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Addi-Chek Filtration, BACTEC, and 10-ml Culture Methods for Recovery of Microorganisms From Dialysis Effluent during Episodes of Peritonitis

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The Addi-Chek (filtration; Millipore Corp., Bedford, Mass.) and BACTEC (radiometric detection of growth in culture media; Johnston Laboratories, Inc., Towson, Md.) systems were compared with the 10-ml culture (centrifugation) method for the recovery of microorganisms from peritoneal dialysate collected from patients with clinical evidence of peritonitis and containing ≥200 leukocytes per mm³. Both alternate methods were comparable, and results were not significantly different from those of the conventional 10-ml culture method. All systems were adversely affected in their capacity to recover organisms when dialysates had been collected during periods of antimicrobial therapy.

Continuous ambulatory peritoneal dialysis (CAPD) is now considered an effective therapy for patients with end-stage renal disease (5). A frequent complication of peritoneal dialysis is peritonitis (7, 10). The diagnosis, treatment, and outcome of peritonitis is dependent, in part, on recovery and identification of the organism(s) from the dialysis effluent of the patient at the onset of infection. Various techniques have been used to enhance recovery of microorganisms from dialysate. They include the culture of larger volumes of peritoneal effluent processed by centrifugation (8) or filtration (7, 9) and the use of blood culture media (3, 6).

In this center, microorganisms are recovered from dialysate by the culture of sediment from 10 ml of centrifuged dialysis fluid (10-ml culture method). The efficacy of this relatively simple recovery system to initially detect and recover microorganisms present in dialysis effluent was assessed by comparison with other, putatively more efficient, more costly and labor-intensive recovery techniques and was based on the use of dialysate specimens containing ≥200 leukocytes (WBC) per mm³, since they represent the majority of dialysates collected at the onset of peritonitis. This report presents our findings on the use of the Addi-Chek filtration and BACTEC system (radiometric detection) methodologies compared with the 10-ml culture method in detection and recovery of microorganisms from peritoneal dialysis effluent.

MATERIALS AND METHODS

In a prospective study covering a 17-month period, 26 patients on continuous ambulatory peritoneal dialysis were monitored for clinical peritonitis, which was defined by one or more of the following: cloudy dialysate, rebound tenderness, and rebound tenderness accompanied by abdominal pain. During this period, 117 dialysate specimens represented by 2-liter bags filled with dialysis effluent were collected. Of the 117 dialysate specimens, 41 with total WBC counts of ≥200 per mm³ were included in the present study. They represented 29 discrete episodes of clinical peritonitis in 19 of the 26 patients (6 of the 19 patients had two or more episodes). Samples of each of the 41 specimens were processed by the three recovery and isolation systems outlined below.

Ten-milliliter culture. Dialysate (10 ml) was centrifuged, and the sediment was used for Gram-stained film and inoculation of the following media: chocolate and 5% sheep blood agar (Scott Laboratories, Inc., Fiskeville, R.I.); and MacConkey agar, thioglycolate broth, and 5% human blood agar containing vitamin K, yeast extract, and hemin for anaerobic growth, which were prepared in house. All cultures were incubated and examined daily for 7 days.

BACTEC. Dialysate (3 to 5 ml) was inoculated into each BACTEC (Johnston Laboratories, Inc., Towson, Md.) blood culture bottle (aerobic, hypertonic, and anaerobic media) and incubated according to manufacturer instructions for up to 7 days. Gram-stained films were prepared from blood culture bottles yielding a positive growth index (radiometric detection).

Addi-Chek. A variable volume (up to 500 ml) of each dialysate specimen was passed through the Addi-Chek (Millipore Corp., Bedford, Mass.) filtration system, which consisted of an enclosed, sterile chamber containing a 0.45-μm (pore size) membrane filter, for entrapment of microorganisms present in the dialysate. Clogging of the membrane filter determined the maximum amount of effluent that could be processed through each filtration unit. Sterile, prepackaged, 100-ml samples of a bacteriologic growth medium, tryptic soy broth, were then added to each Addi-Chek unit, followed by incubation at 35°C for up to 10 days. Organisms were detected by the development of turbidity, and a Gram-stained film was made from the turbid broth. In each system, final recovery and identification of microorganisms were accomplished by standard methods.

