Bacterial Isolation with On-site Inoculation of Ascites Fluid into Hemoculture Bottle in Spontaneous Bacterial Peritonitis

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Objective : *To compare the on-site bacterial inoculation of ascites fluid into hemoculture bottle with routine method in the patients who were preliminary diagnosed of SBP.*
Material and Method : *A retrospective analysis of case records during January-December 2001.*
Results : *A total of 673 specimens from 325 patients were retrieved from the data records at the Department of Microbiology, Siriraj Hospital in 2001. The neutrocytic ascites were found in 163 specimens (94 patients). The routine method and on-site inoculation into the hemoculture bottle were employed in 107 and 56 specimens respectively. Culture-positive neutrocytic ascites was found in the routine method 16 (14.9%) specimens and in the on-site inoculation 26 (46.4%) specimens (p <0.0001).*

Among these samples, the two methods were simultaneously performed in 42 specimens of which 18 paired specimens were eligible for analysis. Positive culture was found in 2 samples in which the routine method and in an additional 5 samples in which on-site inoculation into hemoculture bottle method. Using Kappa analysis (score = 0.328, 95% CI = -0.172 to 0.829) that can be interpreted the on-site inoculation method had a higher yield than the routine technique.

Moreover, 21 cases also had their blood and ascites samples simultaneously collected and cultured. 4 of ten (19%) and 5 of eleven (23.8%) cases were found in the routine and on-site and direct inoculation groups respectively. These finding suggested that the severity of infection in among two groups were similar and unlikely to be the cause of the difference of the positive isolation rate in both groups.
Conclusion : *The on-site and direct inoculation of ascites into hemoculture bottle method had a significantly higher isolation rate than routine method (i.e. 46.4 % versus 14.9 % p < 0.0001) either with separated or paired samples of ascites.*

Keywords : *Ascites fluid culture, Spontaneous bacterial peritonitis*

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Ascites fluid production is associated with several conditions such as chronic liver disease with portal hypertension, congestive heart failure, nephrotic syndrome, pancreatic disease, tuberculosis and malignancy. When ascites are rapidly accumulated and associated with recent onset of fever and generalized tenderness of the whole abdomen, the most common cause is cirrhosis of the liver complicated with primary bacterial peritonitis, the so-called spontaneous bacterial peritonitis (SBP)[1-5]. History taking, physical examination and abdominal paracentesis with ascites fluid analysis[6-10] and culture are usually performed to determine the etiology. The definitive diagnosis is based on (1) clinical evidence, (2) an ascites neutrophil count of more than 250 cells/mm^3 and (3) a positive ascites fluid culture[3,11-12]. However, there is a condition resembling SBP that is characterized by an ascites neutrophil count of more than 250 cells/$mm^3$with a negative culture. Culture Negative Neutrocytic Ascites (CNNA) is

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the name applied to this condition which was found in 35% to 65% of cases with suspected SBP when other known causes such as acute pancreatitis or perforation of visceral organ were ruled out[13]. One probable explanation for the false negative conditions is a low concentration of bacteria in ascites fluid and inadequate ascites culture techniques[14-17]. Runyon et al has studied the yield of several sampling techniques and culture methods of ascites. He finally proposed inoculating ascites fluid into the blood culture bottle, the optimal fluid volume being 10 ml to achieve the highest yield[14, 18-19]. The improved efficacy of the technique was later supported by other experiments that also showed a higher isolation rate with direct bedside inoculation of ascites into blood culture bottles[15-17,19-23].

At Siriraj Hospital, the statistical records of the patients revealed culture-confirmed cases of SBP in only 54 of 5,686 cases (0.95%) from January to December 2001. This prevalence is rather low and may indicate that the use of an effective technique for bacterial detection in ascites fluid is still neglected. In Siriraj Hospital, the results of the above-mentioned studies have not been uniformly or seriously applied. Ascites fluid from patients with suspected SBP wear sent for bacterial culture using the routine method with or without the direct inoculation of ascites into the hemoculture bottle. The authors can not find any local study that confirms the superiority of the direct inoculation method over the routine method. Hence, we lack local clinical data supporting the practice of on-site direct inoculation is lacking. Since variation in specimen sampling and culture in suspected SBP cases exist in Thailand without any local standard, the author were able to undertake a retrospective study to find out whether or not the direct inoculation method is superior to the routine method.

