 100,000 children aged <5 years (102). It should be pointed out, however, that because the culture detection of *K. kingae* remains suboptimal, even when the BCV laboratory method is used, this figure can be considered only a minimal estimate.

Between 1988 and 2013, a total of 143 culture-confirmed cases of invasive *K. kingae* infections were identified at the SUMC, including 141 children younger than 4 years, of whom only two were aged <6 months. A male-to-female ratio of 1.3 has been found in a large nationwide Israeli study comprising over 300 patients (102). In Israel (117) and Sweden (6), invasive *K. kingae* diseases occur throughout the year, peaking during the fall and early winter months and being far less common between February and April (102, 117).

Child Care Facilities, Colonization, and Disease

During the last decades, a growing proportion of children living in countries in the developed world countries have attended out-of-home care facilities. This phenomenon has important public health significance because the risk of transmission of pediatric pathogens and the occurrence of mucosal and invasive infections are significantly increased among day care center attendees (118, 119). Respiratory organisms readily spread by direct contact or through fomites among youngsters who share objects coated with respiratory secretions or saliva (119). Day care center classes are attended by children of similar age, immunological immaturity, and susceptibility to bacterial pathogens. Therefore, once a virulent strain has been introduced in a crowded facility, rapid propagation of the organism and outbreaks of disease may occur.

In a survey comprising 1,277 children younger than 5 years referred to a pediatric emergency department in southern Israel, out-of-home care was strongly and independently associated with *K. kingae* colonization (odds ratio, 9.66 [95% confidence interval, 2.99 to 31.15]; $P < 0.001$) (101). In a prospective cohort investigation, 35 of 48 (72.9%) children attending a day care center who

were cultured fortnightly over an 11-month period showed oropharyngeal *K. kingae* colonization at least once (84). At any given time, an average of 27.5% attendees carried the bacterium, a much larger fraction than that found in the general pediatric population of comparable age (84). Genotyping of the colonizing isolates demonstrated that identical strains were simultaneously or successively carried by multiple attendees, indicating child-to-child spread (105). In an ongoing study, the prevalence of *K. kingae* among children attending elementary schools was less than 3%, but PFGE typing of the recovered isolates showed temporal clustering of clones among classmates, suggesting that transmission of the organism continues well beyond preschool age (Yagupsky, unpublished data).

Outbreaks of invasive *K. kingae* infections, including almost the entire spectrum of the disease (septic arthritis, osteomyelitis, spondylodiscitis, endocarditis, and meningitis), have been reported from French (98), American (113, 120), and Israeli (112, 121) day care facilities in the recent decade. The epidemiological investigation of these events revealed high disease attack rates among attendees, ranging from 14% (120) to 21% (98, 121). Clustering of cases within a 1-month period was noted, as well as an elevated prevalence of *K. kingae* carriage among asymptomatic young contacts of the index patients (up to 45% in a U.S. cluster, as determined by culture on selective medium [120], and 85% in the French outbreak, as demonstrated by an NAA method [98]). The age range of the colonized and infected day care center attendees was 8 to 25 months, coinciding with the period in life of highest *K. kingae* carriage (85, 100) and susceptibility to invasive disease (102). Clinical isolates from infected children were genotypically identical to those detected in the oropharynges of their respective classmates (113, 121, 122).

The strain implicated in the day care outbreak of bone infections that occurred in 2005 in an Israeli facility belonged to the aforementioned highly virulent clone K and to MLST ST-6 (112), and the organism that caused the North Carolina cluster (113) was identical to a strain isolated in 11.3% of healthy carriers (58) and in 11.6% of patients with invasive infections in Israel (110). The global circulation of these organisms shows that outbreaks of infection usually originate in bacterial populations characterized by superb colonization capability, enhanced transmissibility, and remarkable invasiveness (64, 65).

CLINICAL FEATURES OF *K. KINGAE* DISEASE

Invasive *K. kingae* disease usually affects previously healthy children aged less than 4 years (102), whereas older children and adults frequently have predisposing conditions such as failure to thrive (102), congenital heart disease (102), prolonged corticosteroid therapy (102), primary immunodeficiency (123, 124), hematological malignancies (6, 125), liver cirrhosis (126, 127), end-stage renal disease (128), sickle cell anemia (126), diabetes mellitus (21, 128), cardiac valve pathology (9, 129, 130), systemic lupus erythematosus (131–134), rheumatoid arthritis (135), renal transplants (9), solid tumors (82), or AIDS (22, 128, 136, 137).

Children with *K. kingae* infections other than endocarditis commonly present with mild symptomatology, requiring an elevated index of clinical acumen. Many patients are afebrile or have a mildly elevated body temperature and are in good general condition, and the yield of blood cultures is low, suggesting that the bacteremic stage of the disease is transient (18, 102, 117). Recent or concomitant rhinorrhea, pharyngitis, stomatitis, or diarrhea is noted in the majority of cases (102). With the exception of patients with endocardial involvement who develop embolic phenomena (21, 132, 138), a single body system is usually affected.

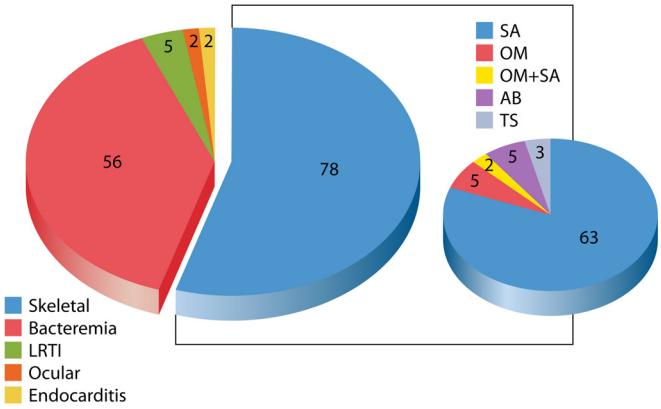

FIG 7 Clinical syndrome in 143 consecutive patients with *K. kingae* infections diagnosed in southern Israel between 1988 and 2013. LRTI, lower respiratory tract infection; SA, septic arthritis; OM, osteomyelitis; AB, abortive; TS, tenosynovitis.

