JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1997, p. 2410–2412 Vol. 35, No. 9
0095-1137/97/$04.00+0

Use of the Isolator 1.5 Microbial Tube for Culture of Synovial Fluid from Patients with Septic Arthritis

P. YAGUPSKY[1]* AND J. PRESS[2]

Clinical Microbiology Laboratories[1] and Pediatric Rheumatology Unit, Pediatric Department,[2] Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Received 3 February 1997/Returned for modification 17 April 1997/Accepted 28 May 1997

Synovial fluid specimens obtained from patients with arthritis were plated onto solid media (conventional cultures) or inoculated into an Isolator 1.5 microbial tube (Isolator cultures), and the yield and time to detection of organisms were compared. Overall, 144 specimens obtained from 137 patients were processed, and 31 (21.5%) cultures obtained from 29 patients were positive by at least one method. *Staphylococcus aureus* was isolated from 12 patients, *Streptococcus pneumoniae* and *Kingella kingae* were isolated from 4 patients each, group G streptococci were isolated from 3 patients, *Staphylococcus epidermidis* and members of the family *Enterobacteriaceae* were isolated from 2 patients each, and *Streptococcus mitis* and *Peptostreptococcus prevotii* were isolated from 1 patient each. Overall, the causative organism was detected in 31 of 31 (100.0%) Isolator cultures and 24 of 31 (77.4%) conventional cultures ($P < 0.02$). Twenty-nine of 31 (93.5%) positive Isolator cultures and 20 of 24 (83.3%) conventional cultures were positive by the second day of incubation. Among the 24 cultures positive by both methods, higher numbers of CFU per milliliter were detected with the Isolator system in 13 cultures and with conventional cultures in 2 cultures ($P < 0.002$). Inoculation of synovial fluid into an Isolator 1.5 microbial tube improves the recovery of organisms causing septic arthritis.

Septic arthritis, the name given to an infectious process involving the joint space, is an important clinical condition which, if neglected, may result in permanent disability (1, 7, 8). Because of the serious implications of delayed diagnosis of septic arthritis, patients with suspected articular infections are usually given empirical antimicrobial therapy based on clinical considerations and cytological and biochemical examination of the joint fluid aspirate (8–11). Detection of the causative microorganism remains important to confirm the diagnosis and to guide the antimicrobial regimen (5, 8). Culture of synovial fluid of patients with suspected septic arthritis, even when performed prior to the initiation of antibiotics, is frequently disappointing (1, 5, 7, 14).

In an attempt to improve the bacteriological diagnosis of children with septic arthritis, we have introduced the inoculation of synovial fluid into an aerobic (NR6A) BACTEC blood culture bottle (Becton Dickinson Diagnostic Instruments Systems, Towson, Md.) as a routine procedure. Adoption of this approach has resulted in the unexpected enhanced detection of *Kingella kingae* and the recognition of this organism as one of the most common etiologies of skeletal infections in young children (15).

In recent years, the Isolator 1.5 microbial tube (Wampole Laboratories, Cranbury, N.J.) has become commercially available for processing blood cultures obtained from pediatric patients (3). The system consists of a tube containing a mixture of saponin, polypropylene glycol, and sodium polyanethole sulfonate that prevents clot formation, causes rapid lysis of erythrocytes and leukocytes, and inhibits complement. The Isolator system has been recognized as a sensitive method for the detection of a wide array of organisms in blood cultures and is particularly useful in the isolation of obligatory and facultative intracellular pathogens, such as mycobacteria, yeasts, and staphylococci (2–4, 6).

A prospective study was carried out between 1 July 1992 and 31 December 1996 to compare the performance of the Isolator 1.5 microbial tube, inoculated with joint tap fluid, with that of conventional cultures for the microbiologic diagnosis of septic arthritis.