Material and Method

A retrospective cohort study was designed to collect data to compare the isolation rates of both methods. Patients who underwent abdominal paracentesis and subsequent ascites culture from January to December 2001 were eligible for the study. The patients' names were collected from the microbiological laboratory and their medical records were retrieved. Inclusion criteria for suspected SBP were clinical manifestation and result of ascites fluid analysis. Entry criteria were generalized abdominal pain, rapid accumulation of ascites and neutrophil counts of more than 250 cells/mm^3. Other signs of infection such as fever, leukocytosis or septic shock and hepatic encephalopathy without any clear precipitating factor were helpful but unnecessary for inclusion. Those who had an inadequate amount of ascites fluid for culture, clinical events of fibrinolysis, disseminated intravascular coagulation or surgical conditions were excluded. The study was approved by the Siriraj Ethics Committee. Fisher's exact test and Kappa statistical analysis were used when the isolation rates of the two culture methods were compared.

Results

A total of 673 specimens from 325 patients were retrieved from the data records at the Department of Microbiology, Siriraj Hospital in 2001. The routine method and on-site inoculation into the hemoculture bottle were employed in 478 and 195 specimens respectively. Males comprised 181 cases (55.7%). The mean age and its standard deviation were 53 6.7 years old. Cirrhosis of the liver was diagnosed in 173 cases (53.2%). Among these patients, the cause of cirrhosis was viral hepatitis B or C infection in 71 cases (21.9%), alcoholic cirrhosis in 89 cases (27.4%) and a combination of viral hepatitis and alcoholic cirrhosis in 13 cases (4%). The etiology of ascites was unknown or unrelated to any disease in 152 cases (46.8%).

The routine method and the on-site inoculation into hemoculture bottle method were employed for 107 and 56 ascites specimens respectively in which the neutrophil count was found to be more than 250 cells/ mm^3. The present study showed that culture-positive neutrocytic ascites were found in 26 (46.4%) specimens that used the on-site inoculation technique and in 16 (14.9%) specimens that used the routine method (Table 1) ($p < 0.0001$).

Among these samples, the two methods were simultaneously performed in 42 specimens of

Table 1. Result of ascites culture in 163 samples by each method

Method	Total	Positive culture (%)	Negative culture or CNNA (%)
Routine method	107	16 (14.9)	91(85.0)
On-site inoculation	56	26 (46.4)	30 (53.6)
Total	163	42 (25.8)	121 (74.2)

CNNA = culture negative neutrocytic ascites

which 18 paired specimens were eligible for analysis according to the entry criteria. Positive culture was found in two samples in which the routine method was used and in an additional five samples in which on-site inoculation into hemoculture bottle method was used (Table 2). Using Kappa statistical analysis, the k score was calculated to be 0.328, which showed the discordance of the bacterial isolation of the two methods. Its 95% confidence interval was -0.172 to 0.829 which can be interpreted to mean that the on-site inoculation method had a higher yield than did the routine technique.

The details of the type of bacteria isolated from the 42 culture-positive samples analyzed by either technique are shown in Table 3. Escherichia coli, Streptococcus group D and enterococci, Klebsiella pneumoniae were the three most common isolated bacteria. Moreover, 21 cases also had their blood and ascites samples simultaneously collected and cultured and 9 of these cases (42.9%) had both positive blood and ascites cultures. The same types of bacteria were isolated from the ascites and blood obtained from the same patients in every case. Four and five cases were found in the routine, on-site and direct inoculation groups respectively as shown in Table 4. These findings suggested that the severity of infection in routine, on-site and direct inoculation groups was similar and unlikely to be the cause of the difference of the positive isolation rate in both groups.

Table 2. Result of ascites culture in 18 paired samples by each method

		Routine method		Total
		+ ve culture	- ve culture	
On-site inoculation	+ ve culture	2	5	7 (38.9%)
	- ve culture	0	11	11 (61.1%)
Total		2 (11.1%)	16 (88.9%)	18 (100%)

Table 3. Type of bacteria isolated from ascites by each method

	Total (%)	Routine method	On-site inoculation
Escherichia coli	16 (34)	7	9
Streptococcus.group D	7 (15)	2	5
Enterococci	7 (15)	3	4
Klebsiella pneumoniae	5 (11)	1	4
Pseudomonas aeruginosa	2 (4)	1	1
Salmonella group B	1	-	1
Salmonella group D	2	1	1
Enterobacter species	1	-	1
Aeromonas hydrophila	1	-	1
Vibrio cholerae non O1	1	1	-
Vibrio vulnificus	1	-	1
Staphylococcus aureus (MRSA)	1	-	1
Proteus mirabilis	1	-	1
Non-hemolytic streptococcus	1	1	-
Total	47 (100)	17	30

Table 4. Type of bacteria simultaneously isolated from blood and ascites in the same patients (n = 21)