The vast majority of medical reports on *K. kingae* consist of descriptions of single cases of disease or small patient series in which rare conditions are, most likely, overrepresented. The large body of information gathered at the SUMC provides a more accurate and unbiased picture of clinical *K. kingae* morbidity (Fig. 7). Between 1988 and 2013, septic arthritis, osteomyelitis, or tenosynovitis represented the prime manifestation of *K. kingae* disease and was detected in 78 of 143 (54.5%) individuals, followed by occult bacteremia in 56 (39.2%), bacteremic lower respiratory infection (laryngotracheobronchitis and pneumonia) in 5 (3.5%), ocular infections (corneal abscess and periorbital cellulitis) in 2 (1.4%), and bacterial endocarditis in 2 (1.4%), including the oldest child (aged 66 months), who suffered from supravalvular aortic stenosis due to Williams' syndrome, and the only adult patient in the series, who also had systemic lupus erythematosus. Other, more rare syndromes, including spondylodiscitis, urinary tract infection (24), meningitis (139), peritonitis (140), and pericarditis (141), were not recorded in this series.

Skeletal System Infections

The role of *K. kingae* as a pathogen of the skeletal system is mostly limited to the young age group (18, 142, 143), whereas occurrence of joint or bone disease in older children or adults is uncommon (6, 25, 127, 135, 144–150). In a study in which conventional cultures, BacT/Alert BCVs, and real-time PCR with universal and pathogen-specific primers targeting the *cpn60* gene were employed, *K. kingae* was implicated in 35 of 46 (76.1%) children younger than 4 years with septic arthritis and in 9 of 17 (52.9%) with osteomyelitis with or without associated joint invasion, and the organism was not detected in any of 20 older children with skeletal system infections (151). In a recent large study, 11 of 15 (73.3%) children with joint or bone infections were positive for *K. kingae* DNA sequences by broad-range PCR and later confirmed with species-specific primers, whereas the organism was not detected in any of 126 PCR-positive specimens from adult patients (152).

Suppurative arthritis constitutes the most common *K. kingae*

infection of the skeletal system in childhood. Sixty-three of the 78 (79.5%) pediatric patients with skeletal system involvement diagnosed at the SUMC since 1988 had joint infections, 5 (6.4%) had osteomyelitis, 2 (2.6%) had both, 3 (3.8%) had tenosynovitis, and 5 (6.4%) presented with *K. kingae* bacteremia and transient skeletal system symptoms, suggesting an abortive infection. In a recent report comprising all 230 children with culture and/or NAA assay-confirmed *K. kingae* infections of the skeletal system diagnosed over a 4-year period in two large European pediatric orthopedic units, 178 (77.4%) had arthritis, 40 (17.4%) had osteomyelitis, and 12 (5.2%) had spondylodiscitis (153).

In an Israeli nationwide study comprising 169 pediatric patients with skeletal system infections, the organism was isolated from a synovial fluid sample in 109 (64.5%), from a bone exudate in 12 (7.1%), and from the blood in 48 (28.4%), emphasizing the importance of the blood culture as a diagnostic tool (102).

Septic arthritis. Since the adoption of conjugate vaccination programs, a rapid drop in the incidence of *H. influenzae* type b arthritis has occurred (154, 155), whereas *K. kingae* has emerged as the most common etiology of joint infections in children 6 months to 3 years of age (13, 14, 16). Although the mutual relationships (competition, symbiosis, etc.) between the different components of the resident upper respiratory tract are complex and the potential antagonism between carried *K. kingae* and *H. influenzae* type b organisms has not been investigated, the increasing importance of *K. kingae* as a skeletal system pathogen does not appear to have resulted from the introduction of the conjugate vaccine, because colonization of *H. influenzae* type b in the prevaccine era was uncommon and the rate was usually lower than 5% (156). Rather, it seems that *K. kingae* is being increasingly implicated in the causation of pediatric septic arthritis because of the use of novel and more sensitive microbiological methods in recent years.

The disease generally involves the large weight-bearing knee, ankle, or hip joints in over 80% of cases (15, 102, 157–160), followed by the wrist, shoulder, and elbow (102, 160–169). However, the metacarpophalangeal, sternoclavicular, tarsal, and sacro-iliac joints (148, 170–173), which are rarely affected by other bacterial species, are involved with unusual frequency in *K. kingae* arthritis (18). Involvement of two joints was observed in 8 of 140 (5.7%) children with *K. kingae* suppurative arthritis (102), a proportion comparable to that found in infections induced by traditional pathogens (174). Inflammation markers measured in the blood, such as the white blood cell count, erythrocyte sedimentation rate, and C-reactive protein (CRP) levels, are frequently normal, while the joint fluid shows $<50.0 \times 10^9$ WBC/liter (171) in more than 20% of patients, meeting the laboratory criterion used to exclude bacterial arthritis. Microscopic examination of the exudate rarely reveals the presence of *K. kingae* (18, 102, 160), probably because of the low bacterial concentration (from 11 CFU/ml to 300 CFU/ml) (175) and the difficulty in visualizing the Gram-negative bacilli against the pink-stained fibrin background. Because of the mild clinical presentation of *K. kingae* arthritis, when the disease involves the hip joint, patients may be mistakenly diagnosed as having transient synovitis (176), a benign inflammatory condition that is managed without antibiotics. Employing a diagnostic predictive algorithm to differentiate between the two conditions (177), 20 of 28 (71.4%) Israeli and Spanish children with culture-proven *K. kingae* of the hip joint would have been considered to have a ≤40% probability of infectious arthritis, and the true nature of the disease was revealed only by BCVs seeded with blood or synovial fluid specimens (178–180). It should be pointed out that this predictive model was developed many years ago when *K. kingae*'s role in pediatric septic arthritis was still unrecognized, and none of the patients included in the original and subsequent validation studies had a proven infection caused by this organism.

