High Prevalence of *Kingella kingae* in Joint Fluid from Children with Septic Arthritis Revealed by the BACTEC Blood Culture System

PABLO YAGUPSKY,¹* RON DAGAN,² CHARLES W. HOWARD,³ MENACHEM EINHORN,² IMAD KASSIS,³ AND ALEXANDER SIMU¹

Clinical Microbiology Laboratory, ¹Pediatric Infectious Disease Unit,² and Pediatric Orthopedic Unit,³ Soroka Medical Center, Beer-Sheva, Israel

Received 19 August 1991/Accepted 24 February 1992

In an effort to improve detection of fastidious organisms, joint fluid aspirates of pediatric patients were inoculated into BACTEC 460 aerobic blood culture bottles, in addition to cultures on solid media. Culture records for the 1988 to 1991 period were reviewed to compare the performance of both methods for the recovery of pathogens. Overall, 216 children underwent a diagnostic joint tap, and 63 specimens grew significant organisms, including *Kingella kingae* in 14. While both methods were comparable for recovery of usual pathogens, with a single exception, *K. kingae* isolates were detected by the BACTEC system only. *K. kingae* appears to be a more common cause of septic arthritis in children than has been previously recognized. The BACTEC blood culture system enhances the recovery of *K. kingae* from joint fluid and improves bacteriologic diagnosis of pediatric septic arthritis.

In the preantibiotic era, most cases of septic arthritis were caused by streptococci and staphylococci (1, 50). After the advent of antimicrobial therapy, *Staphylococcus aureus* remained the most common cause of septic arthritis in childhood, while routine inclusion of chocolate agar media for culture of synovial fluid specimens in the last decades resulted in increased recovery of *Haemophilus influenzae* type b and its recognition as a major etiologic agent of septic arthritis, especially in the 6-month to 2-year age group (1-4, 11, 15-17, 19, 23, 24, 26-28, 30-32, 34, 35, 41-43, 50). Nowadays, despite advances in bacteriological methodology, cultures of synovial fluid remain negative in a substantial fraction of patients with clinical septic arthritis (2, 4, 11, 23, 34, 35, 41, 42).

In an attempt to improve the recovery of fastidious organisms from joint fluid aspirates of children with arthritis, the radiometric BACTEC blood culture system has been used in our institution during the last 3 years. Adoption of this practice has resulted in a dramatic change in the spectrum of the detected microorganisms.

**MATERIALS AND METHODS**

**Background.** The Soroka Medical Center is a tertiary-care, 750-bed university hospital located in the city of Beer-Sheva, southern Israel. Soroka Medical Center is the only hospital in this desertic region of the country and serves a population of about 320,000 inhabitants, of whom 150,000 are children and adolescents younger than 18 years of age (8a).

Three-fifths of the population of the area are of Jewish origin, and the remaining members are Bedouin Arabs who live in small suburban settlements or in transition from seminomadic conditions to permanent settlements.

Pediatric patients who presented to the hospital with arthritis underwent diagnostic aspiration of the involved joint(s) under strict sterile conditions, as recommended elsewhere (22, 48). Synovial fluid specimens were sent for bacteriological culture as well as for cytologic and biochemical determinations.

**Bacteriological methods.** (i) Conventional cultures. Joint fluid aspirates were plated by the medical staff onto sheep blood agar and chocolate agar media at the patients' bedside or were sent to the Clinical Microbiology Laboratory, where specimens were inoculated onto 10 ml of thioglycolate broth and blood agar, chocolate agar, MacConkey's, and New York City media (Hy-Labs Laboratories, Rehovot, Israel). Plates were incubated at 35°C in a CO₂-enriched atmosphere and examined daily for 2 days. Plates showing no growth after the second day were sealed with masking tape and examined once per week for a total of 3 weeks.

(ii) BACTEC cultures. Starting in 1988, the medical staff was instructed to inoculate a portion of the synovial fluid into a BACTEC 460 (Johnston Laboratories, Towson, Md.) aerobic 6B blood culture bottle. Growth indices were radiometrically monitored once per day on day 1; twice per day on day 2; once per day on days 3, 4, and 5; and then once per week for 2 additional weeks. Bottles showing a growth index of >20 U and/or an increment of >10 U between two consecutive readings were sampled. A Gram stain examination was performed, and the broth was subcultured on blood, chocolate, MacConkey's, New York City, and Mueller-Hinton agars. Identification of microorganisms recovered was performed according to standard bacteriological procedures (22). Terminal subcultures of radiometrically negative bottles were not performed.

Records of the Clinical Bacteriology Laboratory for the 43-month period from 1 January 1988 to 31 July 1991 were reviewed to compare the performance of conventional and BACTEC cultures of synovial fluid for the recovery of microorganisms from patients younger than 18 years of age. Paired conventional and BACTEC cultures were not available for all specimens.