RESULTS

The 41 dialysate specimens tested by the three recovery systems represented 29 discrete peritonitis episodes. Of the

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TABLE 1. Culture-positive specimens with various results in the three detection and recovery systems

Specimen	10-ml culture	BACTEC	Addi-Chek	Rx[a]	Prior culture[b]
A	C-NS	NG[c]	NG	+	C-NS
B	NG	NG	*Acinetobacter* sp.	+	*Acinetobacter* sp.
C	NG	NG	C-NS	+	C-NS
D	NG	NG	*P. maltophilia*	+	None
E	NG	α-Streptococci	α-Streptococci	0	None
F	*H. influenzae*	*H. influenzae*	C-NS	0	None
G	α-Streptococci	α-Streptococci, micrococci	α- and γ-Streptococci, micrococci	0	None
H	*Acinetobacter* sp.	*Acinetobacter* sp., *Bacillus* sp.	*Acinetobacter* sp.	0	None
I	*Acinetobacter* sp., *K. pneumoniae*, C-NS	*Acinetobacter* sp., *K. pneumoniae*	*Acinetobacter* sp., *K. pneumoniae*, C-NS	0	None
J	C-NS	C-NS	C-NS, *P. maltophilia*	0	None
K	*K. pneumoniae*	*K. pneumoniae*, C-NS	*K. pneumoniae*, C-NS	0	None
L	NG	C-NS	C-NS	+	α-Streptococci
M	α-Streptococci	α-Streptococci	Clogged filter	0	None

[a] Specimen collected during (+) or in the absence of (0) antimicrobial therapy.
[b] Results of a previous 10-ml culture, if any, before the start of antimicrobial therapy.
[c] NG, No growth.

41 specimens, 24 were culture positive by one or more of the recovery systems. They represented 79% (23 of 29) of the peritonitis episodes included in this study. The 10-ml culture method recovered bacteria from dialysates from a smaller number of peritonitis events ($n = 19$) than did BACTEC ($n = 20$) or Addi-Chek ($n = 22$). However, this difference was not considered statistically significant ($P > 0.05$; chi-square).

A total of 32 microorganisms were recovered from 24 dialysate specimens. No fungi or anaerobes were isolated. The identity of these organisms and the number of dialysis specimens from which they were recovered were: coagulase-negative staphylococci (C-NS), 15; α-streptococci, 4; *Acinetobacter* sp., 3; *Pseudomonas maltophilia*, 2; *Klebsiella pneumoniae*, 2; *Staphylococcus aureus*, 1; γ-streptococci, 1; micrococci, 1; diphtheroids, 1; *Bacillus* sp., 1; and *Haemophilus influenzae*, 1. The types and frequencies of organisms isolated were similar to those reported previously (7, 10). Twenty-four (75%) of the isolates recovered from the dialysates were gram-positive organisms. C-NS were the most prevalent organism group recovered. Gram-negative bacilli accounted for 8 (25%) of the 32 isolates.

Gram-stained films were also prepared from dialysate sediment (10-ml culture method) for the rapid detection of bacteria; three (7%) of the specimens demonstrated organisms on smear. Two of the three specimens were culture positive by all three recovery systems; the third specimen, which was obtained after the start of therapy, was culture negative in each system.

Identical results were obtained among each of the three recovery systems in 28 of the 41 dialysates. Seventeen effluent specimens were culture negative by each system, including Addi-Chek, in which the volumes of dialysate processed ranged from 20 to 400 ml (100 ml or more was processed in 13 of the 17 samples). Fourteen of these specimens were collected from patients after the start of antimicrobial therapy and from whom previous dialysates yielded microorganisms prior to therapy. The remaining three dialysates were obtained at the onset of "sterile" peritonitis episodes and prior to the start of therapy. In 11 of 28 samples, the same organisms were recovered by each system. They included eight C-NS and one each of *S. aureus*, α-streptococci, and diphtheroids. The volumes of dialysate processed by Addi-Chek among these specimens ranged from 12 to 500 ml, with volumes of 100 ml or more in 6 of the 11 samples. Ten of these specimens were obtained before the start of antimicrobial therapy. The remaining specimen was collected during the period of therapy but was still culture positive for the same organism recovered prior to this time.