Although children with *K. kingae* arthritis show a milder symptomatology than patients with joint infections caused by other bacteria, it is disputed whether the patients' clinical features and/or laboratory test results are specific enough to accurately predict the etiology of the disease. In a retrospective study, Basmaci et al. compared the clinical and biological characteristics of 64 French pediatric patients with NAA assays positive for *K. kingae* arthritis and 26 children with *Staphylococcus aureus* infections (181, 182). Patients with *K. kingae* infections were younger than those with staphylococcal joint disease (median age, 1.4 years versus 7.9 years; $P < 0.001$), and their hospital stay was shorter and characterized by fewer complications, yet the two populations did not significantly differ in terms of duration of symptoms, body temperature, white blood cell counts, and concentrations of a variety of acute-phase reactants (181, 182). In a Swiss study, Ceroni et al. compared 30 children with *K. kingae* joint infections and 30 children with arthritis caused by *S. aureus*, *S. pyogenes*, *Streptococcus agalactiae*, and *H. influenzae* type b (183). A clinical and laboratory score combining body temperature of <38°C, serum C-reactive protein (CRP) level of <55 mg/liter, leukocyte count of $<14.0 \times 10^9$/liter, and <150 band forms/mm^3 was built, and the data for the two patient groups at presentation were compared. Whereas 29 of 30 (96.7%) patients with *K. kingae* infections displayed fewer than 2 predictors, 27 of 30 (90.0%) children with arthritis caused by other bacteria presented with 3 or 4 predictors. Consistent with the concept that *K. kingae* behaves like a low-grade-virulence pathogen when invading the skeletal system (160), magnetic resonance imaging (MRI) of 21 patients with osteoarticular infections caused by the organism showed bone and soft tissue reactions less frequently than that of 10 patients with skeletal system infections caused by Gram-positive cocci, whereas epyphyseal cartilage abscesses were present in *K. kingae* disease only (184). These favorable experiences suggest that MRI may enable early differentiation (before culture or NAA tests results are known) between *K. kingae* infections and those caused by traditional pathogens, but this needs independent confirmation. Until the question is definitely settled, it seems prudent to administer wide antibiotic coverage to all children with suspected joint infections, pending definitive bacteriological test results.

Osteomyelitis. *Kingella kingae* affects the tubular tibia, femur, humerus, radius, or ulnar bone in more than half of the cases (102, 185–192), yet involvement of the pelvis (193, 194), calcaneus (102, 195–197), talus (172, 198, 199), sternum (16, 130, 200), or clavicle (201) is also common (18, 102, 153, 172). Epiphysial invasion, which rarely occurs in osteomyelitis of other etiology, is frequently observed in *K. kingae* infections (152, 164, 188, 202, 203).

Kingella kingae osteomyelitis habitually has an insidious onset, and the vast majority of patients are diagnosed after more than 1 week of evolution (102, 130, 170, 172, 203, 204). The unossified epiphysis of infants receive blood supply from a metaphyseal capillary network that is obliterated before the age of 12 months. Therefore, bone infections can easily progress from the growth plate to the epiphysis and articular space, and in a substantial fraction of cases, osteomyelitic foci of the femur or humerus rup-

ture into the adjacent hip or shoulder articulations, causing concomitant arthritis (102). The high-contrast resolution and multiplanar imaging offered by MRI have resulted in detection of large intraosseous ("Brodie") abscesses involving the epiphyses, metaphyses, and cartilage that fistulize through the contiguous joint in 9 of 40 (22.5%) children with *K. kingae* osteomyelitis (153, 204). In two pediatric cases of *K. kingae* osteomyelitis, ascertained by either culture isolation or NAA, examination of the osteolytic lesion revealed a typical Langerhans cell histiocytosis picture, indicating that a systematic search for the organism should be carried out when this rare diagnosis is entertained in young children (189, 205). Of note, despite the frequent diagnostic delay and severe radiological pictures in some cases of osteomyelitis, no evolution to chronicity or significant residual orthopedic disabilities have been reported (18, 152).

Spondylodiscitis. Historically, tubercular invasion of the intervertebral space from a contiguous bony focus was the most common etiology of spondylodiscitis. The declining incidence of pediatric tuberculosis in Western countries in recent years and increasing use of sensitive laboratory methods have resulted in the detection of *K. kingae* in more than one-quarter of children younger than 5 years with hematogenous spondylodiscitis (94, 206–208), whereas isolation of the organism in older children (209, 210) and adults (150, 211) is rare. It is presumed that the bacterium penetrates into the rich vascular network that traverses the vertebral endplates cartilage and enters the annulus in young children during a bacteremic *K. kingae* episode. Because intervertebral disk biopsies and aspirations are rarely performed in children (212, 213) and by the time patients present with spinal symptoms, the bacteremic phase has already passed, it is presumed that many cases of *K. kingae* spondylodiscitis remain undiagnosed. In a recent study, all 10 children with MRI-proven spondylodiscitis had a positive *K. kingae* real-time PCR assay in an oropharyngeal sample, whereas only two blood specimens were positive for the test, and all blood cultures remained sterile (94). Despite the already discussed limitations of this strategy, detection of pharyngeal carriage of the organism by culture or NAA assays may provide reasonable suspicion that *K. kingae* is responsible for the infection, whereas a negative NAA test could practically exclude the organism as the culprit.

Kingella kingae spondylodiscitis generally involves a single intervertebral disk (213), usually at the lumbar spine level and, with decreasing frequency, the thoracolumbar, thoracic, lumbosacral, and cervical spaces (7, 20, 207, 209, 214–218). Patients with this syndrome show gait disturbances, refuse to sit or walk, or complain of back pain. In more advanced or neglected cases, physical examination may reveal neurological signs. Roentgenological and MRI studies show narrowing of the intervertebral space (191, 207). Although intravertebral and spinal subdural abscesses have been detected by MRI (153), if it is adequately treated with antibiotics and paraspinal purulent collections and prompt surgical drainage are performed, *K. kingae* spondylodiscitis runs a benign clinical course. Residual narrowing of the intervertebral space is uncommon, and the disease leaves no permanent neurological deficits (191, 207).

