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Total Volume Culture Technique for the Isolation of Microorganisms from Continuous Ambulatory Peritoneal Dialysis Patients with Peritonitis

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A total volume method of culturing dialysis fluid from continuous ambulatory peritoneal dialysis patients during episodes of peritonitis was developed. Concentrated culture media stored in small blood transfer bags were added directly to the drained dialysate exchange bags by the same technique used to carry out the dialysate exchange. The exchange bag with the added culture medium was incubated at 35°C and observed for turbidity. Seventy-eight dialysis exchange bags from patients without clinical peritonitis (negative controls) and forty-eight dialysis exchange bags from patients with clinical peritonitis were cultured. Bacteria were recovered from all cultures of patients with clinical peritonitis (100% sensitivity) and from five cultures of negative control fluids (94% specificity). Of these isolates, 86% were gram positive, and 14% were gram negative. This technique represents an advance over previously described culture techniques in its ability to isolate the causative organism(s) in cases of peritonitis in continuous ambulatory peritoneal dialysis patients.

Continuous ambulatory peritoneal dialysis (CAPD) has become a well established and acceptable alternative to hemodialysis in the treatment of patients with end-stage renal disease. Despite the many advantages of CAPD over hemodialysis (5, 17), peritonitis continues to be a major complication which increases morbidity and cost (1, 11, 12). For the episodes of peritonitis to be treated effectively, the clinical microbiology laboratory must be able to isolate the causative organism(s). The recovery of microorganisms from CAPD patients with peritonitis lacks sensitivity because the large volume (2 liters) of dialysis fluid in the peritoneal cavity decreases the bacterial concentration in the fluid sent for culture (18). Various methods have been employed to increase the sensitivity of culture. These include centrifugation of the fluid and culture of the sediment (5, 16, 18) and filtration of a large volume of dialysate and culture of the filter (7, 15, 16, 18). Culture methods which do not employ concentration of the dialysis fluid include inoculation of fluid into blood culture bottles (8, 10) and thioglycolate broth (9, 14). The culture-negative rate has been reported to range from 4 to 37% in cases of peritonitis (3, 6, 8–10, 13). Even when concentration and enrichment methods have been utilized, no organisms were isolated in 12 to 28% of infected patients (5, 7–10). Therefore, we developed a technique for the culturing of dialysis fluid from CAPD patients with peritonitis which has resulted in an increased sensitivity over those of previously described techniques. The technique is a total volume culture.

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MATERIALS AND METHODS

Peritoneal dialysis solution. Plastic dialysate bags containing 1, 1.5, or 2 liters of dialysis fluid and 1.5, 2.5, or 4.25% glucose were obtained from Travenol Laboratories, Deerfield, Ill. (Dianeal).

Patients. One hundred twenty-six dialysis exchange bags containing peritoneal dialysis effluent from 41 patients on CAPD were examined over a 15-month period. Controls were dialysis exchange bags obtained from patients on routine visits or follow-up after successful treatment for episodes of peritonitis who did not meet the criteria for peritonitis. Patients with peritonitis were defined as those who had cloudy dialysate and a peritoneal leukocyte count of greater than 100 cells per mm^3, with a predominance of polymorphonuclear leukocytes (10, 16).

Culture procedure. The patient dialysis exchange bags containing effluent were delivered to the microbiology laboratory for culturing on the same day that they were collected from the patients. Bags not processed immediately were refrigerated. A 300-ml blood transfer bag (Transfer Pack; Travenol Laboratories) which contained 50 ml of a fivefold concentrate of sterile thioglycolate broth (Difco Laboratories, Detroit, Mich.) was aseptically attached to the patient exchange bag after decontamination of the connection site with povidone-iodine solution and allowed to fill with well-mixed effluent. The blood transfer bag containing the thioglycolate broth and added effluent was disconnected and incubated at 35°C for growth of anaerobic microorganisms. A second blood transfer bag, which contained 100 ml of a 10-fold concentrate of sterile brain heart infusion (BHI) broth (Difco), was aseptically connected to the patient exchange bag. The BHI broth was mixed with the effluent, and the blood transfer bag was left attached to the exchange bag (Fig. 1). The exchange bag with added media and attached blood transfer bag was incubated at 35°C and