Isolation of *Bacillus* spp., coagulase-negative staphylococci, diphtheroids, and alpha-hemolytic streptococci in one or both types of cultures was considered indicative of contamination. For the purposes of the comparison between
the two culture methods for the detection of true pathogens, cultures yielding contaminants were included among those with negative results. For patients in whom more than one joint tap was done, only results of the first aspiration were considered in the data analysis.

RESULTS

During the 43-month period, 216 cultures of synovial fluid, obtained from children and adolescents, were processed by the Clinical Microbiology Laboratory. Significant microorganisms were recovered from 63 (29.2%) specimens (Table 1).

Pathogens were recovered from 57 of 189 (30.2%) BACTEC cultures and from 21 of 127 (16.5%) conventional cultures (P = 0.008). Pathogens (excluding Brucella melitensis) were detected by BACTEC cultures after an average of 2.5 days (median, 2 days), and 43 of 45 (95.6%) were detected before day 5. Kingella kingae was detected within 1 to 10 days (mean, 3.7 days; median, 3.5 days). Two of 11 B. melitensis isolates were detected on day 7, and the remaining isolates were detected at the end of the second week. Contaminants were detected in 20 BACTEC cultures after a mean of 9.3 days, and only seven of them (35.0%) were detected before day 5. Conventional cultures usually yielded visible bacterial growth after 1 to 2 days of incubation and by the end of the 3-week period were almost uniformly heavily contaminated.

For 27 of 216 specimens, only conventional cultures were performed, and 3 (11.1%) were positive for pathogens (S. aureus, Neisseria meningitidis group Y, and Escherichia coli in 1 specimen each); for 89 only BACTEC cultures were performed, and 26 (29.2%) were positive (S. aureus in 11, B. melitensis in 8, K. kingae in 3, Streptococcus pyogenes in 2, and Streptococcus group C and Streptococcus pneumoniae in 1 specimen each).

For the remaining 100 specimens, were performed by both methods (Table 2). Thirty-four grew organisms considered to be of clinical significance, and the remaining 66 were negative or contaminated. Both methods were comparable for the recovery of most pathogens; however, 10 of 11 K. kingae isolates were recovered from BACTEC cultures only.

K. kingae isolates exhibited the well-known cultural and biochemical characteristics of the species. Two isolates were sent to the Division of Bacterial Diseases, Centers for Disease Control, Atlanta, Ga., and their presumptive identification as K. kingae was confirmed. Two strains of K. kingae were isolated in 1988, four were isolated in 1989, five were isolated in 1990, and three were isolated in 1991, with no seasonal pattern. Blood cultures were performed for all 14 patients in whom K. kingae was recovered from synovial fluid, and all were negative. K. kingae was not recovered from any of more than 80,000 blood cultures processed by the BACTEC system during the entire period of the study, excluding the possibility of laboratory contamination. Involvement of Jewish as well as Bedouin children, living separately in totally different conditions, seems to exclude a common source of exposure.

DISCUSSION

Even when modern bacteriological techniques are used, including antigen detection tests, and blood and other normally sterile body fluids are cultured, bacterial etiology of suspected septic arthritis remains unproved in one-third of cases (1, 2, 4, 5, 11, 15, 23, 27, 28, 30, 31, 34, 35, 41, 42, 47, 50).

It can be argued that in some of these culture-negative cases, the diagnosis of septic arthritis was incorrect, patients received antimicrobial therapy before cultures of joint fluid were obtained, or the arthritis was of a reactive nature (5, 27, 41). The strikingly consistent portion of bacteriologically unproved cases found in different series published worldwide suggests that some cases of septic arthritis may be caused by microorganisms that are not detected by currently available techniques.

The effects of the methodology employed upon the detection of organisms have been exhaustively investigated in blood cultures. The importance of drawing a large blood sample has been clearly demonstrated, as has the crucial role played by inoculation of blood samples into a large volume of broth to dilute complement, antibiotics, antibodies, and other factors that may be detrimental to the growth of microorganisms (49). Although it has long been known that pus exerts a bacteriostatic effect on microbial growth, most clinical microbiology textbooks and reviews do not devote much attention to the methodology required for optimal culture of synovial fluid and other exudates (19, 22, 48).

In 1966, Nelson and Koontz proposed the inoculation of joint fluid into enriched broth to Improve the disappointing recovery rate of organisms, although they did not explicitly mention the use of blood culture systems for this purpose (32). The original recommendation, however, was not repeated in the follow-up report published a few years later (31). In Baron and Finegold's diagnostic microbiology text,
inoculation of blood culture media is advised, especially for cases in which specimens cannot be delivered to the laboratory immediately (3), but it seems that the idea did not gain general acceptance.

