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Culture with BACTEC Peds Plus/F Bottle Compared with Conventional Methods for Detection of Bacteria in Synovial Fluid

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An evaluation was undertaken to determine the utility of the BACTEC Peds Plus/F bottle and the BACTEC 9240 instrument (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) for the detection of clinically significant microorganisms in synovial fluid specimens. The Peds Plus/F bottle was used because in our laboratory the quantity of synovial fluid available for culture is frequently in the range of 0.5 to 3.0 ml. The culture results obtained with the Peds Plus/F bottle were compared to those obtained by a conventional agar plate method for a total of 805 synovial fluid specimens. Microbial growth was produced by 74 cultures (9.2%) from 60 patients, yielding a total of 77 microorganisms. Organisms were classified as pathogens ($n = 62$), contaminants ($n = 12$), or indeterminate ($n = 3$) on the basis of a review of the patients' medical histories. Culture using BACTEC Peds Plus/F bottle detected statistically significantly more pathogens overall (62 versus 51 pathogens [$P = 0.001$]) and statistically fewer contaminants overall (1 versus 11 contaminants [$P = 0.006$]) than culture by the agar plate method. These results indicate the superior performance of the BACTEC Peds Plus/F bottle over the conventional agar plate method for the detection of clinically significant microorganisms from synovial fluid specimens.

Recent studies have demonstrated the utility of blood culture methods over standard agar plate and broth methods for the isolation of microorganisms from synovial fluid (1–6). Two of those studies evaluated older versions of the BACTEC blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) (2, 5, 6). We are unaware of similar evaluations for more recent medium formulations and instrument upgrades for the BACTEC system. The objective of the present study was to evaluate the utility of culture with the BACTEC Peds Plus/F Bottle and the BACTEC 9240 blood culture system for the detection of microorganisms in synovial fluid specimens from patients suspected of having septic arthritis. We used the Peds Plus/F bottle because in our laboratory the quantity of synovial fluid available for culture is frequently in the range of 0.5 to 3.0 ml. This is an accepted specimen inoculum size for the BACTEC Peds Plus/F bottle.

MATERIALS AND METHODS

All synovial fluid specimens were collected from patients at the Mayo Medical Center in Rochester, Minn., who were suspected of having septic arthritis. Specimens that were from patients who declined use of their specimens and medical histories for evaluation (Minnesota Statute 144.335) or that contained an inadequate volume were excluded from the study. The Mayo Medical Center consists of two large teaching hospitals (combined number of beds, ≈1,800) and a large subspecialty clinic.

Synovial fluid was collected with a sterile syringe, and the sample was transported to the clinical microbiology laboratory within a 2-h period. After receipt in the laboratory, an aliquot of synovial fluid was inoculated directly onto 5% sheep blood agar and chocolate agar, which were incubated at 35°C with 5 to 7% CO_2 for 2 days. In addition, approximately 0.25 ml was inoculated into thioglycolate broth, which was incubated at 35°C with 5 to 7% CO_2 for 5 days. Residual volumes of synovial fluid between 0.5 and 3 ml were inoculated into a BACTEC Peds Plus/F bottle, which was loaded into the BACTEC 9240 instrument in the computer-assigned position and incubated for 5 days. The BACTEC 9240 instrument was observed at 4-h intervals for positive signals.

Microorganisms isolated from positive cultures were identified by standard biochemical techniques. All microorganisms were classified as either pathogens or contaminants on the basis of a review of the medical record by two of the authors of the present paper (F.R.C. and R.P.), who are also infectious disease physicians.

For each organism species detected (and overall), comparisons of the detection rates of the two systems were assessed by the sign test. All calculated P values were two sided, and P values of ≤0.05 were considered statistically significant.

RESULTS

A total of 805 synovial fluid specimens met the criteria for inclusion in the study. Results for positive specimens are provided in Table 1. Microbial growth was produced by 74 cultures (9.2%) from 60 patients, yielding a total of 77 microorganisms. On the basis of medical history reviews, 62 of 77 microorganisms were considered pathogens, 12 microorganisms were considered contaminants, and 3 microorganisms were of indeterminate significance.

