JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1996, p. 34–37 Vol. 34, No. 1
0095-1137/96/$04.00+0

Clinical Comparison of Isolator, Septi-Chek, Nonvented Tryptic Soy Broth, and Direct Agar Plating Combined with Thioglycolate Broth for Diagnosing Spontaneous Bacterial Peritonitis

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Received 26 May 1995/Returned for modification 1 August 1995/Accepted 5 October 1995

Spontaneous bacterial peritonitis is a life-threatening complication of cirrhotic ascites. Optimal patient management depends on the isolation of the causal organism from ascitic fluid. To evaluate culture techniques for the diagnosis of spontaneous bacterial peritonitis, we prospectively compared three blood culture systems, the Isolator system, a lysis-centrifugation system, the Septi-Chek system, a biphasic culture system, and a nonvented tryptic soy broth system, all inoculated at the bedside, and our standard method of direct inoculation of specimens after transport to the laboratory onto agar plates and into thioglycolate broth. The results showed that the Septi-Chek and nonvented tryptic soy broth systems each recovered statistically significantly more pathogens than either the Isolator system ($P = 0.0084$) or the standard method ($P = 0.00098$). The Isolator system recovered more pathogens than the standard plate method, but this difference was not statistically significant. Both the Isolator system and the standard plate method recovered more contaminating microorganisms than the Septi-Chek or nonvented tryptic soy broth system. The Isolator system required the most processing time compared with the processing times required by any other method.

Spontaneous bacterial peritonitis (SBP) is a common and frequently recurrent complication of cirrhotic ascites. This potentially fatal condition occurs in 10 to 30% of patients with chronic liver disease and ascites (3, 7, 16). The clinical presentation of SBP can be subtle and its early recognition can be missed, yet once it is considered, an accurate diagnosis must be pursued to allow the institution of treatment with appropriate antibiotics.

The examination of ascitic fluid obtained by diagnostic paracentesis is essential for the diagnosis of SBP. While a polymorphonuclear leukocyte (PMN) count in ascitic fluid of greater than 250/mm^3 provides a presumptive diagnosis, confirmation requires the isolation of the causal microorganism (10). Direct inoculation of ascitic fluid into broth or onto agar plates provides a positive microbiologic diagnosis in less than 50% of suspected cases of SBP (11, 12). Patients with elevated PMN counts in their ascitic fluid but negative cultures, referred to as culture-negative neutrocytic ascites (CNNA), present in an identical manner clinically and biochemically to those with microbiologically confirmed SBP. It has been assumed that these patients have SBP which has escaped detection by these direct plating culture methods (11, 12). This insensitivity likely results from the fact that infected ascites may have less than one microorganism per milliliter (10).

Microorganism concentrations in infected ascitic fluid from patients undergoing chronic ambulatory peritoneal dialysis (CAPD) are similar to those observed in patients with SBP. The inoculation of blood culture bottles with ascitic fluid has proven to be more sensitive for providing a microbiologic diagnosis of both SBP and CAPD than direct plating methods (sensitivities, 80 to 90% compared with 50%, respectively) and results in decreased time for the detection of microorganisms (1, 2, 4, 8–10, 12–14). Furthermore, it has been shown that the immediate or bedside inoculation of ascitic fluid into blood culture bottles produces better results than if blood culture bottles are inoculated after transport of the specimen to the laboratory (9). Most of these studies have used either tryptic soy broth (TSB) (1, 2, 12) or thioglycolate blood culture medium (10) for inoculation. Despite these advances, in most reported series, CNNA continues to occur in a significant number of patients suspected of having SBP.

Techniques which concentrate microorganisms from ascitic fluid obtained from patients with SBP or CAPD maximize their recovery (5, 15, 17, 19). Recently, a lysis-centrifugation blood culturing system (Isolator; Wampole Laboratories, Cranbury, N.J.) has been shown to be superior to large-volume centrifugation systems for recovering both aerobic and anaerobic organisms in CAPD patients with infected ascites (5, 19). One of those studies (5) also reported the Isolator system to be more labor-intensive and prone to contamination. A biphasic blood culture system (Septi-Chek; Becton Dickinson, Cockeysville, Md.), which is less labor-intensive, has been shown to be comparable in sensitivity to filtration techniques (13).

