Evaluation of Modified Laboratory Processes for Isolation of Bacteria and Fungi in Continuous Ambulatory Peritoneal Dialysis Patients with Peritonitis

Continuous ambulatory peritoneal dialysis (CAPD) is a common, safe, and simple method to dialyze waste metabolites in patients with end-stage renal failure (ESRF). Peritonitis is a serious complication of CAPD, leading to increased morbidity and mortality in patients. Peritonitis in the CAPD patient is defined according to the International Society for Peritoneal Dialysis (ISPD) guidelines, which include symptoms and signs, white blood cells (WBC) $> 100/\text{mm}^3$ in dialysate fluid, more than 50% polymorphonuclear cells, and identification of pathogens in the peritoneal fluid (1). Culture-negative peritonitis is estimated to be lower than 20% of all peritonitis episodes according to the ISPD guidelines. However in 2011, the Malaysian National Renal Registry (MNRR) reported culture-negative peritonitis as 29%, which is higher than the international standard (2). Therefore, the purpose of this study was to evaluate different laboratory processes for isolation of bacteria and fungi in CAPD patients with peritonitis.

MATERIAL AND METHODS

Ninety-one non repetitive peritoneal fluids were obtained from CAPD patients with suspected peritonitis, who were admitted to Hospital Serdang from June 2011 to May 2012. Clinical samples were taken from patients before commencing antibiotic therapy. Fifty milliliters of peritoneal fluid was collected in sterile containers, while 10 mL was injected into aerobic and anaerobic blood culture bottles to be processed by BACTEC

(BACTEC; Becton Dickinson, Sparks, MD, USA) method. Five different culture methods were performed as illustrated in Table 1. Meanwhile, a sedimentation method was used in order to evaluate whether concentrating the sample (peritoneal fluid) would improve the sensitivity. This method involved centrifugation of 30 mL peritoneal fluid at 3,000 rpm for 15 min. Then a loop full of sediment from centrifuged PD fluid was plated onto 5% sheep blood agar, chocolate agar in CO_2 jar, MacConkey agar, Sabouraud dextrose agar, and blood agar incorporated with 2% Tween 80 (Sigma-Aldrich, St. Louis, MO, USA). However, it was hypothesized that some intracellular pathogens may not be isolated using routine culturing techniques, which would require lysis of WBC to release sequestered organisms (3,4). Therefore, 10 mL of peritoneal fluid was incubated with 100 μL of Triton X 100 (BDH, Poole, Dorset, UK) for 30 min at room temperature. The tube was then centrifuged at 3,000 rpm for 15 min. A loop full of sediment from centrifuged PD fluid was plated onto culture media mentioned previously, except blood agar with 2% Tween 80. The culture plates were incubated at 37°C in aerobic and anaerobic condition up to 48 hours. Later, the isolated colonies were identified using standard bacterial identification techniques.

RESULTS

Among the 91 patients with suspected peritonitis in Hospital Serdang from June 2011 to May 2012, 45 (49.4%) were confirmed to have peritonitis based on the ISPD guidelines. Thirty-seven patients (40.6%) had symptoms of peritonitis such as abdominal pain, fever, and tenderness, with peritoneal WBC $> 100/\text{mm}^3$, but a positive culture was obtained in only 29 samples. However, 8 patients had symptoms of peritonitis with a peritoneal WBC less than $100/\text{mm}^3$ and had positive culture. The most common symptoms of peritonitis in CAPD patients were abdominal pain (88.9%; 40/45) followed by fever (53.3%; 24/45) and abdominal tenderness (33%; 15/45). Table 1 illustrates the recovery rate of etiological agents by different culturing and processing methods. A significant difference ($p < 0.001$) between direct culture method and the other processing methods used to detect the etiological agents

TABLE 1
Comparison of Different Methods to Detect Etiological Agents in All Peritonitis Cases*

Method	Positive culture n (%)	Negative culture n (%)	Total n
Direct culture	7 (15.5%)	38 (84.5%)	45
BACTEC system	36 (80%)	9 (20%)	45
WBC lysis by Triton X	37 (82.2%)	8 (17.8%)	45
Sedimentation	36 (80%)	9 (20%)	45
Blood agar with 2% Tween 80	37 (82.2%)	8 (17.8%)	45

WBC = white blood cell.

