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Improved Detection of Bacterial Growth in Continuous Ambulatory Peritoneal Dialysis Effluent by Use of BacT/Alert FAN Bottles

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Culture-negative peritonitis is a major complication for patients on continuous ambulatory peritoneal dialysis (CAPD) and precludes organism-specific therapy. The aim of the present study was to compare inoculation of 10 ml of CAPD effluent into BacT/Alert blood culture bottles (FAN [fastidious antimicrobic neutralizing], BacTAlert aerobic [BTA], and BacT/Alert anaerobic [BTAn] bottles) to our conventional method of using 50 ml of concentrated CAPD effluent to inoculate peptone broth bottles (BD bottles) and MacConkey agar and blood agar medium (BA-MAC). The FAN, BTA, and BTAn bottles were monitored automatically in the BacT/Alert blood culture instrument. A total of 207 CAPD effluents were studied, and in 97 bacteria were detected by at least one method. Compared to BTA bottles (79 of 97; 81.4%), BTAn bottles (78 of 97; 80.4%), and BD bottles (88 of 97; 90.7%), the single best broth medium for detecting bacterial growth in CAPD effluents was the FAN bottle (90 of 97 effluents; 92.8%). A total of 125 bacterial species were detected by any method, and the majority (91.8%) of CAPD effluents were infected with a single species. A combination of FAN and BTAn bottles detected 111 of 125 (88.8%) of all organisms, whereas a combination of BD bottles and BA-MAC detected 107 of 125 (85.6%) of all organisms. One or more organisms that would have been completely missed by the conventional method with BD bottles and BA-MAC were detected in 18 CAPD effluents. Of these 18 CAPD effluents, 6 showed no growth by the conventional method with BD bottles and BA-MAC. On the basis of our data, the most sensitive and least labor intensive method was direct inoculation of 10 ml of CAPD effluent into a FAN bottle and a BTAn bottle, which could be automatically monitored by the BacT/Alert blood culture instrument. On the basis of case definitions for peritonitis, the sensitivities and specificities of the methods with FAN and BTAn bottles and with BD bottles and BA-MAC were 81.1 and 98.8% and 74.5 and 96.5%, respectively.

Continuous ambulatory peritoneal dialysis (CAPD) has been shown to be a safe and effective alternative for patients with end-stage renal disease compared to hemodialysis. The number of patients on CAPD is continually expanding (21). Despite improvements such as using a closed system of commercially prepared dialysate effluent, a major complication associated with this procedure is peritonitis. Mortality rates for patients with CAPD-associated peritonitis range from 2% for patients whose average age is 45 years to 25% for patients over age 55 years who have signs of systemic sepsis (21). Fungi, *Pseudomonas* spp., and polymicrobic infection are associated with the highest mortality rates. The most frequently encountered pathogens are coagulase-negative staphylococci, *Staphylococcus aureus*, streptococci, members of the family *Enterobacteriaceae*, nonfermentative gram-negative rods, and gram-positive rods (1, 4, 9). Detection of the etiologic agent causing the CAPD-associated peritonitis relies heavily on culture techniques (2, 3, 10–12, 14, 15, 18, 19, 22). This presents a major challenge, and the lack of sensitivity of culture methods has been attributed to the low load of organisms in the majority of patients (which may be as low as 1 CFU/ml), the presence of antimicrobial agents in the peritoneal cavities of patients receiving treatment, intracellular survival of organisms, peritonitis caused by endotoxin rather than bacterial infection (8), and the use of inappropriate media or incubation conditions (21). Culture-negative peritonitis has been reported in 10 to 50% of patients with CAPD-associated peritonitis (1, 21).