Abortive skeletal infections. Although bacterial infections of the skeletal system follow an unrelenting progress unless treated with antibiotics, self-limited involvement of joints and/or bones in the course of a *K. kingae* bacteremic episode has been reported, suggesting an abortive clinical course (168, 178, 187, 219–222). Patients with this unusual syndrome present with symptomatology suggestive of joint or bone disease, such as localized pain or limited limb motion, but lack objective signs of osteoarthritis, and therefore, antibiotic therapy is not initially provided (219). By the time *K. kingae* is recovered from a blood culture, the febrile condition, as well as the subjective complaints, have mostly subsided. Although a few children made an uncomplicated and full recovery without receiving antibiotic therapy (220, 221), it should be kept in mind that circulating *K. kingae* organisms may invade the cardiac tissues with severe consequences, and therefore, adequate antibiotics should be administered without delay whenever the bacterium is identified in a blood culture (187).

Soft Tissue Infections

A diversity of soft tissue infections, including cellulitis (185), tenosynovitis and dactilytis, (171, 223, 224), bursitis (225), and subcutaneous (6, 225), presternal (199, 200, 226, 227), and intramuscular (111) abscesses, has been described. Detection of the causative organism has been performed by culture of the aspirated exudate (171, 199, 200, 215, 225, 227), by a blood culture (171, 185, 223), or by an NAA assay (111, 200, 224).

Occult Bacteremia

Isolation of *Kingella kingae* from the blood without evidence of endocarditis or another nidus of infection has been reported repeatedly in children (11, 12, 220, 222, 225, 228–231) and exceptionally in adults (6, 126, 129, 232), and this syndrome represents the second most frequent expression of *K. kingae* disease in pediatric patients (18). A disseminated maculopapular rash, resembling that described in systemic meningococcal or gonococcal infections (125, 126, 130, 222, 233), or frank Henoch-Schonlein purpura (234) has been described in a few patients.

Mild to moderate fever is usually recorded, and the mean leukocyte count frequently is less than 15×10.0^9/liter (12, 102). Because of the seemingly benign clinical presentation, the current decision algorithms for managing febrile children aged 6 to 36 months with no apparent clinical source, which are based on the height of body temperature and presence of marked leukocytosis for drawing blood cultures (235), frequently fail to detect occult *K. kingae* bacteremia, and many events probably are undiagnosed. In a survey carried out in a pediatric emergency room in which blood cultures were obtained from febrile children without regard to the height of the fever or the blood leukocyte count result, *K. kingae* represented a common agent of bacteremia in the pediatric population of southern Israel (11).

Endocarditis

The genus *Kingella* is represented by the "K" in the acronym HACEK, which comprises a group of fastidious oral Gram-negative organisms responsible for 6% of all cases of endocarditis in the general population (236–239). *Kingella kingae* appears to cause a higher burden of endocardial infections in children and was the etiological agent of 4 of 51 (7.8%) episodes in a tertiary care pediatric hospital in Israel (240) and of 6 of 85 (7.1%) cases among New Zealand children (241).

Contrary to observations made with other *K. kingae* infections, only three-quarters of all pediatric patients with endocarditis caused by *K. kingae* are younger than 3 years (18, 242). Antecedent poor dental hygiene (243) or dental extractions (244) have been reported in adolescents and adult patients. A native valve is af-

fected in 95% of children (138, 168, 245–252), whereas native (133, 245, 252–255) and prosthetic (21, 23, 243, 256–259) valve involvement have been reported with approximately similar frequency in adult patients. The mitral valve is affected in over 90% of cases (113, 124, 130, 133, 168, 244, 245, 247, 248, 250, 252, 255, 257, 260–266), followed by the aortic valve (6, 23, 243, 246, 249, 253–256, 259, 267, 268), while tricuspid (269) or pulmonary (130) valve endocarditis is exceptional. Involvement of multiple valves has been also described (255, 259). A wide variety of cardiac malformations (9, 124, 130, 133, 252, 262, 269) and rheumatic fever (21, 133, 259) are the usual predisposing factors, but many pediatric patients have no antecedent valvular disease (113, 130, 138, 242, 244, 247–251, 263, 266, 267, 270). A single case of endocarditis in a 4-year-old child occurring a few months after the percutaneous correction of a ventricular septal defect using a transcatheter device has been reported (271). The blood leukocyte count and other markers of inflammation, such as the erythrocyte sedimentation rate and CRP levels, are significantly increased in patients with *K. kingae* endocarditis compared to those recorded in patients with other infections. However, no laboratory result reliably diagnoses or excludes endocardial invasion by the organism (6, 102, 242).

Despite *K. kingae*'s remarkable susceptibility to antimicrobial drugs, serious complications, such as embolism or mycotic aneurism to the femoral (9, 130, 251), brachial (124), or ophthalmic (9) arteries, valvular insufficiency (113, 242, 245, 247, 249, 250, 264, 269), congestive heart failure (133, 249, 272) or cardiogenic shock (21, 130, 258), pulmonary infarction (269), mitral valve rupture (244) or perforation (242, 250, 263), paravalvular abscess formation (249, 259, 265), cerebrovascular accidents (113, 168, 242, 255, 257, 261–263, 265, 266, 272, 273), meningitis (113, 138, 242, 245), brain abscess (251), and embolic arthritis (138) and cellulitis (252), are common. Emergency cardiac surgery has been prescribed for life-threatening complications that do not respond to conservative medical treatment (9, 130, 244, 248–250, 259, 263, 267, 271, 272), many patients require late valvuloplasty (269) or valve replacement (242, 247, 259), and the overall mortality rate of the disease is >10% (9, 18, 21, 113, 133, 242, 266). This unusual clinical aggressiveness is probably related, in part, to delays in the identification of the bacterium (51) rather than failure to isolate the organism because of insufficient length of incubation (273). Because of the severe implications of *K. kingae* endocarditis, it has been strongly advised that individuals from whom the bacterium is isolated from a normally sterile site should undergo a careful and complete echocardiographic assessment (18, 115).