* $p < 0.001$.

in CAPD-associated peritonitis was observed. The average time for detection of etiological agents was similar (39 hours) for all 5 methods (Table 2). The pairwise comparison revealed no significant difference ($p = 0.875$) in time to positivity among 5 different culturing and processing methods. The cost of different culturing methods to identify the etiological agents from peritoneal fluids is compared in Table 3. The cost was calculated for the reagents per sample only, but did not include the cost of any special equipment required for the different methods, such as centrifuge and BACTEC system analyzer for automated analysis and manpower. The BACTEC alert system was the most expensive, while the blood agar with 2% Tween 80 was the cheapest high-yield method (Table 3). Out of the 45 clinical samples, 8 samples (17.8%) were culture-negative and the remaining 37 (82.2%) were culture-positive. Gram-positive bacteria were isolated in 20 cases (44.4%), while gram-negative bacteria in 15 (33.3%) cases and fungi in 2 (4.4%) cases. The most common organism isolated was *Staphylococcus aureus*, while *Escherichia coli* was the most common gram-negative bacteria isolated (Table 4).

DISCUSSION

Although in Malaysia the BACTEC alert system is used in all government hospitals routinely for isolation of bacteria from sterile body fluids, its use for isolation of etiological agents from peritoneal fluid is still uncommon due to its high cost. The BACTEC alert system is requested by physicians only in some

CAPD cases with repeated peritonitis and cloudy peritoneal fluid without a positive culture. In many Malaysian hospitals the direct culturing method is used where the peritoneal fluid is cultured directly on blood agar, chocolate agar, and Sabouraud agar for 48 hours at 37°C, followed by gram staining and biochemical identification of bacterial isolates. In the current study, using the direct culture method, the rate of culture-negative peritonitis was 38/45 or 85%. It was found that, by using the BACTEC alert system and sedimentation methods, the recovery rate of pathogens increased from 15% to 80% (36/45) in comparison to the direct culture method (7/45). Our results were in agreement with the findings from previous investigations that the BACTEC system showed a higher culture positivity (70 – 75%) when compared with a direct culturing method (5–7). The usefulness of non-ionic surfactant agents such as Tween 80 and Triton X to increase the recovery of organisms from body fluids was previously described by Taylor *et al.* (1987) and Gould *et al.*, (1988) (3,8). In the present study, for the first time, non-ionic surfactants were used for isolation of etiological agents in peritonitis cases in Malaysia. Among 45 peritonitis cases, an etiologic agent was identified in 37 (82.2%) samples by WBC lysis-centrifugation and blood agar with 2% Tween 80. In 1 case, peritoneal fluid processed using Triton X and Tween 80 incorporated in blood agar isolated *Klebsiella pneumoniae* but did not yield any growth when processed by other methods such as the sedimentation method, BACTEC alert system, and direct culture. This observation may be explained by the fact that peritoneal fluid with WBC > 100/mm³ from patients with peritonitis may have intracellular organisms in polymorphonuclear leukocytes or macrophages,

TABLE 2
Average Time (Hours) to Positivity Among 4 Different
Culturing Methods*

Methods	Average time	Standard error	Lower bound	Upper bound
Direct culture	39.12	3.50	32.10	45.96
WBC lysis by Triton X	38.83	3.50	31.96	45.70
Sedimentation	39.36	3.50	32.47	46.23
BACTEC	38.44	3.60	31.36	45.51
Blood agar with 2% Tween 80	38.83	3.50	1.96	45.70

WBC = white blood cell.

* $p=0.875$.

TABLE 3
Cost Effectiveness of Different Methods

Method	Cost (US\$)
Direct culture	5
WBC lysis by Triton X	5.6
BACTEC alert system	13.3
Sedimentation method	5.3
Blood agar with 2% Tween 80	3.3

WBC = white blood cell.

TABLE 4
Different Types of Organisms Isolated from Peritonitis

Isolated organisms	Number (n)	Percentage (%)
Gram-positive		
<i>Staphylococcus aureus</i>	7	15.55
Coagulase-negative <i>Staphylococci</i>	4	8.88
<i>Viridans Streptococcus</i>	5	11.10
<i>Streptococcus bovis</i>	1	2.22
<i>Enterococcus faecalis</i>	1	2.22
<i>Enterococcus faecium</i>	1	2.22
<i>Micrococcus luteus</i>	1	2.22
Gram-negative		
<i>Escherichia coli</i>	5	11.11
<i>Klebsiella pneumoniae</i>	4	8.88
<i>Pseudomonas aeruginosa</i>	3	6.66
<i>Acinetobacter baumannii</i>	1	2.22
<i>Burkholderia cepacia</i>	1	2.22
<i>Neisseria spp</i>	1	2.22
Fungal		
<i>Candida parapsilosis</i>	1	2.22
<i>Candida tropicalis</i>	1	2.22
Culture negative	8	17.77
Total	45	100

