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Evaluation of Various Culture Methods for the Diagnosis of Spontaneous Bacterial Peritonitis

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Abstract

Introduction: Spontaneous Bacterial Peritonitis (SBP) is a common and serious complication of patients with liver cirrhosis and ascites, without an apparent intra-abdominal source of infection that can be surgically treated. Its prevalence ranges from 10% to 30%. Earlier mortality rate was reported more than 90% which has now reduced to 30% - 50% with proper diagnosis and prompt initiation of antibiotics.

Aims &Objectives: The present study was done to evaluate the various culture methods for the diagnosis of SBP.

Materials and Methods: Ascitic fluid samples were collected aseptically from 100 cirrhotic patients with ascites. Two different culture methods were employed for the diagnosis of SBP. In first method (Conventional), two millilitre of ascitic fluid was centrifuged and the sediment was used for inoculation of Mac Conkey agar (MA) and blood agar (BA) plates which were than incubated aerobically at 37^{o}C for 18-24 hrs. In the other method, ten ml of ascitic fluid was inoculated directly into blood culture bottles containing 30 ml of Brain Heart Infusion (BHI) broth at the bedside. It was than incubated at 37^{o}C aerobically and thereafter three subcultures were done on BA and MA plates after 24 hrs., 72 hrs. and last on 7th day of incubation. Both plates were than incubated aerobically at 37^{o}C for 18-24 hrs.

Results: The number of positive cultures increased on an average from 16% to 51% with the bedside blood culture bottle inoculation technique.

Conclusion: Conventional bacterial culture method have shown poor diagnostic yield (<50%) even with an elevated PMN count (≥250 cells/mm^{3}). So bed side inoculation method is recommended in order to increase the sensitivity of bacterial culture which will subsequently help in correctly diagnosing SBP, thereby helping in its proper management by administration of right antibiotic therapy in patients.

Keywords: Bedside enrichment culture technique, Cirrhotic patients with ascites, Conventional bacterial culture, SBP.

Introduction

Spontaneous bacterial peritonitis (SBP) is an infection of previously sterile ascitic fluid without an apparent intra-abdominal source of infection that can be surgically treated.[1] It often develops insidiously. It is a common complication in patients with cirrhosis and ascites.[2, 3]

The variants of SBP are

Classic SBP

Classic SBP is diagnosed, when there is a positive ascitic fluid culture for single organism, elevated ascitic fluid PMN count ≥ 250 cells/mm^3 and no evidence of an intra-abdominal surgically treatable source of infection.[3- 9]

Culture Negative Neutrocytic Ascites (CNNA)

CNNA is diagnosed when ascitic fluid culture grows no bacteria, ascitic fluid PMN count is ≥ 250 cells/mm^3 and not even a single antibiotic has been given to the patient. There is also no other explanation for an elevated ascitic PMN count like intra peritoneal haemorrhage, peritoneal carcinomatosis, hepatocellular carcinoma, tuberculosis or pancreatitis.[3-9]

Monomicrobial non-neutrocytic bacterascites (MNB)

MNB is diagnosed when there is a positive ascitic fluid culture for a single organism, an ascitic fluid PMN count <250 cells/mm^3 and no evidence of an intra-abdominal surgically treatable source of infection.[3-9]

Even though the mortality rate was initially reported to exceed 90%, the prognosis has improved with early diagnosis and treatment.[4] Paracentesis is extremely important, as the polymorphonuclear leukocyte (PMN) count in the ascitic fluid plays a vital role in obtaining a diagnosis of SBP. [5] Although all cirrhotic patients with ascites are at risk of SBP, the prevalence of SBP among hospitalized patients (10%) is higher than that observed in outpatients (1.5–3.5%). It is therefore recommended that diagnostic paracentesis should be performed in all cirrhotic patients with ascites who require hospital admission, regardless of whether they exhibit clinical symptom(s) of SBP or not.[5-8] Diagnosis can be done by various culture methods, which include:- [3, 6, 9]

Conventional bacterial culture method

Conventional bacterial culture method includes laboratory analysis of two millilitre ascitic fluid collected in syringes or tubes by inoculation on agar plates. This method has shown a poor diagnostic yield (<50%) with an elevated PMN count (≥250 cells/mm^3).

