Clinical and Microbiological Evaluation of Four Culture Methods for the Diagnosis of Peritonitis in Patients on Continuous Ambulatory Peritoneal Dialysis

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A prospective study was performed to evaluate four culture methods for the diagnosis of bacterial peritonitis in patients on continuous ambulatory peritoneal dialysis. Peritonitis was present in 44 of 85 patient admissions (52%). The overall sensitivity of the culture methods in detecting peritonitis was 66%. The sensitivities of the individual methods were as follows: bag culture method, 61%; blood culture broth method, 51%; filter method, 54%; and plate method, 39%. Our broad definition of peritonitis resulted in lower sensitivities. A combination of the bag and blood culture broth methods detected all positive cultures.

Continuous ambulatory peritoneal dialysis (CAPD) was introduced by Popovich and colleagues in 1976 (6). For patients previously on hemodialysis, CAPD has offered many of them an improvement in the quality of life.

Peritonitis is still the most frequent complication of CAPD, although its incidence has declined since the initial work of Popovich and colleagues (6). The current incidence is estimated at one episode per patient year (9). The decrease can be attributed to the use of plastic dialysis bags, technological advances in the design of catheters and connectors, continuing education on aseptic techniques, and patient motivation (3, 7–9). The majority of cases of peritonitis are caused by bacteria (3). This study was undertaken to evaluate four culture methods for the diagnosis of bacterial peritonitis in patients on CAPD.

(These data were presented in part at the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy [P. W. Doyle, E. P. Crichton, and R. G. Mathias, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 216, 1987].)

MATERIALS AND METHODS

A prospective study was performed on successive patients on CAPD admitted to the renal ward at St. Paul's Hospital over a 7-month period in 1986. Patients were evaluated clinically on admission, and the first dialysis bag collected was sent to the laboratory for evaluation.

Peritonitis was defined as one or more of the following: (i) clinical signs (abdominal pain, tenderness, and/or fever); (ii) cloudy dialysate; and (iii) leukocyte count of >100/mm³ in the dialysate. Patients without peritonitis, as defined above, served as controls.

The dialysis bag was gently mixed in the laboratory, and the volume and gross appearance were recorded. Fluid was removed from the bag aseptically through the needle port with a syringe. Ten milliliters was sent to the hematology department for leukocyte count determination. The following methods were used in setting up the cultures. (i) A total of 5 ml from the dialysis bag was inoculated into one BACTEC blood culture bottle (Johnston Laboratories, Inc., Towson, Md.). The BACTEC bottle used depended on the availability of medium and was one of the following: 6B (aerobic), 8B (hypertonic), or 16B (resin). We did not take the type of bottle into account when the data were analyzed. This method is referred to as the blood culture (BC) broth method.

(ii) A total of 5 to 10 ml from the dialysis bag was then centrifuged at 2,500 rpm for 10 min (Silencer; Western Scientific, Richmond, British Columbia, Canada) and Gram stained, and the sediment was cultured onto plated medium. This method is referred to as the plate method.

For the aerobic culture plate method, the sediment was plated onto a blood agar plate, a MacConkey plate, and a chocolate plate. For the anaerobic culture plate method, the sediment was plated onto a blood agar plate and a colistin-nalidixic acid plate.

(iii) A total of 100 ml of fluid was removed aseptically, and as much as possible was filtered through a 0.45-μm-pore-size analytical filter unit (type A 130-4045; Sybron Corp.). The filter was cultured sludge side up on a blood agar plate.

(iv) The remaining fluid in the dialysis bag was processed in a laminar flow cabinet as follows. The bag wasbled down to 1,000 ml if there was >1,000 ml present. Brain heart infusion broth that was concentrated 10 times was added to the bag with a 100-ml glass syringe, at a ratio of 1 ml of brain heart infusion broth to 10 ml of dialysate. This modification of the total volume culture technique described by Dawson et al. (2) is referred to here as the bag culture method.

Plates were incubated at 35°C for 48 h, with the aerobic plates placed in 5% CO₂ and the anaerobic plates placed in an anaerobic jar. The dialysis bag, BC broth, and the filter were incubated for 5 days at 35°C in 5% CO₂. The BC broth samples were analyzed radiometrically. BC broth and bag cultures were subcultured if they were cloudy. Terminal subcultures were performed on the BC broth and bag cultures. Organisms were identified and susceptibilities were determined by standard methods (4).

Results were coded by one of the investigators and analyzed by using the computer program SPSSX on a mainframe computer. The sensitivity, specificity, positive and negative predictive values, and prevalence were determined by the calculations given in footnote α of Table 1 by using peritonitis (as defined above) as the "gold standard" (12). Differences in culture methods were analyzed statistically for individual organism groups by using the Fisher exact probability two-tail test. Data from the plate method were further
analyzed to determine the effectiveness of the aerobic and anaerobic plates.