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which can be cultured only when they are released. The present result is in accordance with Iyer and Kapoor (2009), who also reported that the usefulness of Triton X and Tween 80 incorporated in blood agar is to identify intracellular microorganisms such as *Klebsiella pneumoniae* in spontaneous bacterial peritonitis (4). Among the methods tested, the highest rates for detection of etiological agents were observed in the WBC lysis-centrifugation method and blood agar with 2% Tween 80 ($n = 37$; 82.2%). By using this method, the rate of culture-negative peritonitis decreased from our historical rate of 29% and the rate of 85% in the current study using the direct culture method to less than 18%. This result is in agreement with the accepted rate of less than 20% culture-negative peritonitis recommended by the ISPD guidelines (9). In the present study of culture-positive cases, 44.45% were gram-positive organisms, 33.3% gram-negative bacteria, and 4.4% fungal species. *Staphylococcus aureus* (7/37) was the most frequently isolated gram-positive bacteria, while *Escherichia coli* (5/37) was the most common gram-negative bacteria, and *Candida* species (2/37) were the only fungal. These results were almost similar to a previous study in Malaysia (10). *Candida* species were isolated from 2 human immunodeficiency virus (HIV) patients with CAPD peritonitis. The present study supports findings from previous studies that CAPD patients with HIV appear to have more frequent *Candida* peritonitis than those without HIV (3,11). Human immunodeficiency virus results in an immunocompromised state rendering patients on CAPD susceptible to *Candida* peritonitis (12).

In conclusion, by using methods other than direct culture, the rate of culture-negative peritonitis decreased to less than 18%, which is slightly lower than the 20% benchmark according to ISPD guidelines. This study suggests the culture of peritoneal fluid sediment onto blood agar with 2% Tween 80 is simple and cost-effective, yielding a positive culture in a timely fashion in patients with CAPD peritonitis. This approach may be applied in developing countries such as Malaysia to reduce the rate of culture-negative cases and improve the etiologic peritonitis diagnosis in CAPD patients economically.

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DISCLOSURES

The authors have no financial conflicts of interest to declare.

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REFERENCES

1. Piraino B, Bailie GR, Bernardini J, Boeschoten E, Amit Gupta A, Holmes C, et al. ISPD guidelines/recommendations. Peritoneal dialysis related infections recommendations. *Perit Dial Int* 2005; 25:107–31.
2. Morad Z, Lee D G, Lim YN, Tan PC. Peritoneal dialysis in Malaysia. *Perit Dial Int* 2005; 25(5):426–31.
3. Taylor PC, Poole-Warren LA, Grundy RE. Increased microbial yield from continuous ambulatory peritoneal dialysis peritonitis effluent after chemical or physical disruption of phagocytes. *J Clin Microbiol* 1987; 25(3):580–3.
4. Iyer RN, Kapoor D. Diagnosis of spontaneous bacterial peritonitis: role of Tween 80 and Triton X in ascitic fluid cultures. *Indian J Med Microbiol* 2009; 27(2):159–61.
5. Dalton MT, Prevost E. CAPD fluid examination using the BAC/TEC 460. *Perit Dial Int* 1986; 6(1):9–11.
6. Lye WC, Wong PL, Leong SO, Lee EJ. Isolation of organisms in CAPD peritonitis: a comparison of two techniques. *Adv Perit Dial* 1994; 10:166–8.
7. Azap OK, Timurkaynak F, Sezer S, Çağır U, Yapar G, Arslan H, et al. Value of automatized blood culture systems in the diagnosis of continuous ambulatory peritoneal dialysis peritonitis 2006. *Transplant Proc* 2006; 38(2):411–2.
8. Gould IM, Reeves I, Chauhan N. Novel plate culture method to improve the microbiological diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 1988; 26(9):1687–90.
9. Li PKT, Szeto CC, Piraino B, Bernardini J, Figueiredo AE, Gupta A, et al. Peritoneal dialysis-related infections recommendations: 2010 update. *Perit Dial Int* 2010; 30(4):393–423.
10. Kamaliah MD, Roziawati Y. Peritonitis complicating acute peritoneal dialysis in Northeast Malaysia 2000. *Southeast Asian J Trop Med Public Health* 2000; 31(3):540–6.
11. Ascioglu S, Rex JH, De Pauw B, Bennett JE, Bille J, Crokaert F, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002; 34(1):7–14.
12. Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis* 2003; 3(11):685–702.

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