Adult and pediatric patients with clinical arthritis, who were treated at the Soroka Medical Center in southern Israel, underwent a diagnostic joint tap. Synovial fluid was aspirated from the affected joint by a strict sterile technique, as recommended (8). Specimens of fluid from the joint aspirate were sent without delay to the Microbiology Laboratory in a sterile syringe sealed with a sterile plastic top. Once in the laboratory, the amount of fluid was measured and recorded, and the specimen was divided into two aliquots. Small volumes of fluid were transferred to an insulin syringe for precise measurement. Half of the specimen was plated onto chocolate-agar plates and Trypticase soy agar plates supplemented with 5% sheep blood supplied by a commercial source (Hy-Labs, Rehovot, Israel) (conventional cultures), and the remaining fluid was inoculated into an Isolator 1.5 microbial tube (Isolator cultures). This pediatric tube was chosen instead of the large adult Isolator tube because, in our experience, only small amounts of synovial fluid are sent for microbiological examination, and according to the manufacturer's recommendation, volumes less than 6 ml should not be placed in the adult tube because of the inhibitory effect of the lytic agents. The Isolator tube was processed in a biological safety cabinet, according to the methodology recommended for blood cultures. The lysate was plated onto solid media similar to those described for conventional cultures. Up to 0.25 ml of synovial fluid or lysate was dispersed on each plate. When volumes smaller than 0.25 ml were received, a single chocolate-agar plate was seeded. Conventional and Isolator cultures were incubated at 35°C in a 5% CO_2-enriched atmosphere and examined daily for 1 week.

Identification of recovered organisms was performed according to conventional microbiological procedures. Identifi-

* Corresponding author. Mailing address: Clinical Microbiology Laboratories, Soroka Medical Center, Ben-Gurion University of the Negev, Beer-Sheva 84101, Israel. Phone: (972)-7-6400507. Fax: (972)-7-6403541.

TABLE 1. Comparative results of Isolator and conventional cultures for 29 patients with culture-proven septic arthritis

Patient[a]	Organism	Result by:				
		Isolator culture		Conventional culture		Gram stain
		CFU/ml	Detection (day)	CFU/ml	Detection (day)	
1a	*Staphylococcus aureus*	500	2	10	2	−
1b	*Staphylococcus aureus*	4	2			ND[b]
2	*Staphylococcus aureus*	52	2			−
3	*Staphylococcus aureus*	50	2	25	2	ND
4	*Staphylococcus aureus*	>1,000	2	>1,000	2	+
5	*Staphylococcus aureus*	>1,000	2	>1,000	2	+
6a	*Staphylococcus aureus*	>1,000	2	>1,000	2	+
6b	*Staphylococcus aureus*	>1,000	2	>1,000	2	+
7	*Staphylococcus aureus*	>1,000	2	12	2	−
8	*Staphylococcus aureus*	>1,000	2	44	2	−
9	*Staphylococcus aureus*	<1	2			−
10	*Staphylococcus aureus*	30	2	152	2	−
11	*Staphylococcus aureus*	ND	2	ND	2	+
12	*Staphylococcus aureus*	>1,000	1	67	1	+
13	*Streptococcus pneumoniae*	52	2	30	2	ND
14	*Streptococcus pneumoniae*	28	1			−
15	*Streptococcus pneumoniae*	>1,000	1	>1,000	1	+
16	*Streptococcus pneumoniae*	88	2	60	1	+
17	*Streptococcus* group G	>500	2	10	2	+
18	*Streptococcus* group G	ND	2			ND
19	*Streptococcus* group G	5	3			−
20	*Kingella kingae*	15	2	10	2	+
21	*Kingella kingae*	300	2	16	2	+
22	*Kingella kingae*	11	2			+
23	*Kingella kingae*	80	1	2	1	+
24	*Salmonella enteritidis*	>1,000	1	>1,000	1	+
25	*Escherichia coli*	56	2	8	2	ND
26[c]	*Staphylococcus epidermidis*	280	2	ND	4	−
27[c]	*Staphylococcus epidermidis*	250	2	44	3	ND
28[c]	*Streptococcus mitis*	400	5	200	5	−
29[c]	*Peptostreptococcus prevotii*	200	2	ND	4	−

[a] a, first (diagnostic) joint tap; b, specimen obtained 24 h after onset of antimicrobial therapy.
[b] ND, colony count not done.
[c] Infected prostheses.

cation of coagulase-negative staphylococci was performed with the API Staph strip (Biomerieux, Marcy l'Etoile, France). Isolation of *Bacillus* spp., diphtheroids, alpha-hemolytic streptococci, and coagulase-negative staphylococci, when not recovered from a prosthetic joint, was considered indicative of contamination. Organisms growing out of the streaked area of the plates were also dismissed as contaminants.