Lower Respiratory Tract Infections

Kingella kingae has been recovered from blood or respiratory cultures of immunocompromised as well as otherwise healthy pediatric and adult patients with laryngotracheobronchitis (18), epiglottitis (82), pneumonia (83, 274), and pleural empyema (275).

Meningitis

Kingella kingae meningitis may result from the hematogenous seeding of the organism in the central nervous system during a bacteremic episode (276–280) or by migration of septic emboli or rupture of a mycotic aneurism in patients with endocarditis (113, 132, 138, 242, 245, 272). The disease has been diagnosed in children (113, 138, 245, 277–280), adolescents (139), and adults (132, 242, 272, 276). The clinical course of *K. kingae* meningitis secondary to endocardial infection is severe and may leave permanent neurological sequelae such as hemiplegia (280) or hemiparesis (272), aphasia (272), and ophthalmoplegia (139) among survivors.

Ocular Infections

Kingella kingae has been recovered from patients with different ocular infections, including palpebral abscess (34), keratitis (281, 282), corneal ulcer (283), endophthalmitis (130, 284), orbital cellulitis (285), and periorbital cellulitis (Yagupsky, unpublished data).

Other Infections

Anecdotal cases of *K. kingae* pericarditis (141), peritonitis (140), urinary tract infection (24), and aptho-stomatitis (286) have been also reported in adult patients.

DIAGNOSIS OF INVASIVE *K. KINGAE* INFECTIONS

Detection by Culture

The isolation of *K. kingae* on bacteriological solid media is clearly unsatisfactory (17, 18, 287, 288). The sensitivity of cultures can be significantly enhanced by inoculating synovial fluid aspirates into aerobic BCVs from commercial systems such as Bactec (17, 289), BacT/Alert (173, 290–292), Hémoline DUO (129, 293), Isolator 1.5 Microbial Tube (175), and in-house-made liquid media (294). In a pioneer study, *K. kingae* was detected in 25 synovial fluid specimens seeded into aerobic Bactec bottles after an average of 4 days, whereas the accompanying culture plates recovered the organism in only two cases (17). Subcultures of positive BCVs onto blood agar and chocolate agar plates grew the organism without difficulty, indicating that routine bacteriological media support *K. kingae*'s growth requirements. This observation confirms that synovial fluid has potent antibacterial properties (295), exerting an inhibitory effect on *K. kingae* organisms. Inoculation of the minute exudate sample aspirated from the joint of a young child in a large volume of liquid medium contained in the BCV diluted the noxious effect of the exudate, improving the growth of fastidious *K. kingae* organisms (17, 18). When synovial fluid samples obtained from Israeli and French children with arthritis were inoculated into Bactec (13) or BacT/Alert (14) vials, *K. kingae* was detected in one-half of the patients with bacteriologically documented infections.

Despite the noticeable improvement in the isolation of *K. kingae* by the BCV method, the culture detection of the organism remains problematic and the question of which blood culture systems and broth media are best for recovering *K. kingae* remains unsolved (296). In an experiment in which BacT/Alert 3D vials were spiked with different concentrations of a variety of fastidious blood-borne pathogens, *K. kingae* organisms remained undetected despite prolonged incubation (297). In an *in vitro* study aimed to identify factors influencing the isolation of *K. kingae* from BacT/Alert BCVs, it was demonstrated, as expected, that the sensitivity of cultures diminishes and the time to detection increases with low bacterial concentrations (298). Strikingly, the pediatric vial was clearly inferior to the adult one in terms of overall recovery and sensitivity at low bacterial concentrations, and the time to detection was more prolonged, whereas addition of blood partially corrected these deficiencies (298).

Using simulated joint fluid cultures, Høst et al. assessed the

performance of different media belonging to two widely used commercial blood culture systems (BacT/Alert and Bactec 9240) for recovering *K. kingae* from skeletal system infections (292). The researchers inoculated BCVs with pooled synovial fluid aspirated from adult patients with noninfectious conditions and seeded with 24 different *K. kingae* strains (292). The investigated liquid media showed substantial differences in terms of sensitivity: all 24 strains were recovered by the BacT/Alert Aerobic and BacT/Alert Pedi-bacT vials, compared to 21 (87.5%) detected in the BacT/Alert FAN aerobic bottles and only 15 (62.5%) detected by the Bactec Peds Plus vials (292). Detection was also significantly earlier with the BacT/Alert vials, implying that this system is possibly superior to the comparator and could potentially improve the detection of *K. kingae*, reducing the fraction of cases of culture-negative septic arthritis. However, these experimental results have not been validated by employing authentic specimens from pediatric patients with arthritis (289, 292).

Detection by Nucleic Acid Amplification Assays

Because of the potential risk for severe complications and long-term functional disability of septic arthritis and osteomyelitis in childhood, prompt laboratory confirmation and early administration of effective antimicrobial therapy are of paramount importance to prevent late sequelae and even death. However, a substantial fraction of pediatric skeletal infections remain bacteriologically unconfirmed, and, in any case, isolation, complete identification of the culprit organism, and antibiotic susceptibility testing require a minimum of 2 to 3 days. In recent years, the use of NAA assays has allowed identification of the etiological agents of joint and bone infections within 24 h (299, 300). This approach consists of DNA extraction from clinical specimens, followed by incubation with "universal primers" that anneal to conserved portions of the 16S rRNA gene, resulting in the amplification of the intervening species-specific sequence (186, 301). The amplification products are then sequenced and the results matched with data deposited in GenBank or other comprehensive genomic databases, or the products are hybridized with organism-specific probes. Alternatively, with clinical samples a PCR procedure may be performed by employing primers that selectively target the most probable bacteria (i.e., *K. kingae* in children younger than 4 years and *S. aureus* in older patients).

