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Microbiological Diagnosis of Peritonitis in Patients on Continuous Ambulatory Peritoneal Dialysis

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The fast and accurate etiological diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis is essential. The concentration of larger volumes of peritoneal fluids may yield more isolates than conventional methods. The removal of antibiotics present in the fluids as a consequence of therapy by washing or using antibiotic-removing resins increases the yield considerably. The use of anaerobic culture media is justified if fecal organisms are suspected as the cause of the infection.

Continuous ambulatory peritoneal dialysis as an alternative for the treatment of end-stage renal failure has become an accepted method in the last few years. Over 6,000 patients in the United States and many more around the world are on this mode of therapy.

Peritonitis has been an important problem associated with this mode of treatment from its institution (8). Although peritonitis rates have decreased in recent years, the accurate diagnosis of peritonitis is still a requirement of a successful continuous ambulatory peritoneal dialysis program. Most methods presently used (4) incorporate a concentration method to increase the chances of detecting an infection caused by a small number of organisms in a large volume of peritoneal dialysis fluid. It is important that the method used be sensitive, accurate, and fast. It should be capable of detecting infections even when antibiotics are present in peritoneal dialysis fluids.

A filtration method for the diagnosis of peritonitis in continuous ambulatory peritoneal dialysis patients has been previously described (5, 7). Filtration can only be used on clear peritoneal fluids, as the cells and fibrin present in cloudy fluids obstruct the filter rapidly. Therefore, centrifugation instead of filtration has been used for cloudy fluids (6). Conventional laboratory methods for the isolation of organisms from peritoneal dialysis fluids (1, 3) usually have a lower recovery rate. Recently, BACTEC (Johnston Laboratories, Inc., Cockeysville, Md.) 6B and 7C blood culture bottles were used (2). In the present paper, we recommend certain modifications to increase the sensitivity and speed of diagnosis.

MATERIALS AND METHODS

Fluid samples. Freshly drained peritoneal dialysis bags from patients suspected of having peritonitis or on antibiotic treatment for previously diagnosed peritonitis were delivered unopened to the laboratory and sampled under sterile conditions. Only visibly cloudy peritoneal dialysis fluids (cell count, >100 μl) were used for this study.

Culturing methods. (i) BHI method. Peritoneal dialysis fluid (10 ml) was centrifuged at 1,800 × *g* for 10 min (Centra 7; IEC Co., Needham Heights, Mass.). A loopful of the sediment was inoculated onto blood agar plates. The rest of the sediment was left in the centrifuge tube, and brain heart infusion (BHI) medium was poured over it. Cultures were incubated and organisms were identified by standard methods.

(ii) Thioglycolate method. The method routinely used in our laboratory has been described elsewhere (6–8). Briefly, it includes using large volumes of peritoneal fluid (two 50-ml portions) and processing clear fluids through a filter apparatus (Addicheck filter, 0.22-μm-pore diameter; Millipore Corp., Bedford, Mass.) or concentrating cloudy fluids by centrifugation at 2,350 × *g* (model V, size 2; IEC Co.); both methods include washing with 100 ml of sterile saline to remove antibiotics present in the peritoneal fluid. Resuspended sediments are inoculated into thioglycolate medium (135/C medium [BBL Microbiology Systems, Cockeysville, Md.] with 0.05% sodium polyanetholesulfonate added) to support aerobic and anaerobic growth and incubated, and organisms are identified by standard microbiological methods. In the present study, centrifugation was used.

(iii) Straight cultures. Two 50-ml portions of peritoneal dialysis fluid were centrifuged at 2,350 × *g* (model V, size 2; IEC Co.) for 10 min. The sediment from each portion was suspended in 3 ml of saline and injected into BACTEC aerobic (6B) and anaerobic (7D) culture bottles.

(iv) Washed cultures. Two 50-ml portions of peritoneal dialysis fluid were centrifuged as described for straight cultures but were washed with 50 ml of physiological saline after centrifugation to remove antibiotics. The sediment from each portion was suspended and injected as described for straight cultures.

(v) Resin cultures. Two 50-ml portions of peritoneal dialysis fluid were prepared as described for washed cultures and injected into BACTEC 16B and 17D antibiotic removal resin bottles without being washed.

Bacterial growth. Bacterial growth was monitored on a BACTEC model 460 instrument, and subcultures were identified by standard methods. Cultures were observed for 14 days.