Bacteria	Routine method (n = 10)	On-site inoculation (n =11)
Escherichia coli	3	1
Streptococcus group D	-	2
Enterococcus species	-	1
Vibrio cholera non O1	1	-
Pseudomonas aeruginosa	-	1
Total	4 (19%)	5 (23.8%)

Discussion

The authors took advantage of the practice variation in ascites fluid culture that still exists in Siriraj Hospital by comparing the isolation rates of pathogenic bacteria from ascites in each method in this retrospective study. A few samples were simultaneously cultured using both methods while most of them were cultured using only one of the techniques. If the direct inoculation into hemoculture bottle method was found to yield a higher isolation rate, then a well-designed prospective trial should be performed to confirm the benefit of this method. Such a study should also aim to set a standard culture procedure, which should include the use of an optimal volume of ascites for collection and proper culture media in order to improve the isolation and detection of pathogenic bacteria in patients with suspected SBP.

The results of this retrospective study demonstrated that the on-site and direct inoculation of ascites into the hemoculture bottle method yielded a significantly higher isolation rate than that of the routine method (i.e. 14.9% versus 46.4%, $p < 0.0001$) both with separated or paired samples of ascites. Previous studies reported by Runyon et al also found higher sensitivity values for the on-site and direct inoculation into the hemoculture bottle method (57%

versus 93%). The reason their study yielded a higher sensitivity value than ours might be due to difference in the severity of illness, patient selection and type of culture media used. The present study used tryptic soy broth instead of BACTEC alarm bottle. Runyon et al reported a sensitivity value of 54% when tryptic soy broth was used for culture at bedside versus 93% using the BECTEC system[3]. The sensitivity of the authors' direct and on-site inoculation was close to that reported by Runyon et al (46.4% versus 54%) for tryptic soy broth. The commercial blood culture media were usually superior to locally made hemoculture bottles because there were nutrients, anticoagulant and opsonin supplemented in the liquid media that inhibited growth of contaminated bacterial flora and further killing of pathogenic bacteria in the liquid media [15]. The on-site direct inoculation method clearly allowed the use of a larger volume of ascites fluid for culture and allowed any pathogenic bacteria to come into immediate contact with a better liquid nutrient. Antibodies, complement, previously used antibiotics and white blood cells in the ascites were also immediately diluted by the liquid media in the hemoculture bottle. Thus, the deleterious effect of the host defenses on pathogenic bacteria in the ascites was quickly brought to a halt after abdominal tapping and direct inoculation into the hemoculture bottle. This is in contrast to the routine method where a smaller volume of ascites was taken for culture several hours may have elapsed before the ascites sample was finally streaked onto the surface of a culture agar plate. Hence, the bacteria in the ascites had much better growth with the on-site inoculation method and thus posed a better chance of survival after abdominal tapping. Also with a larger volume of ascites being used for culture, a higher yield of bacterial isolation could be anticipated. In the present study, the clinical features and mortality rate of the patients with CNNA were similar to those with culture-positive SBP. Hence, the severity of illness in the patients who yielded positive culture were similar to those who yielded negative culture. For the same reason, the higher isolation rates of pathogenic bacteria with the on-site inoculation into hemoculture bottle compared to the routine method was not also explained by the difference in severity of illness. Thus, the authors are inclined to believe that the negative culture found by using either method was due to using a sub-optimal volume of ascites fluid for culture or poor treatment of ascites after abdominal tapping rather than a true difference in bacterial concentration in ascites fluid in both groups of patients.

Previous studies and most literature mentioned that SBP was almost always associated with monomicrobial organism. In the present study, more than one type of bacteria was isolated in five patients who presented with severe sepsis, septic shock or hepatorenal syndrome. More than 10,000 white blood cells/ mm^3 were found in the ascites of these patients with 90 percent or more being neutrophils. Four cases were from the direct inoculation group and one case from the routine method group. Unfortunately, all of them succumbed. Hence, the isolation of more than one isolate from the ascites posed a worse prognosis. For samples that yielded mono-microbial organism, the present study was in accordance with those of several other authors which showed Escherichia coli was the most frequent pathogen isolated, followed by Klebsiella pneumoniae, enterococci and Streptococcus group D[15-21].

The present study had certain limitations most of which were related to the retrospective design. The two methods were not performed simultaneously on the same ascites sample. There were 42 specimens on which the two methods were performed but only 18 of these samples were eligible for the "truly infected" ascites and thus the sample size was too small to give a conclusive result. The optimal volume of ascites fluid for culture was unable to be determined since there were no guidelines established at Siriraj Hospital. If the optimal volume of ascites for culture had been previously determined, the culture negative neutrocytic ascites (CNNA) group might have been smaller than encountered. A prospective study should be planned to explore the benefit of rapid report by direct inoculation in terms of therapeutic outcome and cost-effectiveness if positive culture by this method consumes less time than that of the routine method.