Bed side inoculation method

So, bed side inoculation of 10-20 ml of ascitic fluid into 100 ml blood culture bottles containing enrichment media is recommended in order to increase the sensitivity of the bacterial culture.[3, 6, 9]

Culture methods[6]

Biological body fluids such as pleural, synovial, peritoneal and pericardial fluids are usually sterile but may be invaded and infected with various types of microorganisms, including bacteria. Among all methods currently in use, agar plating or inoculation of samples into agar-based culture media (conventional method) is the most common. However, the risk of false negative results is high because only a small number of microorganisms may be present in the specimens.[10]

Daur et al[10] reported an average increase in the number of positive cases from 9.7% to 23.1% with the enrichment culture technique. Similar results were reported by Simor et al[11] who observed about 10% increase in positive cultures by enrichment culture techniques. Another study comparing the efficacy of bedside inoculation with the conventional method had shown a 40% improvement in sensitivity, increasing the yield from less than half to 80% with the blood culture bottle inoculation containing enrichment media.[6]

Material and Methods

The present study was conducted in the Departments of Microbiology and Medicine, Pt. B.D. Sharma, PGIMS, Rohtak over a period of one year. A total of 100 ascitic

fluid samples collected aseptically with a needle and syringe from cirrhotic patients with SBP were included in the study.

Collection of Samples

Ascitic fluid aspirates approximately 20 ml was collected taking all aseptic precautions from each patient of SBP with a sterile syringe from the peritoneal cavity. Ten ml of ascitic fluid was inoculated directly into blood culture bottles containing 30 ml of Brain Heart Infusion (BHI) broth at the bedside. Samples were transported to Department of Microbiology immediately.[12]

Ascitic fluid culture by conventional method

In conventional method, two ml of ascitic fluid was centrifuged at 1500 rpm for 10 minutes and the supernatant was discarded while the sediment was used for Gram staining and inoculation of Mac Conkey agar (MA) and blood agar (BA) plates. Both MA and BA plates were incubated aerobically at 37°C for 18-24 hrs.[3, 12, 13]

Ascitic fluid culture by bed side inoculation in BHI Broth

In this method, ascitic fluid samples received in the microbiology laboratory in blood culture bottles were incubated at 37°C aerobically and three subcultures were done on BA and MA plates after 24 hrs., 72 hrs. and 7th day of incubation. Both MA and BA agar plates were then incubated aerobically at 37°C for 18-24 hrs..[3, 12, 13]

Identification of the Isolates

Any growth in the agar plates was identified by standard conventional microbiological methods.[13,14] It was based on information from primary plates in conjunction with colony morphology, Gram staining and biochemical reactions.[14]

Results

GNB constituted majority of the organisms recovered by both conventional and direct bedside inoculation techniques being 81.25% and 82.35 % respectively. (Table-1) Fifty one organisms were isolated in pure culture by direct bedside inoculation in BHI broth. Only 16 organisms were isolated in pure culture by conventional method. Organism which was isolated by conventional method was subsequently also isolated in pure culture by direct bed side inoculation method. *E. coli* was the most common organism isolated followed by *S. aureus* and *Klebsiella* spp.. (Table-2)

Classic SBP constituted majority of cases isolated by both conventional and by direct bedside inoculation method in BHI broth. *E.coli* constituted 61.53% of organisms isolated from classic SBP cases by conventional method and 57.14% of organisms isolated from classic SBP cases by direct bedside inoculation in BHI broth. (Table-3)

Table 1: Different Bacteria Grown By Conventional and Direct Bedside Inoculation in Bhi Broth

S. No.	Bacteria	Conventional Method		Direct bedside inoculation in BHI broth	
		No.	%	No.	%
1.	GNB	13	81.25	42	82.35
2.	GPC	3	18.75	9	17.64
Total		16	100	51	100

Table 2: Different Bacteria Grown By Conventional And Direct Bedside Inoculation In Bhi Broth

S. No.	Bacteria	Conventional Method		Direct bedside inoculation in BHI broth	
		No.	%	No.	%
1.	*E. coli*	9	56.25	28	54.9
2.	*S. aureus*	2	12.5	6	11.76
3.	*Klebsiella* spp.	1	6.25	4	7.84
4.	*P. aeruginosa*	1	6.25	4	7.84
5.	*Enterococcus* spp.	1	6.25	3	5.89
6.	*Citrobacter* spp.	1	6.25	3	3.92
7	*Proteus* spp.	0	0	2	3.92
8.	*Acinetobacter* spp.	1	6.25	1	1.9
Total		16	100	51	100