A great deal of effort has been expended to optimize the culture methods for the detection of viable organisms from CAPD effluents. A recent review by von Graevenitz and Amsterdam (21) indicates that major factors include the volume of effluent cultured, lysing of leukocytes, prolonged incubation to ensure detection of difficult-to-grow organisms, and the methods used to neutralize antimicrobial agents. Generally, the larger the volume of effluent cultured, the higher the detection rates. However, comparative studies indicate that the minimum volume that should be tested is 10 ml (5, 13, 15, 21). The method chosen should include an enrichment broth with antiphagocytic and lytic properties (3, 18, 19). Both centrifugation (12, 13, 20) and filtration (11–14, 20) have been tested as means of concentrating bacteria from CAPD effluents. Semiautomated blood culture systems have been used for the detection of peritonitis in CAPD effluents. Both Septi-Chek (6) and BACTEC (13, 15) systems have been evaluated. Generally, methods that use these blood culture systems were equivalent or better than centrifugation methods (6, 21). Prolonged incubation (7 days) of blood or chocolate agar, inoculated directly or with subcultures of enrichment broths, is recommended to allow growth of organisms that may be inhibited by the dialysis effluent or antibiotics present in the dialysate (21) or that may be released slowly from phagocytic cells. Despite these improvements, culture-negative peritonitis remains a significant challenge for effective care of patients on CAPD (1, 21).

The aim of this study was to determine how effective the BacT/Alert blood culture system would be for culturing CAPD effluents compared to our current method of culturing concentrated CAPD effluent in Becton-Dickinson peptone broth bottles (BD bottles) and on two solid media (blood agar [BA] and

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MacConkey agar [MAC]). In addition, we also wanted to determine the optimal medium configuration by testing FAN (fastidious antimicrobic neutralizing) bottles, BacT/Alert anaerobic (BTAn) bottles, and BacT/Alert aerobic (BTA) bottles. In addition to supporting the growth of a wide range of microorganisms, FAN medium includes Fuller's earth, which adsorbs antimicrobial agents or other toxic factors that may otherwise inhibit growth. Recovery of organisms from CAPD effluents by the BacT/Alert blood culture bottles was compared to that by the conventional method that consisted of concentrating 50 ml of CAPD effluent and inoculating it into BD bottles and onto agar media (BA and MAC).

MATERIALS AND METHODS

Patient population. The CAPD effluents used in this study were from CAPD patients who used closed-bag systems containing 1 liter of dialysis effluent. All CAPD effluents submitted to the laboratory were included in this study. In some instances, the CAPD effluent was submitted as part of a routine hospital visit, a presurgical workup, or a follow-up to therapy. As such, some CAPD effluents were from patients with no clinical evidence of peritonitis. CAPD effluents were sent to the laboratory within 2 h of the patient's visit to the hospital and were either examined immediately, or, if they were collected after the laboratory closed at 10 p.m., they were held at 4°C and examined the following morning. A total of 192 patients were assessed and 207 effluents were evaluated (i.e., 15 effluents were taken from the same patient).

CAPD effluent processing methods. The current method for sampling the CAPD effluent was based on a concentration method. Briefly, the contents of the dialysate bag were mixed thoroughly, the tubing was disinfected with alcohol, the first 10 ml of effluent that drained from the bag was discarded, and then 50 ml of effluent was collected and placed into a 50-ml conical centrifuge tube. Once the effluent was obtained from the CAPD effluent bag, all handling of the effluent was performed in a class IIB biosafety cabinet. This effluent was centrifuged at $2,500 \times g$ for 15 min to pellet the cellular material as well as the bacteria. The supernatant was removed, and the pelleted material was resuspended in 1 ml of residual supernatant of the CAPD effluent. This inoculum was then used to prepare a slide for microscopy as well as to inoculate a BD bottle (peptone broth; Becton-Dickinson Microbiology Systems, Cockeysville, Md.), a BA plate, and a chocolate agar (CA) plate.

The test method was performed with uncentrifuged CAPD effluent. Briefly, the mixing and disinfection of the contents of the bag were as described above, and a second 50-ml aliquot was collected in a sterile container. This CAPD effluent was drawn up with a needle and syringe and was then inoculated into the BacT/Alert test bottles; the residual effluent was stored at 4°C and was then discarded after 7 days. Ten milliliters of CAPD effluent was inoculated into each of BTA, BTAn, and FAN blood culture bottles. These bottles were then placed into the automated BacT/Alert blood culture instrument (Organon Teknika Inc., Scarborough, Ontario, Canada) and were monitored for bacterial growth for 7 days. Cell counts were performed for all CAPD effluents (unconcentrated) by using a hemocytometer counting chamber.