RESULTS

A total of 85 specimens were collected. Peritonitis, as defined, was present in 44 of 85 patient admissions (52%) (Table 1). Of these patients, 37 of 44 had clinical peritonitis (84%) (abdominal pain, tenderness, or fever), 32 of 43 had cloudy dialysate (74%), and 28 of 34 had a leukocyte count of >100/mm³ (82%).

The remaining 41 patients served as negative controls. Several patients had multiple admissions (with or without peritonitis), and these were represented as separate events in the study.

In the presence of peritonitis (as defined above), 29 of 44 cultures were positive (66%) and 15 of 44 were negative (34%). The culture-negative patients showed the following. (i) Peritonitis was diagnosed in two patients who presented with marked clinical symptoms, cloudy dialysate, and a leukocyte count of 100/mm³. Pretreatment cultures of samples from both patients were negative. They improved on empiric therapy with cefazolin and with cefazolin plus tobramycin. Both patients were presumed to have bacterial peritonitis. (ii) One patient had tuberculous peritonitis and received 1 day of antituberculosis therapy. (iii) One patient had been on cefazolin for 5 days prior to admission, and peritonitis was resolving at that time. (iv) Two patients had catheter site infections only, without true peritonitis. One of these patients had localized tenderness at the catheter site with an otherwise unremarkable dialysate. The other patient had a slightly bloody dialysate with a leukocyte count of >100/mm³ and no clinical findings of peritonitis. (v) In two patients peritonitis associated with the insertion of a new dialysis tube resolved without therapy. (vi) In seven patients there were no physical signs of clinical peritonitis, and the patients had a cloudy dialysate, an increased leukocyte count, or both. All seven of these patients improved without therapy.

Five cultures were positive in patients without peritonitis. Three grew Staphylococcus epidermidis, one grew a Corynebacterium spp., and one was a mixed growth of gram-negative rods. The S. epidermidis and Corynebacterium spp. were found by only one culture method. It was felt that the specimen with mixed gram-negative bacilli was contaminated with fecal flora after collection.

The efficacy of individual culture methods in diagnosing peritonitis is summarized in Table 2. The bag method had the highest sensitivity at 61% and a negative predictive value of 65%.

A Gram stain of the centrifuged dialysate sediment was positive for only three patients with peritonitis. Staphylococcus aureus grew in two specimens and S. epidermidis grew in the other. The sensitivity of the Gram stain was 7%.

### TABLE 1. Culture results in the diagnosis of peritonitis

<table>
<thead>
<tr>
<th>Culture results</th>
<th>No. of diagnoses of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peritonitis</td>
</tr>
<tr>
<td>Positive</td>
<td>29 (a)</td>
</tr>
<tr>
<td>Negative</td>
<td>15 (c)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
</tr>
</tbody>
</table>

* Sensitivity = a/(a + c) × 100 = 66%; specificity = d/(b + d) × 100 = 88%; positive predictive value = a/(a + b) × 100 = 85%; negative predictive value = d/(c + d) × 100 = 71%; prevalence = (a + c)/(a + b + c + d) × 100 = 52%.

### TABLE 2. Efficacy of culture methods in the diagnosis of peritonitis

<table>
<thead>
<tr>
<th>Culture method</th>
<th>No. of specimens tested</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC broth</td>
<td>63</td>
<td>39</td>
<td>98</td>
<td>94</td>
<td>60</td>
<td>52</td>
</tr>
<tr>
<td>Plate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>85</td>
<td>39</td>
<td>98</td>
<td>94</td>
<td>60</td>
<td>52</td>
</tr>
<tr>
<td>Aerobic</td>
<td>85</td>
<td>39</td>
<td>98</td>
<td>94</td>
<td>60</td>
<td>52</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>85</td>
<td>25</td>
<td>98</td>
<td>92</td>
<td>55</td>
<td>52</td>
</tr>
<tr>
<td>Filter</td>
<td>63</td>
<td>54</td>
<td>92</td>
<td>91</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Bag</td>
<td>54</td>
<td>61</td>
<td>96</td>
<td>95</td>
<td>65</td>
<td>57</td>
</tr>
<tr>
<td>Overall</td>
<td>85</td>
<td>66</td>
<td>88</td>
<td>85</td>
<td>71</td>
<td>52</td>
</tr>
</tbody>
</table>

* Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

Table 3 demonstrates the number of concordant positive cultures. The BC broth method had the highest number of positive cultures at 24 (30%), while the bag method had the highest rate of positive cultures at 37%. All cultures positive on the anaerobic plates also grew on the aerobic plates. Therefore, the aerobic plate data also reflect results for the plate method overall.