In a comparative study, Rosey et al. inoculated BCVs and seeded samples of synovial fluid onto solid medium, resulting in the isolation of *K. kingae* in 6 of 94 (6.4%) children, whereas a combination of conventional and real-time PCR with broad-spectrum primers disclosed 15 additional cases. These results clearly demonstrated the superiority of the molecular methods and indicated that a substantial proportion of culture-negative pediatric arthritis may be attributed to *K. kingae* (302).

Verdier et al. conducted a large study in which conventional cultures on solid medium and use of BCVs confirmed the diagnosis of suppurative arthritis or osteomyelitis in exudate specimens from 64 of 171 (37.4%) children, of which 9 grew *K. kingae* (303). The 107 culture-negative specimens were then subjected to a PCR procedure targeting the universal 16S rRNA genes and 15 samples were positive, all for *K. kingae* (303). Juretschko et al. compared the yield of Bact/Alert FAN or PEDs-F BCVs with that of real-time PCR in children with osteoarticular infections (158). The BCVs were positive in only 5 of the 21 (23.8%) joint fluid aspirates in which *K. kingae* was detected by the NAA assay (158).

In recent years, NAA tests that target *K. kingae*-specific DNA sequences such as *cpn60* (encoding the chaperonin 60 protein) or the RTX toxin-encoding genes have been introduced into clinical practice with the aim of improving detection (15, 16, 88–90, 92, 151, 160). The relative merits of universal and *K. kingae*-specific primers were compared *in vitro* by Cherkaoui et al., who found that the PCR assay targeting the *rtxA* gene had a sensitivity of 30 CFU, one order of magnitude higher than that of the seminested broad-range 16S rRNA gene amplification (91). These experimental results were confirmed in an *in vivo* study by Ferroni et al. (151), who found that in 9 of 44 (20.5%) cases of PCR-proven *K. kingae* infections of the skeletal system, amplification with species-specific primers targeting the *cpn60* gene succeeded, while the universal 16S rRNA gene primers failed. Because of the predominant role of *K. kingae* in children younger than 4 years with joint or bone infections, the authors recommended the use of the *K. kingae*-specific primers as the first-line test in this population segment, reserving the use of broad-spectrum primers for cases with negative species-specific PCR results.

Chometon and coworkers sequentially analyzed bone and joint specimens using the BCV method, conventional PCR with broad-spectrum primers, and real-time PCR with primers derived from the *K. kingae cpn60* gene, increasing the detection rate from 29% to 41% and 45%, respectively (16). These results placed *K. kingae* as the prime agent of pediatric skeletal system infections, being responsible for approximately 80% of all suppurative arthritis and osteomyelitis cases in children aged less than 3 years (16).

Ilharreborde et al. enrolled 89 children with presumptive joint infections, inoculated synovial fluid aspirates into BCVs, and analyzed the specimens by real-time PCR employing primers that amplify *K. kingae*'s *cpn60* gene (15). The diagnosis of suppurative arthritis was established by culture in 36 samples (40%), of which 7 grew *K. kingae*, whereas the NAA test disclosed 24 additional cases of *K. kingae* infection among the 53 children in whom the exudate cultures were negative. Of note, the PCR assay detected *K. kingae* DNA sequences in all 7 culture-positive samples and was negative in all those in which other microorganisms were recovered by culture, confirming the specificity of the molecular assay. Altogether, *K. kingae* was detected in 31 (52%) of 60 bacteriologically proven cases (15).

In a large study conducted in a French pediatric orthopedic unit, bone and joint exudates from children with suspected skeletal system infections were inoculated into BacT/Alert BCVs and subjected to amplification with primers that amplify the species' *cpn60* gene. *Kingella kingae* was detected by BCVs and/or NAA tests in 35 of 46 (76.1%) children aged <4 years with septic arthritis and in 9 of 17 (52.9%) children in the same age range with osteomyelitis or concurrent septic arthritis and osteomyelitis (151). The standard culture on solid medium failed to recover the organism in all 44 patients, while the BCVs were positive in only 5 specimens (including four exudates and one blood culture), and in 39 of the 44 patients (88.6%) *K. kingae* infection was diagnosed by NAA only (151).

A sequential approach was employed by Lévy et al. in patients with culture-negative osteoarticular infections. Initially, the skeletal system exudate was subjected to amplification of the 16S rRNA gene, and the amplicons resulting from positive tests were sequenced (152). *Kingella kingae* represented 8% of the organisms thus identified and was found exclusively in children. Positive results for *K. kingae* obtained with the broad-range PCR primers

TABLE 1 Performance of culture methods and nucleic acid amplification assays for detecting *Kingella kingae* in children with skeletal system infections[a]

Yr	Reference	Culture method	No. with positive *K. kingae* culture/total	PCR method	Target gene		No. with positive PCR/total
					C-PCR	RT-PCR	
1998	301	C	0/1	C	16S rRNA gene	NA	1/1
2003	186	C + BCV	13/18	C	16S rRNA gene	NA	18/18
2005	303	C + BCV	9/24	C	16S rRNA gene	NA	24/24
2007	302	C + BCV	6/21	C + RT	16S rRNA gene	16S rRNA gene	20/21
2007	16	C + BCV	17/39	C + RT	16S rRNA gene	*cpn60*	39/39
2008	200	BCV	2/4	C + RT	16S rRNA gene	*cpn60*	4/4
2008	206	C	0/1	C	16S rRNA gene	NA	1/1
2009	15	C + BCV	7/31	RT	ND	*cpn60*	31/31
2009	88	C	0/2	C + RT	16S rRNA gene	*rtxA* + *rtxB*	2/2
2009	158	C + BCV	5/18	RT	ND	?	18/18
2010	89	C	0/23	RT	ND	*rtxA* + *rtxB*	23/23
2011	90	ND	2/20	RT	ND	*rtxA*	20/20
2013	151	C + BCV	5/44	RT	ND	*cpn60*	43/44
2013	152	C	?	C + RT	16S rRNA gene	*cpn60*	11/11
2014	160	C + BCV	2/24	C + RT	ND	*rtxA*	27/27
2014	304	C	0/3	RT	ND	*cpn60*	3/3
2014	305	C + BCV	0/1	RT	ND	*cpn60*	1/1
Total			68/274 (24.8%)				388/390 (99.5%)

[a] Abbreviations: C, conventional; BCV, blood culture vial; RT, real time; ND, not done; NA, not applicable.

were confirmed in all cases by amplification of specific *cpn60* gene sequences (152).