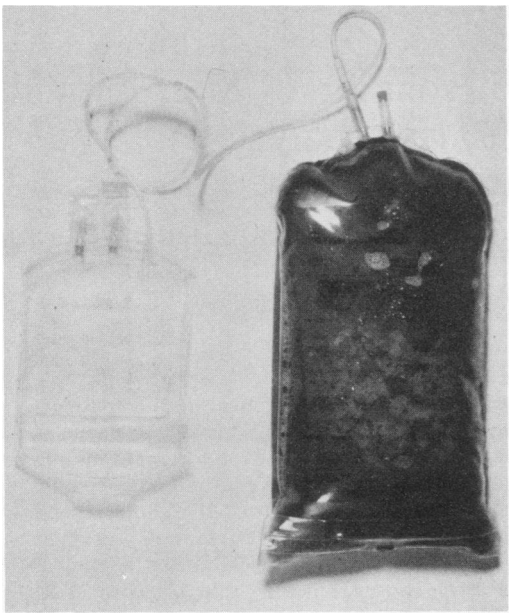

FIG. 1. After connection, blood transfer bag on the left emptied of BHI broth and patient exchange bag on the right with added media.

observed daily for the development of turbidity. After the development of turbidity, samples of fluid were withdrawn from the injection port on the bag with a sterile needle and syringe. The fluid was Gram stained and plated on appropriate media for isolation and identification of the microorganisms. Identification was by established procedures. Bags showing no growth were discarded after 7 days of incubation.

Seeding of dialysis bags. Dialysis exchange bags containing effluent collected from CAPD patients without peritonitis were incubated at 35°C for 5 days before use in seeding experiments and were designated patient peritoneal dialysate. Commercial dialysis solutions (Dianeal; Travenol Laboratories) which had not been used by patients were designated unused dialysis solutions. *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* were grown overnight in BHI broth. The overnight culture (1 ml) was diluted 1:1,000 in BHI broth and added to plastic bags which contained patient peritoneal dialysate or unused dialysis solutions with or without added media. The final bacterial concentration was approximately 10^2 CFU/ml. The bags were incubated at 35°C, and samples were removed at 0, 24, 48, and 72 h for plate counts of bacterial growth.

RESULTS

Growth of bacteria in peritoneal dialysis fluids. *S. epidermidis* and *P. aeruginosa* are commonly isolated from CAPD patients with peritonitis, and therefore, the seeding experiments were designed to determine whether these two organisms would survive and multiply in dialysis fluid which contained added BHI broth or no additional media. Both sterile unused dialysis solutions and asymptomatic patient peritoneal dialysates in plastic bags were utilized. Results of these experiments are shown in Table 1. *S. epidermidis* was unable to survive in unused dialysis solutions without added media and also failed to multiply in patient peritoneal dialysate without added media. However, *S. epidermidis* was able to multiply in either fluid when media was added, increasing by 5 logs in unused dialysis solutions and 6 logs in patient peritoneal dialysate after 72 h of incubation. *P. aeruginosa* was able to survive and grow in both unused dialysis solutions and patient peritoneal dialysates with or without added media, with only a 1 or 2 log difference in bacterial counts between fluids with media or without media (Table 1). Within 24 h of incubation, both *S. epidermidis* and *P. aeruginosa* increased to at least 10^7 CFU/ml in fluids to which media were added (Table 1). No difference in the growth or survival of either organism was found when dialysis solutions with different concentrations of glucose were utilized.