To evaluate culture techniques for the diagnosis of SBP, we prospectively compared the bedside inoculation of the Isolator system, the Septi-Chek system, and a nonvented tryptic soy broth (NVTSB) blood culture bottle (Difco Laboratories, Detroit, Mich.) with our standard procedure, inoculation of specimens after transfer to the laboratory onto agar plates and into thioglycolate broth.

(This study was presented in part at the American Gastroenterological Association, American Association for the Study of Liver Diseases Meeting, San Francisco, Calif., 10 to 13 May 1992.)

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TABLE 1. Summary of patient characteristics and laboratory test results for patients with SBP, BA, and CNNA

Patient		Diagnosis	PMN count/mm^3	Microorganism	Culture positivity of ascites with:				Simultaneous culture of blood
					Blood culture systems[b]			Combined agar plates and thioglycolate broth	
Age (yr)	Sex[a]				Isolator	Septi-Chek	NVTSB		
83	F	SBP	6,882	*Escherichia coli*	−	+	+	−	ND[c]
70	F	SBP	2,482	*Klebsiella pneumoniae*	+	+	+	−	−
86	M	SBP	2,010	*Escherichia coli*	+	+	+	−	+
42	F	SBP	2,970	*Enterobacter aerogenes*	−	+	+	−	ND
54	M	SBP	4,390	*Klebsiella pneumoniae*	−	+	+	+	+
61	M	SBP	6,283	*Klebsiella pneumoniae*	+	+	+	−	ND
52	F	SBP	15,908	*Streptococcus* sp., viridans group	+	−	+	−	−
42	M	SBP	22,256	*Streptococcus* sp., viridans group	−	+	+	−	−
40	M	SBP	1,800	*Streptococcus agalactiae*	+	+	+	−	−
63	F	SBP	1,216	*Streptococcus* sp., viridans group	+	+	+	−	ND
44	M	SBP	612	*Klebsiella pneumoniae*	−	+	+	−	+
70	F	BA	39	*Klebsiella pneumoniae*	−	+	−	−	−
68	M	BA	14	*Streptococcus* sp., viridans group	−	+	+	−	+
54	M	CNNA	12,008		−	−	−	−	−
69	M	CNNA	567		−	−	−	−	−

[a] F, female; M, male.
[b] A total of 30 ml of ascites was evenly distributed among Isolator, Septi-Chek, and NVTSB bottles.
[c] ND, not done.

MATERIALS AND METHODS

From January 1990 until November 1991, all patients with parenchymal liver disease and ascites admitted to the Liver Unit at Rochester Methodist Hospital underwent (i) routine diagnostic paracentesis at the time of admission and (ii) repeat paracentesis when SBP was suspected clinically during hospitalization (e.g., fever, abdominal pain, encephalopathy, diarrhea, and/or leukocytosis). Patients already receiving antimicrobial therapy at the time of initial diagnostic paracentesis and patients with secondary bacterial peritonitis were excluded from the study.