The Fisher exact test was used to assess the statistical significance of the observed differences between discordant Isolator and conventional synovial fluid culture pairs.

A total of 144 synovial fluid specimens obtained from 137 different patients (including 92 children and 45 adults) were cultured. Twenty-four cultures were obtained from patients with prosthetic joint devices, and the remaining cultures were obtained from patients with primary arthritis. Total specimen volumes ranged between 0.3 and 6 ml, with a median of 1 ml. Thirty-one (21.5%) synovial fluid cultures obtained from 29 patients (21.2%) were positive by at least one method. Twenty-five of these culture-positive patients suffered from primary septic arthritis, and the remaining 4 had an infected prosthetic device. In 113 cultures (78.5%) drawn from 108 (78.8%) patients, no organisms were recovered by either of the two methods used. The Gram stain examination revealed the causative organisms in 14 of the 25 (56.0%) cultures in which the test was performed.

The bacteriologic results for the 29 culture-positive patients, including concentration of organisms, time for detection, and Gram stain, are summarized in Table 1. In two Isolator cultures and three conventional cultures, quantitative results were not recorded. Overall, all 31 positive cultures were detected by the Isolator 1.5 microbial tube (sensitivity, 100.0%), compared to 24 conventional cultures (sensitivity, 78.9%) ($P < 0.02$). Close examination of conventional culture plates frequently revealed that bacterial growth was inhibited in the area in which most of the synovial fluid specimen was deposited. Among those 24 cultures positive by both methods, higher numbers of CFU per milliliter were detected with the Isolator system in 13 cultures and with conventional cultures in 2 cultures ($P < 0.002$).

The times to detection of positive cultures were similar by both methods. Twenty-nine of 31 (93.5%) Isolator cultures and 20 of 24 (83.3%) conventional cultures were positive by the second day of incubation ($P > 0.05$). Overall, the contamination rates were 7.8% for the Isolator system and 6.4% for conventional cultures ($P > 0.05$).

Attempts to obtain a bacteriologic diagnosis in patients with suspected septic arthritis are frequently unsuccessful, and between one-third and two-thirds of synovial fluid cultures do not detect the causative organisms (1, 5, 7, 14). Examination of conventional plates seeded with synovial fluid specimens frequently shows that purulent exudates exert an inhibitory effect upon bacterial growth, which may explain the overall low sen-

sitivity of synovial fluid cultures in patients with joint infections (12). It seems natural then that blood culture systems in which strategies to overcome the detrimental activity of host defenses are implemented may also prove to be useful for culturing other normally sterile body fluids (13). Inoculation of synovial fluid into the large volume of broth probably dilutes some of these inhibitory factors, explaining the improved detection of synovial fluid cultures performed with the BACTEC system compared to routine plating of joint tap fluid onto solid media (15).

Because the inflammatory response in the infected joint space is usually characterized by accumulation of leukocytes in the order of $>10^5$/ml of synovial fluid, we speculated that active phagocytosis may contribute to the failure to recover bacteria noted in a large fraction of patients with septic arthritis (8). Introduction of the Isolator system was thus considered to be a rational choice when trying to improve the isolation of the causative organisms. Although the numbers presented herein are small, adoption of this practice seems to have enhanced bacterial isolation in cases that would have been missed by conventional cultures. Examination of the quantitative data shows that in those specimens for which precise colony counts were performed, plates seeded with the Isolator lysate usually yielded the largest number of colonies, suggesting that lysis of the leukocytes releases already phagocytized but still viable organisms (2, 4).

In addition, the present study shows that concentration of bacteria in the synovial fluid of patients with septic arthritis may be of low magnitude. It appears that the highest concentration of organisms may be found in patients with infections caused by gram-positive organisms and especially by *Staphylococcus aureus*. The concentration of organisms observed may have contributed to the overall low sensitivity of the Gram stain examination of the joint fluid observed in this and other series of patients with septic arthritis, as well as the frequent failure to detect the causative organism when small volumes of specimen are cultured (5).