Isolation of microorganisms from CAPD fluids. Table 2 lists the organisms that were isolated from 53 cultures of patient dialysis fluid by the total volume culture technique. The most frequently isolated organism was coagulase-negative staphylococci (48.3%). *Staphylococcus aureus* was the next most common isolate (19%). The most common gram-negative isolate was *P. aeruginosa* (3.5%). Gram-positive organisms represented 86.3% of the isolates, and gram-negative organisms represented 13.7%. Mixed cultures were found in 5 of 53 cultures (9.4%). Two mixed cultures were due to coagulase-negative staphylococci and *Streptococcus mitis*, one was due to coagulase-negative staphylococci and *P. aeruginosa*, one was due to coagulase-negative staphylococci and *Bacillus* sp., and one was due to *Escherichia coli* and *Streptococcus faecalis*.

Correlation of culture results with clinical peritonitis in CAPD patients. Table 3 shows the results of both control cultures and cultures from patients with clinical peritonitis. In all of the patients who presented with symptoms of peritonitis, a causative organism was isolated. No false-negative cultures were found from the patients with peritonitis, resulting in 100% sensitivity of the total volume culture technique. From five control cultures, organisms (false-positive cultures) were also isolated for a specificity of 94%. The isolates from control cultures were *Bacillus* sp. in two

TABLE 1. Growth of bacteria in peritoneal dialysis fluids with or without added BHI broth

Peritoneal dialysis solution	Growth of bacteria (CFU/ml)							
	S. epidermidis (hours of incubation)				*P. aeruginosa* (hours of incubation)			
	0	24	48	72	0	24	48	72
Unused dialysis solution								
Alone	6.8×10^2	0	0	0	6.0×10^2	6.8×10^2	1.6×10^6	1.4×10^7
BHI broth added	6.8×10^2	8.8×10^7	1.4×10^8	1.2×10^7	6.9×10^2	2.2×10^7	8.8×10^7	1.6×10^8
Patient peritoneal dialysate								
Alone	5.2×10^2	9.6×10^2	3.9×10^2	3.3×10^2	9.0×10^2	1.2×10^4	6.6×10^5	1.1×10^7
BHI broth added	5.2×10^2	4.1×10^7	1.1×10^8	7.6×10^8	9.0×10^2	8.5×10^7	2.3×10^8	6.0×10^9

cultures, coagulase-negative staphylococci in one culture, *S. mitis* in one culture, and a mixed growth of both coagulase-negative staphylococci and *S. mitis* in one culture.

DISCUSSION

Peritonitis is a major complication of CAPD. As CAPD use becomes more widespread, the microbiology laboratory has a considerable diagnostic burden to detect the causative organisms in cases of peritonitis. The aim of this study was to develop a technique for culturing peritoneal dialysis fluid from patients on CAPD therapy which would result in a substantial increase, over previously published reports, in the isolation of microorganisms during episodes of peritonitis. By seeding patient dialysis bags containing effluent to which media had been added with either *S. epidermidis* or *P. aeruginosa*, it was found that detectable growth of both bacterial species occurred by 24 h of incubation (Table 1). Several recent reports have looked at growth of gram-positive and gram-negative bacteria in unused dialysis solutions and patient peritoneal dialysates without added media with results similar to ours (Table 1), but these were not compared with results from used dialysis solutions to which media had been added (4, 19). Since rapid substantial growth occurred in the patient peritoneal dialysate-containing media, we used this technique to culture peritoneal dialysate from CAPD patients with peritonitis and control patients without peritonitis. Coagulase-negative staphylococci were the most frequent isolates from CAPD patients in our study, followed by *S. aureus* and *S. mitis* (Table 2). These results are similar to those in previously published studies (3, 7–9, 13, 16, 18). Members of the family *Enterobacteriaceae* are usually found in 10 to 20% of the cases, and *Pseudomonas* sp. is found in 5 to 10% of the peritonitis episodes (7, 10, 16–18). In our study, members of the *Enterobacteriaceae* accounted for 8.6% of the infections, and *P. aeruginosa* accounted for 3.5% (Table 2). One unusual isolate in our study was *Acinetobacter calcoaceticus*. *Acinetobacter* spp. have been isolated in small numbers in only a few other studies (8, 9, 18). *Corynebacterium* sp. was isolated from one patient with peritonitis. Other studies have isolated *Corynebacterium* sp. from 1 to 7% of peritonitis cases (8, 9, 17). Although we isolated *Bacillus* sp. alone in two cultures which were from patients without peritonitis, *Bacillus cereus* has been found to be the causative agent in several cases of peritonitis in CAPD patients (2, 7). Anaerobic bacteria have rarely been isolated from dialysis fluid (8, 16, 18), and we did not recover any from the 126 dialysis bags in this study.