In a study employing a multiplex PCR assay designed for the simultaneous diagnosis of *K. kingae* and *S. aureus* infections, *K. kingae*'s *cpn60* gene was amplified from synovial fluid specimens obtained from three of six children younger than 5 years with arthritis, while cultures of the exudate on solid medium were negative in all cases (304). Of note, none of the 105 synovial fluid samples aspirated from adult patients was positive for *K. kingae* (304).

In a recent report, the NAA test targeting the *rtxA* gene performed in the synovial fluid aspirate of a child with arthritis was still positive 5 days after onset of antibiotic treatment, indicating that the diagnosis of *K. kingae* infection may be possible in patients with partially treated disease (305).

The available published experience comparing the performances of the culture approach and NAA methods for detecting *K. kingae* skeletal infections is summarized in Table 1. These results convincingly demonstrate that the isolation of *K. kingae* remains inadequate, even when specimens are seeded into BCVs, and that the molecular diagnostic tools increase the detection rate by 4-fold. The NAA methods substantially improve the diagnosis of *K. kingae* disease, reduce the frustrating fraction of unconfirmed infections, and show that the organism is the leading bacterial etiology of skeletal infections in young pediatric patients.

ANTIBIOTIC SUSCEPTIBILITY

Kingella kingae has been traditionally considered highly susceptible to antibiotic drugs that are administered to patients with presumptive or proven bacterial diseases (306, 307), whereas β-lactamase production has been uncommonly reported in the species (136, 187). The enzyme, a TEM-1 β-lactamase, is usually encoded on a plasmid (308), but it has been found to be chromosomally integrated in a strain recovered from the oropharynx of a 2-year-old French child with culture-negative arthritis and from her older brother (309). The presence of β-lactamase in *K. kingae* isolates increases the ampicillin MIC to the range of 0.25 mg/liter to 8 mg/liter, with a MIC_{50} of 2 mg/liter and a MIC_{90} of 4 mg/liter. The enzyme does not hydrolyze cephalosporin drugs, and its activity is inhibited by clavulanic acid (309).

In a large study in which β-lactamase production and genomic clonality were investigated in a large sample of *K. kingae* isolates from Israeli individuals with clinical infections and asymptomatic pharyngeal carriers, the enzyme was detected in only 2 of 190 (1.1%) invasive isolates from patients with bacteremia, arthritis, osteomyelitis, or endocarditis and in 68 of 428 (15.9%) randomly chosen carriage organisms ($P < 0.001$), suggesting that the *K. kingae* strains that express β-lactamase are less capable of invading the bloodstream and deep host tissues and confirming that improved colonization ability does not necessarily imply enhanced virulence (60). These findings also imply that antibiotic resistance may confer a biological advantage to *K. kingae* organisms inhabiting in the pharynx in early childhood, overlapping the period in life of enhanced prevalence of oropharyngeal colonization and highest antimicrobial drug consumption (18, 85, 101, 310).

Production of β-lactamase among Israeli isolates was found to be limited to four distinct PFGE clones, and all members of these clones expressed the enzyme (60). A recent study confirmed the clonality of the trait in isolates from Reykjavik (Iceland) and Minneapolis, MN (USA) (61). Mating experiments indicated that despite the fact that the plasmid is conjugative, heterologous *K. kingae* strains were unable to keep it and produce β-lactamase for more than a few passages (308), meaning that additional requirements from the recipient cell are needed to retain the plasmid, thus limiting the possibility of its transfer to additional strains and explaining the strict clonality of β-lactamase production observed worldwide.

The proportion of β-lactamase-producing *K. kingae* isolates

varies widely among countries. It is exceptionally detected in continental Europe, even in countries with high antibiotic consumption such as Spain and France, and in the Montreal area of Canada, but is widespread among carried and invasive isolates from Minneapolis, MN, and invasive isolates in Reykjavik, Iceland (61). Because data on the local prevalence of the enzyme and geographic distribution of producing clones are scarce and incomplete, routine testing of all invasive *K. kingae* isolates for β-lactamase production is strongly advised (61).

Strikingly, the strain responsible for β-lactamase production in Minnesota is identical by PFGE and MLST to that detected in Iceland and exhibits the same *rtxA* and *por* gene alleles (61, 308). The genotypic identity of *K. kingae* isolates derived from these two remote and relatively secluded geographic locations suggests person-to-person spread among the large community of Minnesota residents of Icelandic descent and the ancestral homeland population, although the direction and the time of the transoceanic strain's journey are unknown.

With rare exceptions, *K. kingae* is susceptible to aminoglycosides, macrolides, (191), trimethoprim-sulfamethoxazole (311), fluoroquinolones, tetracycline, and chloramphenicol (18, 130, 312–315). *Kingella kingae* exhibits relatively high MICs to oxacillin (MIC_{50}, 3 μg/ml; MIC_{90}, 6 μg/ml), 40% of invasive isolates are clindamycin nonsusceptible, and all strains are highly resistant to glycopeptide antibiotics, a serious concern in regions where joint and bone infections caused by community-associated methicillin-resistant *S. aureus* are prevalent and clindamycin or vancomycin is initially prescribed to children with skeletal system infections, pending culture and antibiotic susceptibility testing results (306, 316, 317).