Bacterial culture and identification. Broth media (BD, BTA, BTAn, and FAN bottles) with detectable growth were subcultured onto BA, CA, and MAC plates. All media were incubated aerobically (5% CO_2 for CA plates) at 35°C for 7 days. Organisms were identified by using Microscan panels (Dade Diagnostics Corp., Mississauga, Ontario, Canada) or standard microbial identification procedures (7). If mixed cultures were detected and discordant types of microorganisms were detected from various bottles, appropriate selective media were used to attempt to recover all organisms from all bottles. All plates and broth media were incubated for 7 days, at which time if the broth media were still negative, a terminal blind subculture onto CA was performed.

Definition of peritonitis. The guidelines for defining peritonitis reviewed by von Graevenitz and Amsterdam (21) were used in this study. Patients were considered to have peritonitis if they met at least two of the following criteria: (i) signs and symptoms of peritonitis (rebound tenderness, abdominal pain, fever, chills, nausea, vomiting, and diarrhea), (ii) a cloudy dialysate effluent (leukocyte count, $\geq 100 \times 10^6$/liter), and (iii) a positive culture (and/or a positive Gram stain).

Medical charts were reviewed for all patients whose CAPD effluent was culture positive but who did not have an elevated leukocyte count to make a clinicial assessment of signs and symptoms as well as to determine if antimicrobial therapy was given. Medical charts were also reviewed for patients whose CAPD effluents had elevated leukocyte counts but who were culture negative to determine whether these patients had true peritonitis. Repeat CAPD effluents from the same patient within 7 days were excluded from analyses related to the sensitivity and specificity of the diagnostic approach.

TABLE 1. Correlation of growth detected by each method

Concordance and detection method[a]	No. of CAPD effluents		
	Monomicrobic	Polymicrobic	Total
Total concordance	62	0	62
Partial concordance	1	8	9
Discordance:			
FAN bottles only	5	0	5
FAN and BD bottles only	5	0	5
FAN, BTAn, and BD bottles only	4	0	4
FAN, BTA, and BD bottles only	5	0	5
BTA bottles only	3	0	3
BTAn bottles only	1	0	1
BD bottles only	1	0	1
BTAn and BD bottles only	1	0	1
BTA, BTAn, and BD bottles only	1	0	1
Total	89	8	97

[a] The total number of positive CAPD effluents was 97, and the total number of bacteria detected (from all CAPD effluents) was 125. Total concordance indicates that all organisms were detected by all systems. Partial concordance indicates that all systems showed bacterial growth but that different organisms were detected. Discordance indicates that bacterial growth was detected only in some systems.

RESULTS

A total of 207 CAPD effluents were submitted over a 1-year period, and of these 97 had detectable bacterial growth (Table 1). Six CAPD effluents were culture positive, but had leukocyte counts of $< 100 \times 10^6$/liter. Of these, three were considered not to be clinically significant and the patients did not receive antibiotic therapy. One CAPD effluent was from a presurgical patient and the other two CAPD effluents were from patients who had no signs or symptoms of peritonitis for whom the effluent was submitted as part of a routine workup for hospitalized dialysis patients. The other three effluents grew coagulase-negative *Staphylococcus* spp., *S. aureus*, and *Staphylococcus saccharolyticus*. These isolates were deemed clinically significant because the patients had symptoms of peritonitis and received antimicrobial therapy for these organisms.

The effectiveness of the various types of media for detecting bacterial growth is given in Table 1. The most sensitive medium type was the FAN bottle, and this was consistent for CAPD effluents that had elevated polymorphonuclear cell counts as well as those that did not. The average time to detection is presented in Table 2. For some bottles, growth was detected as soon as 5 h after being placed in the BacT/Alert instrument. For each of the bottle types analyzed with the automated BacT/Alert instrument, there were two to three effluents for which growth was not detected by the instrument but viable organisms were grown from the terminal blind subculture (Table 2). The organisms missed were significant isolates and could not be dismissed as contaminants. Polymicrobic growth occurred for 8% of the positive bottles and in most cases resulted in partial concordance, in that all of the bottles inoculated showed growth, but the types of organisms detected varied among the bottle types (Table 1). Discordance was apparent for 26.8% (26 of 97) of all positive bottles. Total concordance was detected for 63.9% of all positive bottles (Table 1). The FAN bottles detected the greatest number (84.8%) of bacteria (Table 3). If the traditional method with BD bottles and BA and MAC plates had been used, 107 organisms would have been detected, whereas 117 organisms would have been detected if the FAN and BTAn bottles had