In conclusion, the present study pointed to a higher yield by the on-site and direct inoculation into hemoculture bottle method. The preliminary data paved the way for a well- designed prospective study in the future that could confirm the benefits of this method and establish guidelines for ascites culture that would improve the outcome of isolation and culture in patients with SBP.

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ผลการเพาะเชื้อจากน้ำในช่องท้องโดยใช้ขวดเพาะเลี้ยงเชื้อจากเลือด และเพาะเชื้อที่ข้างเตียงในผู้ป่วยท้องมานจากโรคเยื่อบุช่องท้องอักเสบติดเชื้อแบบปฐมภูมิ

สุมิตรา เจริญหิรัญยิ่งยศ, เชิดศักดิ์ ธีระบุตร, อมร ลีลารัศมี

คณะผู้ศึกษาได้ศึกษาย้อนหลังจากเวชระเบียน ณ โรงพยาบาลศิริราช โดยเปรียบเทียบอัตราการเพาะเชื้อแบคทีเรียจากน้ำในช่องท้องของผู้ป่วยที่เป็นโรคเยื่อบุช่องท้องอักเสบติดเชื้อปฐมภูมิแบบเฉียบพลัน และมีท้องมาน ระหว่างวิธีการเพาะเชื้อแบคทีเรียทางห้องปฏิบัติการแบบปกติ และวิธีการเพาะเชื้อที่ข้างเตียงโดยฉีดตัวอย่างลงในขวดเพาะเชื้อจากเลือดหลังจากเจาะได้น้ำตัวอย่างจากช่องท้อง และนำส่งห้องปฏิบัติการตั้งแต่วันที่ 1 มกราคม 2544 ถึง 31 ธันวาคม 2544 แล้วพบว่าตัวอย่างที่ส่งเพาะเชื้อในช่วงเวลาดังกล่าวมี 673 ตัวอย่างจากผู้ป่วย 325 ราย น้ำตัวอย่างจากผู้ป่วยที่มีอาการเข้าได้กับท้องมานจากตับแข็งและตรวจพบนิวโตรฟิลมากกว่า 250 เซลล์ต่อ ลบ.มม. ในน้ำมีจำนวน 163 ตัวอย่าง (ร้อยละ 24.2) เป็นตัวอย่างที่เพาะเชื้อด้วยวิธีปกติ และวิธีการเพาะเชื้อที่ข้างเตียงโดยฉีดน้ำตัวอย่างลงในขวดเพาะเชื้อจากเลือดจำนวน 107 และ 56 ตัวอย่างตามลำดับ การเพาะเชื้อได้ผลบวกจากวิธีเพาะเชื้อด้วยวิธีปกติใน 16 ตัวอย่าง (ร้อยละ 15) และจากวิธีการเพาะเชื้อที่ข้างเตียง โดยฉีดน้ำตัวอย่างลงในขวดเพาะเชื้อจากเลือดใน 26 ตัวอย่าง (ร้อยละ 46.4) (ค่า $p < 0.0001$) มีการส่งน้ำในช่องท้อง 18 ตัวอย่างไปเพาะเชื้อทั้งสองวิธีในผู้ป่วยรายเดียวกันและพบว่า วิธีเพาะเชื้อตามปกติได้ผลบวกใน 2 ตัวอย่าง (ร้อยละ 11) และวิธีการเพาะเชื้อที่ข้างเตียงโดยฉีดน้ำตัวอย่างลงในขวดเพาะเชื้อจากเลือดได้ผลบวกใน 7 ตัวอย่าง (ร้อยละ 39) ค่าสัมประสิทธิ์ความสอดคล้องของทั้งสองวิธีได้ 0.328 และช่วงความเชื่อมั่นร้อยละ 95 อยู่ระหว่าง - 0.172 ถึง 0.829 ซึ่งแสดงว่าค่าที่ได้จากวิธีเพาะเชื้อทั้งสองวิธีมีความแตกต่างกัน คณะผู้ศึกษาสรุปว่า วิธีการเพาะเชื้อที่ข้างเตียงโดยน้ำฉีดตัวอย่างลงในขวดเพาะเชื้อจากเลือดให้ผลสูงกว่าและเสนอให้ศึกษาแบบล่วงหน้าเพื่อยืนยันผลการศึกษาครั้งนี้