Culture with the BACTEC Peds Plus/F bottle detected statistically significantly more pathogens overall than culture by the conventional method (62 versus 51 pathogens [$P = 0.001$]). In contrast, culture by the conventional method detected statistically significantly more contaminants overall than culture with the BACTEC Peds Plus/F bottle (1 versus 11 contaminants [$P = 0.006$]).

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TABLE 1. Yield of microorganisms from the conventional agar and broth method compared with that by the BACTEC method

Microorganism (no. of patients)	Total	No. of microorganisms detected with:			*P* value
		BACTEC Peds Plus/F bottle only	Conventional agar and broth method only	Both methods	
Pathogens[a]					
All microorganisms (45)	62	11	0	51	0.001
Staphylococcus aureus (20)	29	1	0	28	1.0
Staphylococcus spp., coagulase negative (11)	14	2	0	12	0.5
Streptococcus, group B (3)	4	3	0	1	0.25
Streptococcus, group C (2)	3	1	0	2	1.0
Streptococcus, group G (1)	3	0	0	3	
Viridans group streptococci (3)	3	2	0	1	0.5
Enterococcus species (1)	2	1	0	1	1.0
Pseudomonas aeruginosa (1)	1	0	0	1	
Morganella morganii (1)	1	0	0	1	
Yersinia enterocolitica (1)	1	0	0	1	
Enterobacter cloacae (1)	1	1	0	0	1.0
Contaminants[a]					
All microorganisms (12)	12	1	11	0	0.006
Staphylococcus spp., coagulase negative (4)	4	0	4	0	0.125
Bacillus species (2)	2	0	2	0	0.5
Burkholderia pickettii (2)	2	0	2	0	0.5
Staphylococcus aureus (1)	1	0	1	0	1.0
Viridans group streptococci (1)	1	1	0	0	1.0
Gram-negative bacillus (not identified) (1)	1	0	1	0	1.0
Corynebacterium species (1)	1	0	1	0	1.0
Indeterminate					
All microorganisms					
Staphylococcus spp., coagulase negative (2)	2	1	1	0	1.0
Staphylococcus aureus (1)	1	1	0	0	1.0

[a] Pathogen versus contaminant on the basis of a medical history review.

DISCUSSION

Treatment for septic arthritis, especially that associated with joint arthroplasties, is often prolonged. Therefore, reliable information about pathogen isolation from synovial fluid is essential so that proper antimicrobial therapy can be provided.

Conventional culture methods, which involve the inoculation of synovial fluid directly onto agar media, have been shown by several investigators to lack sensitivity compared to the inoculation of synovial fluid into blood culture media (1–6). This lack of sensitivity may relate to several factors. First, the quantity of microorganisms in synovial fluid is often quite low. Culture of larger amounts of synovial fluid, which is possible by inoculation of a specimen into blood culture bottles instead of onto agar plates, should theoretically result in higher levels of recovery. Second, inhibitors, including antibiotics, in synovial fluid may inhibit the growth of microorganisms in culture. von Essen and Holtta (3) and Yagupsky and Press (5) independently observed the inhibitory effect of synovial fluid on bacterial growth near the area on culture plates where most of the synovial fluid specimen was deposited. Resins, such as those that are present in the BACTEC Peds Plus/F bottle, and the dilution effect of placing an inoculum into a liquid medium in blood culture bottles may decrease the inhibitory effects of inhibitors, including antibiotics. Third, microorganisms may be phagocytized by white blood cells in synovial fluid and therefore may not be recovered by culture. Release of phagocytized organisms by lytic agents such as saponin, which are contained in most blood culture medium formulations, may be possible.