TABLE 2. Detection of bacterial growth in CAPD effluents

Detection method	No. (%) of CAPD effluents showing bacterial growth (any cell count[b])	Time to detection of growth[a]			
		Avg	Median	Minimum	Maximum
BTA bottles[c]	79 (81.4)	19.6 ± 15.9	15.2 h	1 day	110 h
BTAn bottles[c]	78 (80.4)	24.0 ± 27.1	15.2 h	5.2 h	192 h
FAN bottles[c]	90 (92.8)	18.4 ± 15.2	15.1 h	5.0 h	139 h
BD bottles (BA and MAC)[d]	88 (90.7)[e]	1.6 ± 1.1 day	1 day	1 day	6 days
FAN and BTAn bottles	93 (95.8)[e,f]	NA[g]			
FAN and BTA bottles	94 (96.9)[f]	NA			
BTA and BTAn bottles	85 (87.6)	NA			

[a] Time to detection of growth is measured in hours for BTA, BTAn, and FAN bottles and in days for BD bottles (BA and MAC plates). These averages exclude the bottles that were not detected by the blood culture instrument.

[b] A total of 207 effluents were assessed, of which 97 showed bacterial growth. Leukocyte counts in CAPD effluents of $\geq 100 \times 10^6$ cells/liter were considered to be elevated. A total of 122 CAPD effluents had elevated leukocyte counts, of which 91 had detectable bacterial growth.

[c] Organisms detected by blind subculture were not included in time-to-detection calculations. The following organisms were missed by the blood culture instrument and were detected only by blind terminal subculture on day 7: two isolates from BTA bottles (*P. aeruginosa*, and *S. aureus*), three isolates from BTAn bottles (*C. albicans*, *Pseudomonas aeruginosa*, and *S. aureus*), and three isolates from FAN bottles (*Acinetobacter* sp. and *P. aeruginosa* [two isolates]).

[d] Four of these were from subculture on day 5 or 6.

[e] When recovery rates for BD bottles (BA and MAC) were compared with those for FAN and BTAn bottles by McNemar's chi-square test (with Yates' correction), the two-tailed *P* value was 0.1306, which is considered not significant.

[f] When recovery rates for FAN and BTAn bottles were compared with those for FAN and BTA bottles by McNemar's chi-square test (with Yates' correction), the two-tailed *P* value was 0.1226, which is considered not significant.

[g] NA, not available.

been used. Fifteen organisms that were detected in the BTAn and FAN bottles only were not detected in the BD bottle or the BA or MAC plates, whereas only four organisms found in the BD bottle and BA and MAC plates were not found in the BTAn and FAN bottles ($P = 0.0218$ by McNemar's two-tailed test). Six CAPD effluents that were missed by the FAN bottles showed bacterial growth in other bottles. Of these, two (which grew *Staphylococcus epidermidis* and alpha-hemolytic streptococci) were not considered clinically significant and the patients did not receive antimicrobial therapy, whereas the other four (which grew *Candida albicans-Bacteroides fragilis*, *Bacillus* sp., and two alpha-hemolytic streptococci) were considered clinically significant and the patients received antimicrobial therapy. The two patients whose effluents grew organisms that were considered not clinically significant did not have elevated leukocyte counts, whereas the other four whose effluents grew organisms that were considered clinically significant had elevated leukocyte counts.