In the last decade, use of blood culture systems for the detection of organisms from joint fluid aspirates from patients with suspected arthritis has been recommended by Finnish investigators (20, 39, 47). Experience with this strategy remains, however, limited. Von Essen and Holta have shown that inoculation of synovial fluid into conventional blood culture bottles resulted in positive joint fluid cultures in 10 of 41 episodes of septic arthritis that were negative by conventional culture methods (47). The usefulness of this approach was particularly noticeable for those patients who had been previously treated with antimicrobial agents. False-negative culture rates were reduced by 40% by use of this novel technique (47). In addition, culture of joint fluid aspirates in blood culture bottles allowed recovery of a wider range of organisms, including fungi, coagulase-negative staphylococci, S. aureus, streptococci, Neisseria spp., and K. kingae in one case (20, 39, 47).

The results of the present study show that use of the BACTEC blood culture system improves the recovery of microorganisms from synovial fluid aspirates of children and adolescents with arthritis. For cultures for which both conventional and BACTEC culture results were available, if conventional cultures had been omitted, only 3 of 34 (8.8%) positive cultures would have been missed, and if the BACTEC cultures had been omitted, 16 of 34 (47.1%) positive cultures would have been missed (Table 2).

It should be noted that according to the guidelines in use in our institution for the evaluation of children with arthritis, every synovial fluid specimen is sent for bacteriological culture. Inclusion of synovial fluid specimens from patients with diseases other than septic arthritis (i.e., trauma, reactive arthritis, or collagen disorders) will necessarily lower the fraction of positive cultures. The overall recovery rate of pathogens in the present study (29.2%) is thus lower than that reported in most series of pediatric septic arthritis.

Close examination of results reveals that the availability of the blood culture system is limited to the isolation of K. kingae. In our pediatric population, the organism appears to be second only to S. aureus and much more common than streptococci and H. influenzae type b as the etiologic agent of septic arthritis. When both conventional and BACTEC cultures were performed, the frequency of detection of K. kingae was equal to that of S. aureus. This finding is especially striking against the background of the scarcity of reports of infections caused by this organism. K. kingae has been isolated from the respiratory tract of 1.2% of healthy persons (21). The true prevalence of the organism is probably higher because of the difficulties in distinguishing it from other components of the resident flora (21). K. kingae has not been reported as part of the normal flora or as a cause of infection in lower animals, and thus, the source of human infection appears to be endogenous (36). It has been postulated that, similar to the mechanism described for N. meningitidis infections, K. kingae initially colonizes and infects the mucous membranes of the nasopharynx. Subsequent blood stream invasion results in localization of the organism in remote sites, especially heart valves and joint and disk spaces (29, 44).

K. kingae has been uncommonly isolated from persons with endocarditis, osteomyelitis, septic arthritis, and diskitis and as a rare cause of bacteremia, oropharyngeal infections, meningitis, abscesses, and pleural empyema (18, 29). To the best of our knowledge, there have been fewer than 30 published cases of septic arthritis due to K. kingae (6–10, 12–14, 25, 29, 33, 36–40, 44–46). In the series of 221 pediatric patients with septic arthritis reported by Nelson and Koontz (31) and in that comprising 95 patients reported by Welkon et al. (50), K. kingae was not mentioned among the isolated organisms. The two isolates of Moraxella spp. among the 111 patients reported by Barton et al. (4), however, raise the question of misidentified K. kingae. It should be kept in mind that in all these series, bacterial etiology remained undetermined in a large number of cases. The results of the present study strongly suggest that undetected K. kingae infections may be responsible for a substantial portion of these culture-negative cases.

The reasons for the superiority shown by the BACTEC system over conventional cultures in solid media for the isolation of K. kingae are not clear. Since only small volumes of fluid can be accommodated in a petri dish while up to 5 ml can be inoculated into a BACTEC blood culture bottle, a volume-related effect cannot be ruled out. K. kingae isolates, with only one exception, failed to grow on the primary plates but were detected in BACTEC bottles within 2 to 3 days. The organism, however, grew in subcultures on blood and chocolate media without difficulty, sometime after overnight incubation, excluding the possibility that routine solid media do not support growth of K. kingae. It is postulated that purulent joint fluid exerts an inhibitory effect upon the organism. Dilution of a detrimental factor(s) in a large volume of broth below their inhibitory concentration substantially enhanced the chances to recover K. kingae. If the dilutional effect is in fact crucial for the successful recovery of the organism, it may be postulated that for this purpose, any blood culture system should perform as well as the BACTEC system.

It is concluded that K. kingae is a much more common cause of septic arthritis than has been previously recognized. Use of the BACTEC blood culture system contributes significantly to the detection of this pathogen from synovial fluids of children with septic arthritis.

REFERENCES
DETECTION OF K. KINGAE IN PEDIATRIC SEPTIC ARTHRITIS