TREATMENT

Because no specific protocols for managing invasive *K. kingae* disease have been formulated, patients have been empirically administered a wide array of antimicrobial regimens developed for treating infections caused by other bacterial pathogens. The empirical drug regimens for septic arthritis and osteomyelitis in young children generally combine the parenteral administration of a penicillinase-stable β-lactam antibiotic such as oxacillin and a broad-spectrum second-generation (cefuroxime) or third-generation (ceftriaxone) cephalosporin (307, 316, 317). This initial antibiotic therapy is usually swapped to intravenous ampicillin, once *K. kingae* is convincingly identified and β-lactamase production is ruled out, or to cefuroxime. A favorable clinical response and decrease of serum CRP levels below 20 mg/liter guide switching to oral antibiotics and help to determine the duration of therapy (317). Traditionally, patients with *K. kingae* arthritis have been administered antibiotics for a total of 2 to 3 weeks. Recent studies, however, suggest that a short 10-day course of sequential parenteral and oral antibiotic drugs is adequate for uncomplicated joint infections caused by Gram-positive pathogens, but the experience with this novel approach for treating *K. kingae* arthritis is still limited (317). Although repeated aspirations and lavage of the joint space have been performed in a few children (220), most patients can be adequately managed with antibiotics only (18).

Children with *K. kingae* osteomyelitis have received antibiotic therapy for 3 to 6 weeks and those with spondylodiscitis from 3 to 12 weeks (18). Patients with occult *K. kingae* bacteremia have generally been prescribed penicillin or cephalosporin drugs by the intravenous route, which were switched to oral antibiotics once the clinical condition stabilized and endocardial involvement was excluded. The duration of antibiotic therapy has ranged from 1 to 2 weeks with excellent results (18). Patients with *K. kingae* endocarditis are administered a high dose of intravenous β-lactam antibiotic as monotherapy or combined with an aminoglycoside drug for 4 to 7 weeks (18). In the past, patients with central nervous system infections caused by *K. kingae* were treated with a penicillin drug alone (139) or in combination with chloramphenicol (278, 280), but currently, a third-generation cephalosporin (cefotaxime or ceftriaxone) as monotherapy or in combination with an aminoglycoside for 10 days to 4 weeks is preferred (276, 277, 279).

PREVENTION

As is the case with other pathogens of respiratory origin, the population of asymptomatic carriers at any given time is huge compared to that of diseased individuals. Despite a substantial prevalence of *K. kingae* carriage in children, the rate of attack of invasive disease is relatively low (93, 102), and therefore, there is no indication to eradicate the organism from the colonized mucosal surfaces of healthy individuals. However, the risk of acquisition of *K. kingae* with progression to a severe and even life-threatening infection appears to be greatly increased among youngsters in day care. When the available information on the 6 outbreaks of disease occurring in day cares is pooled, a total of 18 of 122 (14.8%) classmates developed a documented or presumptive *K. kingae* infection, including fatal endocarditis and meningitis, within a 1-month period (98, 112, 113, 120, 121). Under these circumstances, prophylactic antibiotics aimed to eradicate colonization in contacts and prevent further cases of disease were usually offered to the population at risk. The choice of rifampin relied on the exquisite susceptibility of *K. kingae* (98, 312), the fact that the antibiotic is secreted in saliva and respiratory secretions and therefore high antibiotic concentrations may be achieved in the oropharyngeal mucosa, and the successful experience with the drug in the suppression of respiratory colonization by *H. influenzae* type b and *N. meningitidis* and prevention of invasive infections in day care facilities (318, 319). Rifampin at a dosage of 20 mg/kg/day in two divided doses for 2 days, alone (98, 120), or in combination with amoxicillin (80 mg/kg/per day) in two divided doses for two (113) or 4 days (112), was administered. The effectiveness of these regimens, however, was usually limited. On average, eradication of pharyngeal carriage among colonized day care attendees who completed the prescribed regimen was 81.1%, ranging from only 31.2%, as determined by a sensitive NAA (98), to 100%, as established by culture (121), and renewed colonization by the original strain was subsequently observed in a few children. It should be underscored that persistence of the organism was not caused by acquisition of resistance by the infecting *K. kingae* strain to the administered antibiotic regimens (98, 120). Inadequate compliance and/or failure to provide antibiotic prophylaxis to young siblings could have resulted in partial elimination of the reservoir, which was followed by renewed spread of the strain in the day care population (98, 319). Because antibiotics have been relatively unsuccessful in eliminating *K. kingae* carriage, the mandatory administration of prophylaxis to healthy contacts in the day care setting has been disputed (93). It should be noticed, however, that after administration of antibiotic prophylaxis, no new cases of disease occurred in the index facilities, even when only incomplete suppression of the causative strain was achieved (98, 112, 113, 120,

121). It is possible that reduction of colonization by antibiotic administration, even if temporarily, was enough to prevent transmission to additional attendees. Alternatively, prolonged mucosal carriage could have elicited an effective antibody response, decreasing the individual risk of developing an invasive infection and inducing protective herd immunity.

CONCLUDING REMARKS

As the result of the adoption of sensitive detection techniques, especially the BCV and NAA methods, and increasing acquaintance of clinical microbiology laboratories with its identification, *K. kingae* has emerged as an important etiology of invasive pediatric infections in the last decades. Appreciation of the pathogen as a frequent cause of bacteremia and the prime cause of skeletal system infections in young children, as well as endocarditis in pediatric and adult patients, has spurred research on the pharyngeal carriage of *K. kingae* by the healthy population, the interpersonal transmission of the bacterium, and the role of colonization in the pathogenesis of invasive disease. Investigation of the epidemiological and molecular aspects of asymptomatic *K. kingae* colonization has partially elucidated the complex mechanisms involved in the persistence and dissemination of the bacterium in the population, showing striking similarities between *K. kingae* and traditional bacterial members of the upper respiratory mucosal microbiome. It is to be expected that further research will reveal genomic traits responsible for dissimilarities in the colonization fitness, invasiveness, and tissue tropism exhibited by different *K. kingae* strains, disclose host factors that promote the transition from asymptomatic carriage to invasive disease, and identify bacterial antigens able to induce a protective immune response.

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