Culture results, which varied according to the system used, are presented in Table 1. Thirteen specimens yielded different results; included are the relation of specimen collection to antimicrobial therapy and culture results of any previous specimen collected during the same peritonitis episode. Among those specimens (B, C, and D) exclusively positive by Addi-Chek, the volumes of dialysate processed (80, 70, and 170 ml, respectively) were not significantly different (i.e., larger) compared with the remaining specimens (6 to 150 ml, with volumes exceeding 50 ml in four specimens), for which results with Addi-Chek were comparable to those with BACTEC or the 10-ml culture method.

The overall rates of isolate recovery for each system are listed in Table 2. Specimens were grouped according to total WBC count and administration of antimicrobial therapy. The differences in recovery rates among the three systems were not significant ($P > 0.05$; chi-square).

DISCUSSION

Any discussion of peritonitis should be considered in light of the definition of this condition. Application of more

TABLE 2. Recovery of isolates according to cell count and antimicrobial therapy

Recovery system	No. (%) of culture-positive dialysates with:			
	200–499 WBC per mm^3		≥500 WBC per mm^3	
	Rx0[a] (3)[b]	Rx+[a] (13)[b]	Rx0 (18)[b]	Rx+ (7)[b]
10-ml culture	0	0	17 (94)	2 (29)
BACTEC	0	0	18 (100)	2 (29)
Addi-Chek	0	2 (15)	17 (94)	3 (43)

[a] Dialysis specimens collected during (Rx+) and in the absence of (Rx0) antimicrobial therapy.
[b] Numbers of specimens per category.

stringent criteria for defining peritonitis than used in this study (i.e., more than one qualifying criterion) would almost certainly yield a greater percentage of culture-positive dialysate samples. Other factors, such as the presence of chemical irritants in the dialysis fluid, may contribute to cases of sterile peritonitis and give rise to negative culture results (6). Moreover, it has been suggested (10) that the large volume of dialysate in the peritoneal cavity dilutes the organism to such a degree that it may escape detection, particularly by conventional methods.

Alternate methodologies have been devised to compensate for low numbers of organisms in dialysis effluent. They include the use of special recovery media or systems (BACTEC) or the processing of large volumes of dialysate (Addi-Chek filtration). The advantage, if any, of using these more costly and labor-intensive techniques over a conventional method (10-ml culture) for the initial detection and recovery of organisms from dialysate at the onset of peritonitis, was assessed in a selected group of specimens. The dialysates examined were limited to those specimens containing large numbers of WBC ($\geq$200 per mm^3), since these types of specimens are representative of those obtained at the start of peritonitis.

With 10-ml culture, detection of bacteria coincided with their recovery on various media, usually within a 48-h period of incubation. As documented by others (2, 4, 7), inclusion of a Gram-stained film of the dialysate was of minimal value in the rapid detection of bacteria; only 7% of our specimens were positive by smear. In most instances, the bacterial concentration is too low for detection by this method (1, 7). Final recovery of isolates by the two alternate systems could be delayed by an additional 24 h or more; however, the time of initial detection of bacteria by radiometric means (BACTEC) or turbidity (Addi-Chek) was comparable to that of the 10-ml culture method.