Of the 207 CAPD effluents evaluated, 15 were considered replicates. Of the 192 nonreplicate effluents, 106 were from patients considered to have true peritonitis and 86 were from patients who did not have peritonitis, according to the case definition (see Materials and Methods). On the basis of these case definitions, the sensitivity and specificity of the FAN bottle-BTAn bottle method were 81.1 and 97.7%, respectively. For the current method with BD bottles and BA and MAC plates, the sensitivity and specificity were 74.5 and 96.5%, respectively. For the CAPD effluents evaluated in this study, the rate of CAPD effluent culture negativity was 17.9%. In 1994, patient assessments indicated that culture-negative peritonitis accounted for 20.6% of clinically significant peritonitis events, whereas by using the combined BTAn and FAN bottles, the rate decreased to 16.7% in 1995 (unpublished data).

DISCUSSION

Peritonitis in patients living with CAPD is a serious life-threatening complication. Optimizing the detection of the etiologic agent(s) of the peritonitis is an important diagnostic step. Our data demonstrated that the automated BacT/Alert blood culture system could be successfully used to detect bacterial growth in CAPD effluents. For the 192 nonreplicate CAPD samples, of which 106 were from patients with clinical peritonitis (according to our case definition), the sensitivities and specificities of the FAN bottle alone, the FAN and BTAn bottles, the FAN and BTA bottles, and conventional method with BD bottles and BA-MAC plates were 78.3 and 98.8%, 81.1 and 97.7%, 79.2 and 98.8%, and 74.5 and 96.5%, respectively. The culture-negative peritonitis rate in the current analysis was 17.9%. This is similar to the 14 to 23% culture-negative peritonitis rate range reported for the Network 9 study (1). Although the FAN bottle-BTAn bottle combination was not significantly better than the FAN bottle-BTA bottle combination at detecting growth of organisms in CAPD effluents (Tables 2 and 3), to ensure adequate conditions for the detection of anaerobes, we recommend use of the FAN bottle-BTAn bottle combination.

The FAN bottle alone (85.6% positive) was more effective than the BD bottle alone (80.0%) for detecting growth of a larger number of organisms (Table 3). Although it was apparent that no single bottle could detect all organisms from all positive CAPD effluents, the single best bottle was the FAN bottle. The range of organisms detected was similar to those detected in previous studies, with gram-positive organisms dominating (68 of 125; 54.4%). Coagulase-negative staphylococci (27 of 68) and *S. aureus* (21 of 68) were the most frequently isolated gram-positive bacteria, and *Escherichia coli* (12 of 39) was the most frequently isolated gram-negative bacterium. The organisms that were detected by the FAN bottles but that were missed by the routine BD or BTA bottles were not rare pathogens; rather, they were relatively hardy organisms such as *S. aureus*, *E. coli*, and *Acinetobacter* sp. (Table 3). The improved recovery of bacteria by the FAN bottles likely reflects the ability of Fuller's earth to neutralize either antibiotics or other toxic compounds in CAPD effluents.

Despite the improved recovery of organisms from CAPD effluents with the FAN and BTAn blood culture bottles, there was still a large proportion of patients who had clinical peritonitis but in whose effluents no microorganisms were detected (17.9% rate of culture-negative peritonitis). The outstanding question is whether these patients with clinical symptoms and elevated leukocyte counts in their CAPD effluents truly have peritonitis due to microorganisms or whether the inflammation