Previously reported studies which have compared blood culture methods to standard agar- and/or broth-based methods are summarized in Table 2. The results of the present study showed that statistically significantly more microorganisms were isolated from synovial fluid specimens by culture with the Peds Plus/F bottle than by culture by the conventional agar plate method. von Essen and Holtta (3) demonstrated that the inoculation of synovial fluid into blood culture bottles containing supplemented brain heart infusion broth resulted in the detection of the microbial agent responsible for 10 of 41 cases of septic arthritis in which the agent was not detected by conventional agar plate methods. In a subsequent study, von Essen (4) used a similar manual blood culture method and noted that one-third of the specimens from patients not receiving antibiotics and one-half of the specimens from patients receiving antibiotics were positive by culture with blood culture media only and not by culture on conventional solid agar plates. Yagupsky and Press (5) demonstrated that statistically significantly more microorganisms were recovered from synovial fluids when another manual blood culture system, the Isolator 1.5 microbial tube (Wampole Laboratories, Cranbury, N.J.), was used than when conventional agar plate methods were used.

More recent studies have evaluated the utility of automated or semiautomated blood culture systems for the detection of microorganisms from synovial fluid. Yagupsky and colleagues showed that for pediatric patients the number of pathogens recovered by culture with BACTEC 460 aerobic blood culture bottles (Becton Dickinson Diagnostic Systems [formerly Johnston Laboratories, Towson, Md.]) was comparable to the

TABLE 2. Summary of reported studies which compared blood culture techniques to standard agar- and/or broth-based methods for isolation of microorganisms from synovial fluid

Author (reference)	Blood culture system used	Patient demographics	No. of specimens positive/total specimens evaluated (%)	Results	Secular microorganism trends[a]
von Essen (4)	Hemobact system (Orion Diagnostica, Espoo, Finland) or Signal (Oxoid, Basingstroke, United Kingdom)	Mean age, 42 ± 21 yr; patients with prosthetic (secondary) and natural (primary) infections included	NA[b]; information presented on 155 culture-positive specimens; no information available on culture-negative specimens	One-third of specimens from patients not receiving antibiotics and one-half of specimens from patients receiving antibiotics were positive by blood culture method only vs culture on conventional solid media (sheep blood, chocolate, anaerobic, and thioglycolate agars)	Natural: (i) *S. aureus*; (ii) *Staphylococcus* species, coagulase-negative; and (iii to v) beta-hemolytic streptococci, *Streptococcus pneumoniae, Salmonella* spp. Prosthetic joint infection: (i) *S. aureus*; (ii) *Staphylococcus* species, coagulase-negative; (iii) beta-hemolytic streptococci; and (iv, v) *Escherichia coli*, other streptococci (not specified)
Yagupsky et al. (6)	BACTEC 460 aerobic blood bottles (Becton Dickinson Diagnostic Instrument Systems)	All pediatric patients (age, <18 years)	63/216 (29.2)	BACTEC bottles equivalent to solid media (sheep blood, chocolate, MacConkey, and/or New York City agar) and/or thioglycolate broth, except BACTEC bottles recovered more *K. kingae* isolates.	Natural: (i) *S. aureus*, (ii) *K. kingae*, (iii) *Brucella melitensis*, (iv) *Streptococcus pyogenes*, and (v) *S. pneumoniae*
Fuller et al. (2)	BACTEC Plus 26 and 27 system (Becton Dickinson Diagnostic Instrument Systems)	NA	1,101 sterile body fluids specimens evaluated; no. of synovial fluid specimens not specified; overall rate of recovery of bacteria, 21%	BACTEC Plus 26 and 27 system recovered statistically significantly more isolates than solid media (chocolate, sheep blood, and anaerobic agar) and thioglycolate and tryptic soy broth	NA
Yagupsky and Press (5)	Isolator system (Wampole Laboratories)	Pediatric to adult patients; ratio, 2:1	31/144 (21.5)	Isolator system recovered statistically significantly more isolates than culture with solid media (chocolate and Trypticase soy-sheep blood agar)	Natural and prosthetic infections combined: (i) *S. aureus*, (ii) *S. pneumoniae*, (iii) *Streptococcus* group G, (iv) *K. kingae*, and (v) *Salmonella enterica* serovar Enteritidis
Bourbeau et al. (1)	BacT/Alert system (Organon Teknika)	NA	50/361 (13.8)	BacT/Alert bottles recovered statistically significantly more isolates than solid media (blood, chocolate agar) and thioglycolate broth; BacT/Alert FAN aerobic and anaerobic bottles superior to BacT/Alert standard aerobic and anaerobic bottles	NA
von Essen and Holtta (3)	Hemobact Aerobe and Hemobact Anaerobe system (Orion Diagnostica)	37 adult patients and 3 pediatric patients	47/201 (23.4)	10 more cases of infection diagnosed by blood culture method than by culture with solid media (sheep blood, chocolate, and brucella agar [brucella agar with anaerobic incubation])	(i) *S. aureus*; (ii) *Staphylococcus epidermidis*; and (iii to v) *Streptococcus* group G, *Enterococcus* spp., and *S. pneumoniae*