A 10-ml aliquot of ascitic fluid was inoculated at the bedside directly into each of the following: (i) a 10-ml Isolator microbial tube (Wampole Laboratories), (ii) a Septi-Chek blood culture bottle (70 ml; Roche Diagnostics), and (iii) an NVTSB (anaerobic) blood culture bottle (100 ml; Difco Laboratories). At the time of the study, these blood culture systems were used for diagnosing bloodstream infections at the Mayo Clinic and we wished to determine which of these was useful for diagnosing SBP in our patients. After initial centrifugation according to the manufacturer's instructions, the Isolator concentrate was inoculated onto tryptic soy agar with 5% sheep blood (SBA), chocolate blood agar (CBA), inhibitory mold agar (IMA), brain heart infusion agar (BHI), and Sabouraud dextrose agar (SAB). The SBA and CBA plates were incubated for 7 days at 37°C with 5 to 10% CO_2 and were examined daily for growth. The IMA, BHI, and SAB plates were incubated at 30°C for 21 days. The Septi-Chek system consists of a TSB bottle to which an agar slide with chocolate, MacConkey, and malt agars was attached upon arrival in the laboratory. The Septi-Chek and NVTSB bottles were incubated at 37°C for 7 days and were visually examined daily for evidence of growth. After examination, the Septi-Chek bottle was inverted to reinoculate the attached agar slide. Negative NVTSB bottles were blindly subcultured at 2 and 7 days for aerobic and anaerobic growth. These agar plates were subsequently incubated for 2 days at 37°C.

The standard method of ascitic fluid culture in our laboratory was also performed on each specimen. Ascitic fluid in a sterile container was transported immediately to the laboratory, where several drops of fluid were inoculated directly onto each of three agar plates (SBA, eosin methylene blue, and colistin nalidixic acid agar) and 0.5 ml was placed in thioglycolate broth supplemented with rabbit serum. These were incubated at 37°C with 5 to 10% CO_2 for 7 days and were examined daily for growth. Identification of organisms was performed by conventional laboratory methods (18).

The definition of SBP required all of the following: isolation of a clinically significant microorganism from ascitic fluid, a PMN count in ascitic fluid of greater than 250/mm^3, and the absence of another source of infection. The definition of CNNA required all of the following: ascitic fluid cultures with no growth of potentially pathogenic microorganisms, PMN count in ascitic fluid exceeding 500/mm^3, the absence of a secondary source of infection, no antibiotic therapy within 7 days, and no evidence of peritoneal carcinomatosis, tuberculosis, pancreatitis, cholecystitis, or hemorrhage into the ascites. The definition of bacteriascites (BA) included all of the following: isolation of a potentially pathogenic microorganism from ascitic fluid, a PMN count in ascitic fluid of less than 250/mm^3, and the absence of a secondary source of infection. An isolate was regarded as a contaminant or not clinically significant if it was a microorganism which is not usually associated with SBP and/or is a common contaminant, it was present at <10 CFU/ml of fluid inoculated onto agar plates from either the Isolator concentrate or the unprocessed specimen (standard plate method), and there was no clinical evidence of peritonitis without antibiotic therapy. Microorganisms that are not usually associated with SBP or that could be considered contaminants and that are frequently encountered in our laboratory include *Bacillus* spp., *Corynebacterium* spp., *Propionibacterium* spp., *Lactobacillus* spp., coagulase-negative staphylococci, and viridans group streptococci. For each patient, the clinical history, dates of antibiotic therapy, and PMN count in ascitic fluid were recorded. Blood cultures were also obtained from most patients at the time of diagnostic paracentesis.

Fischer's exact test was used to compare the number of pathogens (clinically significant microorganisms) recovered among the blood culture systems (Isolator, Septi-Chek, and NVTSB). McNemer's test was used to compare the number of pathogens recovered by each blood culture system with the number recovered by the standard culturing method (combined agar plates and thioglycolate broth).

RESULTS

Of the cultures of the 203 specimens submitted from 108 patients with parenchymal liver disease, 28 cultures grew microorganisms. Eleven of the 108 patients had SBP and 2 had CNNA. Fifteen of the 28 microorganisms isolated were assessed as contaminants and included coagulase-negative staphylococci ($n = 7$), *Corynebacterium* spp. ($n = 2$), a *Propionibacterium* sp. ($n = 1$), a *Paecilomyces* sp. ($n = 1$), a viridans streptococcus group ($n = 1$), *Alcaligenes* spp. ($n = 2$), and a *Candida* sp. ($n = 1$).