Despite the significant contribution to the bacteriologic diagnosis made by the Isolator system, the positivity rate of synovial fluid cultures found in the present study is in the lower range of that found in other series (1, 5, 7, 14). It should be mentioned that in our institution, all synovial fluid specimens are routinely sent for bacteriologic culture, and, therefore, the population included patients with a variety of clinical conditions, whereas other studies included joint fluid from patients with suspected septic arthritis only. It is possible, however, that an unknown fraction of patients with septic arthritis was also missed by the Isolator system. Although inoculation of synovial fluid into an Isolator 1.5 microbial tube enhances the recovery of microorganisms from patients with septic arthritis, additional studies are needed to determine the optimal method or combination of techniques needed to optimize the bacteriological diagnosis of joint infections.

REFERENCES

1. **Barton, L. L., L. M. Dunkle, and F. H. Habib.** 1987. Septic arthritis in childhood. A 13-year review. Am. J. Dis. Child. **141:**898–900.
2. **Bille, J., L. Stockman, G. D. Roberts, C. D. Horstmeier, and D. M. Ilstrup.** 1983. Evaluation of a lysis-centrifugation system for recovery of yeasts and filamentous fungi from blood. J. Clin. Microbiol. **18:**469–471.
3. **Carey, R. B.** 1984. Clinical comparison of the Isolator 1.5 microbial tube and the BACTEC radiometric system for detection of bacteremia in children. J. Clin. Microbiol. **19:**634–638.
4. **Chandrasekar, P. H., and W. J. Brown.** 1994. Clinical issues of blood cultures. Arch. Intern. Med. **154:**841–849.
5. **Fink, C. W., and J. D. Nelson.** 1986. Septic arthritis and osteomyelitis in children. Clin. Rheum. Dis. **12:**423–435.
6. **Gill, V. J., C. H. Park, F. Stock, L. L. Gosey, F. G. Witebsky, and H. Masur.** 1985. Use of the lysis-centrifugation (Isolator) and radiometric (BACTEC) blood culture systems for the detection of mycobacteremia. J. Clin. Microbiol. **22:**543–546.
7. **Goldenberg, D. L., and A. S. Cohen.** 1976. Acute infectious arthritis. A review of patients with nongonococcal joint infections (with emphasis on therapy and prognosis). Am. J. Med. **60:**369–377.
8. **Goldenberg, D. L., and J. I. Reed.** 1985. Bacterial arthritis. N. Engl. J. Med. **312:**764–771.
9. **Ho, G.** 1993. Bacterial arthritis, p. 2003–2023. *In* D. C. McCarty and W. J. Koopman (ed.), Arthritis and allied conditions, 12th ed. Lea & Febiger, Philadelphia, Pa.
10. **Molteni, R. A.** 1978. The differential diagnosis of benign and septic joint disease in children. Clinical, radiologic, laboratory and joint fluid analysis, based on 37 children with septic arthritis and 97 with benign aseptic arthritis. Clin. Pediatr. **17:**19–23.
11. **Shmerling, R. H., T. L. Delbanco, A. N. A. Tosteson, and D. E. Trentham.** 1990. Synovial fluid tests. What should be ordered? JAMA **264:**1009–1014.
12. **Von Essen, R., and A. Holtta.** 1986. Improved method of isolating bacteria from joint fluid by the use of blood culture bottles. Ann. Rheum. Dis. **45:**454–457.
13. **Washington, J. A., II, and D. M. Ilstrup.** 1986. Blood cultures: issues and controversies. Rev. Infect. Dis. **5:**792–802.
14. **Welkon, C. J., S. S. Long, M. C. Fisher, and P. D. Alburger.** 1986. Pyogenic arthritis in infants and children: a review of 95 cases. Pediatr. Infect. Dis. **5:**669–676.
15. **Yagupsky, P., R. Dagan, C. W. Howard, M. Einhorn, I. Kassis, and A. Simu.** 1992. High prevalence of *Kingella kingae* in joint fluid from children with septic arthritis revealed by the BACTEC blood culture system. J. Clin. Microbiol. **30:**1278–1281.