TABLE 2. Organisms isolated from CAPD patient dialysate with total volume culture

Organism	No. of isolates	% of Isolates	% of Total
Gram positive			86.3
Staphylococci (coagulase negative)	28	48.3	
Staphylococcus aureus	11	19.0	
Streptococcus mitis[a]	5	8.6	
Streptococcus faecalis[b]	2	3.5	
Bacillus sp.[c]	3	5.2	
Corynebacterium sp.	1	1.7	
Gram negative			13.7
Pseudomonas aeruginosa[d]	2	3.5	
Escherichia coli	1	1.7	
Proteus mirabilis	1	1.7	
Klebsiella ozaenae	1	1.7	
Citrobacter sp.	1	1.7	
Serratia marcescens	1	1.7	
Acinetobacter calcoaceticus	1	1.7	

[a] Two *S. mitis* organisms were isolated in mixed culture with coagulase-negative staphylococci.
[b] One *S. faecalis* isolate was from a mixed culture with *E. coli*.
[c] One *Bacillus* sp. was isolated in mixed culture with coagulase-negative staphylococci.
[d] One *P. aeruginosa* organism was isolated with coagulase-negative staphylococci.

TABLE 3. Comparison of culture results with clinically diagnosed infections

Culture results	No. of patients with peritonitis[a] (%)	No. of controls[b] (%)
Positive	48 (100)	5 (6)
Negative	0 (0)	73 (94)

[a] Dialysate was cloudy and contained more than 100 leukocytes per mm^3. $n = 48$.
[b] Clinically well patients. $n = 78$.

The total volume culture technique detected organisms in five peritoneal dialysates from CAPD patients without peritonitis. Since the organisms isolated could all have been potential pathogens, only consultation with the physician to determine whether the patient had signs or symptoms of peritonitis could be used to identify these as contaminants. Quantitative methods have been used to try to determine the difference between contaminants and potential pathogens in CAPD patient dialysates. Rubin et al. (15) compared colony counts of specimens from peritonitis and nonperitonitis cases which were culture positive. Although colony counts tended to be higher in culture-positive peritonitis cases, there was some overlap. They concluded that colony counts alone could not be used to clearly separate the two groups. Therefore, quantitation methods cannot always distinguish between a contaminant and a pathogen. In any technique utilized to analyze CAPD patient dialysates for microorganisms, consultation with the physician may be necessary to identify a positive culture as a contaminant.

Although antibiotic therapy is usually started empirically after the diagnosis of peritonitis in CAPD patients, isolation and identification of the causative organism(s) are still important to determine whether antibiotic therapy is appropriate and to rule out unusual etiological organisms. Recently, Grefberg et al. (8) reported isolation of organisms from peritonitis cases in CAPD patients which had multiple resistance to antibiotics.

In summary, we have developed a total volume method for culturing dialysis fluid from CAPD patients during episodes of peritonitis. In all of our patients with clinically diagnosed peritonitis, we were able to isolate a causative organism. Therefore, the total volume culture technique demonstrated 100% sensitivity in our patient population. This culture method represents increased sensitivity over previously published reports.

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