TABLE 3. Organisms detected by each method

Organism[a]	No. (%) of organisms detected by the following culture method[b]:						
	Total	BTA bottles	BTAn bottles	FAN bottles	BD bottles	BA plates	MAC plates
Staphylococcus aureus	21	18	19	21	19	20	19
Staphylococcus epidermidis	20	18	18	18	18	15	18
Coagulase-negative staphylococci	10	5	4	7	9	6	6
Micrococcus species	1	1	1	1	1	0	0
Stomatococcus mucilaginosus	1	1	1	1	1	0	0
Alpha-hemolytic *Streptococcus* spp. (non-*Streptococcus pneumoniae*)	10	9	7	6	8	5	1
Streptococcus pneumoniae	1	1	1	1	1	1	1
Streptococcus species	1	0	1	1	1	0	0
Group D *Streptococcus* (non-*Enterococcus*)	1	1	1	1	1	1	0
Enterococcus faecalis	2	2	2	2	2	1	0
Streptococcus agalactiae	1	1	0	1	0	1	0
Escherichia coli	12	9	9	12	9	8	11
Gardnerella vaginalis	1	1	1	1	1	0	0
Klebsiella pneumoniae	5	5	5	5	4	1	4
Klebsiella oxytoca	4	2	2	4	2	0	1
Enterobacter cloacae	2	1	2	2	2	0	1
Proteus vulgaris	1	1	1	1	1	1	1
Acinetobacter species	5	2	0	5	3	1	1
Morganella morganii	3	3	3	3	2	1	1
Flavobacterium species	1	1	0	1	1	1	1
Pseudomonas aeruginosa	5	3	3	5	5	2	4
Moraxella cararrhalis	1	1	0	1	0	1	1
Bacillus species	2	1	0	1	0	0	0
Gram-positive rods (facultative)	3	1	1	2	1	1	1
Bacteroides fragilis group	4	1	4	1	3	0	0
Clostridium perfringens	1	0	1	0	1	0	0
Gram-positive rods (non-spore-forming anaerobes)	1	1	1	1	1	0	0
Candida albicans	3	3	2	2	1	2	1
Candida parapsilosis	1	2	1	1	1	1	1
Peptococcus species	1	0	1	0	1	0	0
Total	125	92 (73.6)	94 (75.2)	106 (84.8)	101 (80.8)	70 (56.0)	73 (58.4)

[a] A total of 125 organisms were detected (by any method) from 97 CAPD effluents.
[b] FAN and BTAn bottles combined would have detected 117 of 125 (93.6%) of all organisms, the method with BD bottles and BA and MAC plates combined would have detected 107 of 125 (85.6%) of all organisms (by McNemar's chi-square test with Yates' correction, the two-tailed P value is equal to 0.0218), the FAN and BTA bottles combined would have detected 111 of 125 (88.8%) of all organisms. By McNemar's chi-square test with Yates' correction, there was no significant difference between the FAN-BTAn bottle and FAN-BTA bottle combinations (the two-tailed P values is 1.000).

is due to another cause (e.g., lipopolysaccharide [8, 15, 17]). Elevated leukocyte counts in the dialysates of patients just starting CAPD has been described (15). Differentiating inflammation due to other causes from true microbial infection is an important issue, since antimicrobial therapy may have little value in these patients and indeed may be detrimental, because it could contribute to the development of increased resistance by the normal flora of patients on CAPD. Whether PCR analysis of such CAPD effluents would be able to detect the rRNA of microorganisms and help answer this question remains to be studied. The false-negative culture results obtained with the instrument (Table 2) indicate that blind subculture of BacT/Alert CAPD cultures should be performed on day 5; otherwise, significant pathogens may be missed.

Our data support data from other studies that have demonstrated improved sensitivity when blood culture bottles are used to test peritoneal or CAPD effluents (6, 21). In addition to the increased sensitivity, the inoculation of 10 ml of CAPD effluent without the centrifugation step but with the automated monitoring of the bottles decreases the overall labor costs and frees laboratory technologists to perform other functions. Gram staining was noncontributory for the majority of CAPD effluents. Among the culture-positive effluents, only 24 of 197 (24.7%) had a positive Gram stain result. All of the positive Gram stain results were for CAPD effluents with elevated leukocyte counts. As a cost-effective approach, we recommend that Gram staining not be performed unless the leukocyte count is elevated. Indeed, even when the leukocyte count was elevated, the Gram stain result was negative for 73.6% of CAPD effluents tested. This is consistent with recent reports of Gram stains being positive for only 9 to 40% of CAPD effluents from patients with documented peritonitis (9).

In conclusion, the use of BacT/Alert blood culture bottles provides an effective and less labor intensive approach to the detection of microorganisms in CAPD effluents. Our data support the suggestion by Keane et al. (9) that antimicrobial removal devices may be useful for maximizing the culture yield. To ensure optimal bacterial detection, use of the combination of a FAN bottle and a BTAn bottle is recommended. Future evaluations are required to assess whether an anaerobic FAN bottle would provide an effective single-bottle alternative.

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