RESULTS

It was expected that not all peritoneal dialysis fluids would yield positive cultures, as the study included patients who had just entered the hospital for diagnosis of peritonitis and therefore were previously untreated as well as patients already on therapy. A total of 160 successive cloudy-fluid

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TABLE 1. Numbers of positive cultures from 160 dialysis fluid samples from 51 patients

Culture	No. of cultures	No. (%) of positive cultures
BHI	110	50 (45.5)
Thioglycolate	110	60 (54.5)
Straight	160	80 (50.0)
Aerobic		77
Anaerobic		3
Washed	160	109 (68.1)
Aerobic		105
Anaerobic		4
Resin	50	36 (72.0)
Aerobic		34
Anaerobic		2

samples from 51 patients were processed during this study. During therapy, single cultures were obtained from some patients, and serial cultures were obtained from others. The numbers of positive cultures obtained with the different methods are shown in Table 1. The various organisms isolated from 107 positive dialysis fluid samples during this study are as follows (number of isolates): *Staphylococcus epidermidis* (17), *S. aureus* (18), *Streptococcus bovis* (9), *Neisseria* sp. (1), *Escherichia coli* (26), *Morganella morganii* (5), *Klebsiella pneumoniae* (2), *Proteus vulgaris* (6), enterococci (7), *Hafnia* sp. (4), *Acinetobacter* sp. (2), *Pseudomonas aeruginosa* (5), *Enterobacter* sp. (5), *Serratia marcescens* (6), *Candida* sp. (19), and *Bacteroides fragilis* (3). (Organisms recovered from the same peritoneal fluid sample by different methods are listed only once. Some fluids contained multiple organisms.) The distribution of organisms was similar to that previously reported (3, 7) for large numbers of peritonitis cases. The total number of strains exceeded the number of positive cultures, as several peritoneal fluids contained multiple isolates. No preference was shown by any species for any of the methods used (except that obligate anaerobes were isolated only on media supporting strict anaerobic growth).

DISCUSSION

A method suitable for the diagnosis of peritonitis in peritoneal dialysis patients should (i) allow for the processing of larger volumes of peritoneal dialysis fluid, if necessary; (ii) use a sensitive culture medium which supports the growth of both simple and fastidious organisms; (iii) include a provision for the removal of antibiotics present in the peritoneal dialysis fluid; (iv) yield the fastest results from the largest number of patients; and (v) include the possibility of recovering anaerobic organisms, if necessary.

Our results suggest the following conclusions. The processing of large volumes of fluid was only slightly superior to that of smaller volumes, as shown by a comparison of positive BHI cultures with positive straight cultures (45.5% versus 50.0%, respectively). In fact, if one does not count the anaerobic cultures in the straight cultures (BHI does not support anaerobic growth), the difference (2.6%) may not be significant. The reason for this is not clear from the present experiments. The usefulness of culturing larger volumes has previously been shown (6). Previous studies (1, 2, 5, 6) were done with a variety of patients, whereas the present study was done only with patients having cloudy fluid. The number of infecting organisms may be higher in patients having already developed acute infections, and therefore the advantage of culturing larger volumes may not be evident.

The removal of antibiotics by washing or by other methods (resin cultures) increased the isolation rate.

The BACTEC culture method is a suitable alternative to conventional culture methods and yields higher numbers of positive cultures. The time necessary for the detection of positive cultures was 1 to 2 days and was not significantly different for any of the methods, except that the anaerobic cultures took longer to grow.

The use of anaerobic cultures increased the isolation rate only marginally, but if fecal contamination of the abdominal cavity is suspected, anaerobic media should be included in the procedure.

Although the cost of the above-described methods is considerable, one has to weigh the cost of laboratory diagnosis against the possibility of unnecessary therapy and prolonged hospitalization.

LITERATURE CITED

1. **Fenton, P.** 1982. Laboratory diagnosis of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. J. Clin. Pathol. **35**:1181–1184.
2. **Gopaul, D., and P. Cordy.** 1984. Bacterial isolation from peritoneal fluids in CAPD patients. Can. J. Med. Technol. **46**:186–188.
3. **Knight, K. R., A. Polak, J. Crump, and R. Maskell.** 1982. Laboratory diagnosis and oral treatment of CAPD peritonitis. Lancet **ii**:1301–1304.
4. **Rubin, S. J.** 1984. Continuous ambulatory peritoneal dialysis: dialysate fluid cultures. Clin. Microbiol. Newsl. **6**:3–5.
5. **Rubin, J., W. A. Rogers, H. M. Taylor, E. D. Everett, B. F. Prowant, L. V. Fruto, and K. D. Nolph.** 1980. Peritonitis during continuous ambulatory peritoneal dialysis. Ann. Intern. Med. **92**:7–13.
6. **Vas, S. I.** 1980. Microbiological examinations of fluids during peritoneal dialysis. Peritoneal Dialysis Bull. **1**:2–3.
7. **Vas, S. I.** 1981. Microbiological aspects of peritonitis. Peritoneal Dialysis Bull. **1**(Suppl.):11–16.
8. **Vas, S. I.** 1983. Microbiological aspects of peritoneal dialysis. Kidney Int. **23**:83–92.