A summary of patient characteristics and laboratory test results, including PMN counts and the specific microorganisms (pathogens) recovered, are summarized in Table 1. Table 2 provides a comparison of all culture results by each culture system. The Septi-Chek system and the NVTSB culture recovered statistically significantly more pathogens than the standard combined plate and thioglycolate broth method. Although the Isolator system recovered more pathogens than the standard method, this was not a statistically significant difference. The Septi-Chek system and NVTSB bottles also recovered statistically significantly more pathogens than the Isolator

TABLE 2. Comparison of culture results by culture system

Diagnosis (no. of patients)	No. of patients with positive culture results by:			
	Isolator	Septi-Chek	NVTSB	Combined agar plates and thioglycolate broth
SBP (11)	6	10	11	1
BA (2)	0	2	1	0
Total (true positives) (13)	6[a,b]	12[a,c]	12[b,d]	1[c,d]
Contaminating organisms (false positives) (15)	6	3	1	10

[a,b] Significantly different on the basis of Fischer's exact test ($P = 0.0084$).
[c,d] Significantly different on the basis of the McNemer test ($P = 0.00098$).

system. Also provided in Table 2 are the relatively high contamination frequencies associated with the standard method and the Isolator system compared with those with the Septi-Chek system and NVTSB bottles. Table 3 provides a comparison of the sensitivity, specificity, and negative and positive predictive values for each system. Of the four culture methods, the Septi-Chek system and NVTSB bottles were the most sensitive and specific; i.e., they recovered the largest number of pathogens and the fewest number of contaminants.

DISCUSSION

In the current study, we determined that the bedside inoculation of either a Septi-Chek blood culture bottle or an NVTSB blood culture bottle is more sensitive for determining the causative microorganism for SBP or BA than the bedside inoculation of an Isolator blood culture tube or the in-laboratory inoculation of agar and thioglycolate broth. We used bedside inoculation of blood culture receptacles because this was the most effective method for diagnosing SBP, as demonstrated previously by Runyon et al. (9). SPB is a relatively rare infection. The current study was conducted over a 19-month period at Rochester Methodist Hospital, a large referral hospital which is part of the Mayo Medical Center. During that period of time, SBP was diagnosed in only 13 patients. Because of the low number of evaluable patients over the study period, we were unable to answer the additional question of the effectiveness of bedside inoculation compared with that of in-laboratory inoculation of blood culture receptacles.

Both the Septi-Chek system and NVTSB bottles had sensitivities (both 92.3%) comparable to that reported by Runyon (8) for the bedside inoculation of aerobic and anaerobic BACTEC bottles containing TSB (91%) (8) and exceeded the sensitivity of a TSB blood culture bottle (sensitivity, 77%; Liquoid; Hoffmann-LaRoche, Basel, Switzerland) studied by Castellote and colleagues (2). The Septi-Chek blood culture system requires minimal handling of the specimen; the addition of the agar slide allows direct testing of the isolate from the agar colony, which in our experience decreases the time to the identification of microorganisms when it is used in blood culturing. Unfortunately, the low overall frequency of isolates in the current study did not permit an acceptable comparison of organism identification times.

Despite its efficacy in previously reported studies with peritoneal dialysates (5, 19), the Isolator lysis-centrifugation system in our study proved to be a relatively insensitive method for determining the causative microorganisms of SBP or BA. In one of the prior studies, the one by Woods and Washington (19), only dialysates from patients with suspected CAPD-associated peritonitis were evaluated by the Isolator system, the aerobic BACTEC bottle, and large-volume centrifugation. The results showed that the Isolator system recovered more organisms than the BACTEC blood culture bottle and the centrifugation method. It is possible that such patients, in contrast to those with SBP or BA, may have higher microorganism concentrations in their peritoneal fluid and, therefore, that less sensitive methods for the detection of microorganisms may be acceptable. Also, in that series more than one-half of the microorganisms isolated were *Staphylococcus* spp. including coagulase-negative staphylococci and *S. aureus*. These organisms are more commonly associated with CAPD-associated peritonitis than with SBP or BA, in which enteric organisms are more commonly the causative agents. Previous blood culture studies have demonstrated that *Staphylococcus* spp. are more frequently isolated by the Isolator system than by broth blood culturing systems (6). In another study, a study by Elston and colleagues (5), the Isolator system isolated more microorganisms than large-volume centrifugation. Like the study of Woods and Washington (19), peritoneal dialysates from patients undergoing CAPD were analyzed, and a large percentage of the isolates were staphylococci. In the current study, the lower level of sensitivity of the Isolator system, together with the greater processing time and higher contamination rate, makes it less suitable for routine culturing of ascitic fluid.