All the organisms that were isolated were placed in groups (Table 4). There were 43 isolates from the 34 positive specimens. Some specimens were not processed by all four methods. By using the Fisher exact probability two-tail test, it was found that Streptococcus spp. were isolated by the bag culture method significantly more often than by the plate method (P < 0.05). The BC broth method appeared to be better than the other methods for isolating members of the family Enterobacteriaceae, but the difference was not significant. All other differences between methods were not significant.

In specimens from patients with peritonitis for which all culture methods were performed, there were five cases in which organisms were isolated by only one method. The bag culture isolated Streptococcus faecalis twice and Pseudomonas vesicularis once. The BC broth method identified one

### TABLE 3. Concordant positive cultures

<table>
<thead>
<tr>
<th>Culture method</th>
<th>No. of cultures positive by the following methods:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BC broth</td>
</tr>
<tr>
<td>BC broth</td>
<td>24</td>
</tr>
<tr>
<td>Plate</td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>18</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>12</td>
</tr>
<tr>
<td>Filter</td>
<td>22</td>
</tr>
<tr>
<td>Bag</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
</tr>
</tbody>
</table>

* Rate = Total number of cultures positive for the method/total number of cultures for the method.
Six patients with peritonitis were on antimicrobial therapy at the time that specimens were taken. Specimens from two of these patients were culture negative and were mentioned above. The culture of a specimen from one patient grew an Acinetobacter sp. in the aerobic plates, bag, and filter, despite receiving 1 day of appropriate therapy (tobramycin); the culture grown by the BC broth method and the anaerobic plates were negative. Cultures of specimens from three patients grew organisms that were resistant to the therapy that they were receiving.

**DISCUSSION**

In this study we used a modification of the total volume culture technique described by Dawson et al. (2). We did not use thioglycolate broth because of the low yield of anaerobic organisms in their study (2) and in our own previous results at St. Paul’s Hospital (data not shown). Dawson et al. (2) added broth via blood transfer bags. We found that these bags were difficult to obtain and awkward to use. We used a 100-ml glass syringe. We also standardized the addition of the concentrated brain heart infusion broth to dialysate at a ratio of 1/10 ml, and this improved our yield in culture in preliminary investigations. Dawson et al. (2) found the bag culture method to be sensitive. We confirmed their findings and also compared them with other methods.

Studies have been performed by various methods to increase the sensitivity of the culture for samples from patients on CAPD. These methods have included filtration, centrifugation, disruption of phagocytes, and the inoculation of blood culture and thioglycolate broths (5, 10, 11, 13, 14). These studies have shown similar isolation rates of individual organisms as we have. Ryan and Fessia (11) recently found the Septi-Chek system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.) to be an effective method, and we are evaluating this system in our laboratory.

In this study we evaluated four culture methods in the diagnosis of bacterial peritonitis in patients on CAPD. Using the definition of peritonitis given above as the “gold standard,” our overall sensitivity was 66%.

If one considers only the two patients with presumed bacterial peritonitis, who responded clinically to empiric antimicrobial therapy, and the patient with tuberculous peritonitis as false-negative cultures, then the sensitivity is 91%. In the seven culture-negative patients with positive dialysate findings and no physical signs of peritonitis, the dialysate returned to normal without therapy. We did not perform eosinophil counts on these specimens to detect eosinophilic peritonitis (1). These cases could represent a self-limited viral infection or some other cause. Our definition of peritonitis was broader than that used in other studies. If we had defined peritonitis as two or more of the criteria we listed (as others have done), then the overall sensitivity would have been higher (8).

*S. epidermidis* and *Corynebacterium* spp. were always isolated by multiple culture methods in patients with peritonitis. They were isolated by only one culture method if peritonitis was not present.

If we assess the overall efficacy of the four culture methods by a combination of the sensitivity, the ability to culture individual organism groups, and the ability to isolate organisms that other methods fail to isolate, we find the best two methods are the bag culture and the BC broth methods. In fact, a combination of the BC broth and the bag culture methods would have detected all clinically relevant organisms isolated in this study. The effect of concomitant antibiotic therapy did not change this conclusion.

All organisms identified by the filter method were found by other methods. It was therefore superfluous. The aerobic plates were valuable for rapid identification and sensitivity testing. The anaerobic plates were not helpful. Other investigators have also found a very low yield from anaerobic plates (2, 14). We would only recommend the use of anaerobic plates if bowel perforation or anaerobes are specifically suspected.

In conclusion, based on the results of this study, the combination of the BC broth, the bag culture, and the aerobic plate methods is the quickest and most effective technique for the detection of clinically significant isolates and in the assessment of contamination. Further studies are needed to determine the optimal ratio of brain heart infusion broth to dialysate in the bag culture technique. Further evaluation of other blood culture broth methodologies for the culture of dialysate is also necessary. Emphasis should be placed on the volume of dialysate required and the advantage of centrifugation and disruption of phagocytes prior to inoculation. These techniques should all be scrutinized carefully with regard to cost-effectiveness.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