[a] The top five pathogens are ranked in descending order except for the study by von Essen (4), in which the organisms ranked iii to v occurred at equal frequencies for natural joint infections and organisms ranked iv and v occurred at equal frequencies for prosthetic joint infections, and the study by von Essen and Holtta (3), in which the organisms ranked iii to v occurred at equal frequencies.

[b] NA, not available.

number recovered by culture with solid media. However, *Kingella kingae* isolates, which accounted for 14 of 63 (23%) isolates overall, were exclusively recovered from the BACTEC bottles (6). Of interest, *K. kingae* was also a frequent isolate in the study of Yagupsky and Press (5) mentioned above. Those two studies, unlike ours and the other studies listed in Table 2, predominantly evaluated pediatric patients and were conducted in a single geographic region, Israel. Fuller and colleagues (2) compared the results for pathogen detection by culture with BACTEC Plus 26 and 27 media (Becton Dickinson Diagnostic Instrument Systems) versus those by culture by conventional agar plate methods; they evaluated a variety of sterile body fluids other than blood. Although the total number of synovial fluid samples studied was small, more pathogens were recovered by culture with BACTEC bottles than by culture by the conventional agar plate method (2).

Bourbeau and colleagues (1) evaluated the utility of the BacT/Alert blood culture system (Organon Teknika Corporation, Durham, N.C.) for the detection of clinically significant microorganisms in a variety of sterile body fluids other than blood. They noted a statistically significant increase in the number of pathogens recovered from the FAN bottle of the BacT/Alert culture system compared with the number recovered by routine culture. The routine culture method used by those investigators, like our method, incorporated both agar plates and thioglycolate broth. However, if the synovial fluid specimen volume was >1.0 ml, the specimen in the study of Bourbeau et al. (1) was centrifuged, resuspended in 1.0 ml of supernatant, and then inoculated onto plates and into thioglycolate broth (1).

The results of the present study also demonstrated that statistically significantly fewer contaminating microorganisms were isolated by culture with the Peds Plus/F bottle than by culture by the conventional agar plate method. We speculate that the agar plates were more susceptible to contamination, especially during processing of the specimens in the laboratory. Moreover, agar plates are not a closed system, unlike blood culture bottles. Additional contamination could occur during the periodic viewing of the plates during the incubation period. Among the studies described above, the isolation of contaminating microorganisms was described in only one. In contrast to our study, von Essen (4) observed that the majority of contaminating microorganisms (19 of 21) were recovered only from the blood culture system used and not from the plate used for the agar-based method. Organisms like coagulase-negative staphylococci are commonly encountered as contaminants, but they may cause septic arthritis, especially in patients with joint arthroplasties. When these organisms are isolated, the clinician must decide whether prolonged antibiotic therapy and/or removal of the joint arthroplasty is warranted. In some cases, additional specimens for culture must be obtained by invasive procedures in an effort to determine whether an organism is clinically significant. The results of the present study suggest that use of the Peds Plus/F bottle should decrease the frequency of isolation of contaminating microorganisms in synovial fluid specimens. Further studies are required to confirm this finding.

In summary, we have demonstrated the superior performance of culture with the BACTEC Peds Plus/F blood culture bottle compared to conventional plating methods for the detection of clinically significant microorganisms in synovial fluid specimens.

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