Based on our criteria for inclusion of dialysate specimens in the present study, the bacterial recovery rate was comparable for all three systems and approached 80% (20/25) when the dialysate contained $\geq$500 WBC per mm^3 (Table 2). The conventional method (10-ml culture) proved to be a satisfactory system for recovery of microorganisms from these dialysates. In two previous reports (2, 4) low rates of recovery were obtained with microbiologic media and methods similar to those of the 10-ml culture system; however, in those studies no distinction was made between cloudy specimens (effluent containing increased numbers of WBC) and clear dialysates. Depending on the phase of peritonitis, bacterial counts may vary (1). Consequently, the selection of only cloudy dialysis effluent could mask any advantage of the alternate systems, e.g., the processing of large volumes (11), in recovery of microorganisms from dialysate. Addi-Chek and BACTEC appeared nominally more efficient than the 10-ml culture method; several additional specimens were culture positive by these two systems (Table 1). However, there was no statistically significant advantage for either alternate approach. Use of the alternate methods did, however, increase the proportion of specimens containing multiple organisms (Table 1). This may indicate a greater sensitivity of the alternate methods in detection of organisms present in dialysate at lower concentrations.

The relative effectiveness of each recovery system was apparent in a portion of the specimens collected during antimicrobial therapy (Table 1). Here Addi-Chek was better than the 10-ml culture method in the recovery of isolates from dialysis effluent, although it was not always clear as to whether the organism recovered was related to the clinical condition (specimens D and F). Results with BACTEC were essentially identical to those of the 10-ml culture system.

Previous studies have demonstrated an advantage in using BACTEC (6) or filtration (9) compared with conventional culture practice to recovery microorganisms from dialysate; however, these earlier findings must be reevaluated in view of the effect of inoculum size and culture media (4) on rates of bacterial recovery. In the BACTEC evaluation by Luce et al. (6), the conventional method used 0.05 ml of dialysate as the inoculum. The present study used 10 ml of dialysate (sediment) for conventional processing of specimens, which was comparable to that used for the BACTEC system. Whereas the Addi-Chek system is capable of processing larger volumes of fluid, the actual volume of dialysate filtered was limited to 20 ml or less in five dialysates (see specimen M), owing to clogging of the filter. This is attributed to large amounts of fibrin and cells in the effluent (8). The processing of larger volumes of fluid by Addi-Chek resulted in no significant advantage (and was more labor intensive) compared with the 10-ml culture method. In 28 of 41 specimens, the processing of large volumes (up to 500 ml) of dialysate by Addi-Chek yielded culture results identical to those of the BACTEC and 10-ml culture methods; and of those specimens (B, C, and D) exclusively positive by Addi-Chek, the volumes filtered were not significantly different from the amounts processed in the remaining specimens (Table 1). Moreover, the culture media used with Addi-Chek were unsuitable for the recovery of more fastidious organisms (specimen F) and could not be used for the culture of anaerobes.

Recovery rates (approaching 100%) were excellent among all systems for specimens containing $\geq$500 WBC per mm^3 and drawn prior to the start of antimicrobial therapy (Table 2). In only three peritonitis episodes were specimens culture negative (by each system) in the absence of antibiotics. These three dialysate samples contained 200 to 499 WBC per mm^3. Indeed, antimicrobial therapy was the single most important factor influencing the recovery of organisms from dialysate. The rates of recovery among the three systems were dramatically decreased for specimens taken during the time of therapy (Table 2). (None of the dialysate specimens were washed prior to culture.)

Vas and Law (11) recently reported the inclusion of a wash step for the removal of antibiotics to be more effective in enhancing recovery of organisms from dialysate than the use of either blood culture media (BACTEC) or increased specimen volume (centrifugation). Moreover, a significant increase in the recovery of organisms from dialysate was achieved when they combined these techniques and used a large volume sediment from centrifugation along with BACTEC 16B and 17D resin media (for removal of antibiotics). This combination was a significant improvement over a single recovery system and may greatly decrease the number of sterile dialysates.

Our experience comparing a conventional method (10-ml culture) with several alternate recovery systems indicates the 10-ml culture method to be a satisfactory means of recovering microorganisms from selected dialysate specimens without the need for a more labor-intensive and costly technique such as the BACTEC or Addi-Chek system. Clearly, the ideal approach to rapid detection and recovery of microorganisms from continuous ambulatory peritoneal dialysis patients is dependent on several factors in which the clinical definition of the condition of the patient, ongoing therapy, and microbiologic methodology play a role.

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