One may speculate that the poor performance of the Isolator system in our study could be related to effective dilution of the specimen as the result of the distribution of the concentrate to selective fungal (IMA and SAB) as well as bacterial agar plates (SBA and CBA). Bacteria may grow poorly or not at all on the selective fungal agar plates. However, in our previous studies (6) we have demonstrated that the Isolator system recovers more bacteria overall than either the Septi-Chek system or NVTSBs when they are used for diagnosing bloodstream infection and are processed in the same way as they were in the current study.

Our study confirms the observations of others (11, 12) that conventional methods of culturing of ascitic fluid by the laboratory inoculation of agar plates and broth are inadequate in this clinical setting. The lack of sensitivity of this method may be related to the low volume of the inoculum and the delayed time to inoculation. However, neither of these variables was specifically evaluated in our study.

Despite the relatively high levels of sensitivity achieved with the Septi-Chek system or NVTSB bottles compared with those achieved with other culture methods for diagnosing bacterial peritonitis, cases of CNNA continue to occur and require the use of broad-spectrum antibiotic coverage for both gram-negative bacilli and gram-positive cocci. In addition, further inves-

TABLE 3. Comparison of the accuracy of culture methods for diagnosing microorganisms causing SBP or BA[a]

Culture method	Percent		Positive predictive value (%)	Negative predictive value (%)
	Sensitivity	Specificity		
Isolator[b]	46.1	96.8	50.0	96.3
Septi-Chek[b]	92.3	98.4	80.0	99.5
NVTSB[b]	92.3	99.5	92.3	99.5
Combined agar plates and thioglycolate broth[c]	7.7	94.7	9.1	93.7

[a] The reference standard was any clinically significant positive culture by any culture method.
[b] Specimen inoculated at the patient's bedside.
[c] Specimen inoculated after transport of specimen to the laboratory; agar plates included blood agar, eosin methylene blue, and colistin nalidixic acid agar.

tigation including abdominal computerized tomography is required to exclude secondary causes in these patients. Cases of CNNA are assumed to represent cases of SBP for which the causative microorganisms have not been isolated. The best study demonstrating the clinical and biochemical similarities between SBP and CNNA used culture methods significantly less sensitive than those used currently (7). It is not clear if the CNNA cases that we identified in our study represent cases of bacterial infection of ascitic fluid not diagnosed by the culture techniques that we used. Patients who demonstrate a prompt clinical and ascitic fluid PMN response to antimicrobial therapy probably have SBP in which the very low organism concentration or the fastidious nature of the organism prevent its recovery and identification or in which the antimicrobial effects of the ascitic fluid may inhibit the growth of the microorganism in the laboratory.

We conclude that for diagnosing spontaneous bacterial peritonitis the bedside inoculation of either the Septi-Chek system or NVTSBs is superior to the bedside inoculation of the Isolator system or inoculation of the combination of plate media and thioglycolate broth after transport of the specimen to the laboratory. Newer automated blood culturing systems often use TSB as the culture medium in blood culture bottles. It is possible that these systems could produce results similar to those provided by the Septi-Chek system and NVTSB bottles, both of which also contain TSB.

ACKNOWLEDGMENT

We thank Roberta Kondert for efforts in the preparation of the manuscript.

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