Table3: Different Bacteria Grown By Conventional And By Direct Bedside Inoculation Method In Classic Sbp And Mnb Cases

S. No.	Bacteria	Conventional method (n=16)				Direct bedside inoculation in BHI broth (n=51)			
		Classic SBP		MNB		Classic SBP		MNB	
		No.	%	No.	%	No.	%	No.	%
1.	*E. coli*	8	61.53	1	33.33	24	57.14	4	44.44
2.	*Klebsiella* spp.	1	7.69	0	0	3	7.14	1	11.11
3.	*P. aeruginosa*	0	0	1	33.33	3	7.14	1	11.11
4.	*Acinetobacter* spp.	1	7.69	0	0	1	2.38	0	0
5.	*Citrobacter* spp.	1	7.69	0	0	1	2.38	2	22.22
6.	*Proteus* spp.	0	0	0	0	2	4.76	0	0
7.	*S. aureus*	1	7.69	1	33.33	5	11.90	1	11.11
8.	*Enterococcus* spp.	1	7.69	0	0	3	7.14	0	0
Total		13	100	3	100	42	100	9	100

Discussion

In this study, the number of positive cultures increased on an average from 16% to 51% with the enrichment culture technique. In the bed side inoculation method, subculturing after 24 hrs of incubation recovered 26 organisms while subculturing after 72 hrs of incubation yielded further 15 organisms. Finally 10 more organisms were detected from the remaining blood culture bottles containing BHI broth after performing subculture on the 7th day of incubation. Daur et al[10] has reported an increase in positivity rate from 9.7% to 23.1%. Similar results were reported by Simor et al[11] who observed isolation rate of 11.2% and 19.8% for conventional method and enrichment technique respectively. Various authors have reported a similar increase in positive culture rate by using enrichment culture technique.[6, 15-17]

GNB predominated 81.25% by conventional method and 82.35% by direct inoculation in BHI broth. Almost all the studies of ascitic fluid cultures have shown predominance of GNB ranging from 28.17% to 100%.[15,18-24] Amongst the GNB, *E.coli* was the commonest bacteria 56.25% and 54.9% followed by *Klebsiella* spp. and *P. aeruginosa* at 6.25% and 7.84% each by conventional method and by direct inoculation in BHI broth respectively. Bankar et al[3] has also reported *E.coli* as the commonest bacteria- 57.89% and 40.38%, followed by *P.aeruginosa* 21.07 % and 11.54% by conventional method and by direct bedside inoculation technique respectively. Various studies have isolated *E.coli* from 22.22% to 75% from ascitic fluid.[19-21, 23, 24] Other organisms isolated from ascitic fluid in the present and other studies were *P.aeruginosa* and *Acinetobacter* spp..[18-24]

In the present study, majority of the cases (49%) were that of CNNA i.e. ascitic fluid PMN count ≥250 cells/mm^3 and culture negative. This was followed by 42% cases of Classic SBP. MNB was encountered in only 9% cases. This pattern was almost similar to recent study by Bhat et al[25] in 2013 where CNNA constituted 57.1%, Classic SBP 35.8% and MNB 7% of total spontaneous ascitic fluid infection (SAI) cases. In another study by Bankar et al[3] where Classic SBP was commonest at 53.45% followed by 36.21% of MNB. However the study from Larkana[18] did not have any case of MNB, but prevalence of Classic SBP was 54% and of CNNA was 46% which was almost similar to

findings in this study. Culture positive cases of SBP constituted 51% of total cases. Globally, CNNA have been estimated to occur in 30 to 60% of patients with SBP.[2] This could also be the result of poor culturing techniques or late- stage resolving infection.[2]

Limitations: None

Conclusion

Conventional bacterial culture method have shown poor diagnostic yield (<50%) even with an elevated PMN count (≥250 cells/mm^3). So direct bed side inoculation method in enrichment media is recommended in order to increase the sensitivity of bacterial culture which will subsequently help in correctly diagnosing SBP cases, thereby helping in its proper management by administration of right antibiotic therapy in patients. It will also prove to be much more cost effective than automated microbial detection systems especially in resource limited settings like various peripheral health institutions where automated equipment's are out of scope of the budget for procurement.

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