



Original Research Article

Comparison of three culture methods for diagnosis of Spontaneous Bacterial Peritonitis (SBP) in adult patients with cirrhosis

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ABSTRACT

Keywords

Spontaneous Bacterial Peritonitis (SBP); Cirrhosis; tryptic soy broth (TSB)

Ascitic fluid infections are a frequent complication among patients with cirrhosis and ascites, of which Spontaneous Bacterial Peritonitis (SBP) is the most common, potentially fatal, yet reversible cause of deterioration in patients with advanced cirrhosis with ascites. One hundred consecutive hospitalized patients of cirrhosis with ascites were included in this prospective study. Ascitic fluid was analysed by culture and the comparison of culture was made between the conventional method, direct bedside inoculation in tryptic soy broth (TSB) and direct inoculation on blood agar incorporated with 2% Tween 80. Statistical analysis was done by Chi Square test. Out of 100 patients of cirrhosis with ascites, 58% were SBP cases and 42 % were non-SBP cases. All culture positive cases of SBP showed growth in TSB (100%). SBP is a serious complication in patients with advanced cirrhosis. Apart from conventional method, direct bedside inoculation in TSB is highly sensitive for the diagnosis of SBP.

Introduction

Spontaneous Bacterial Peritonitis (SBP) is the most common, potentially fatal, yet reversible cause of deterioration in patients with advanced cirrhosis with ascites.¹ SBP is defined as the infection of previously sterile ascitic fluid without an apparent intra-abdominal source of infection.²

There are reports mentioning the clinical aspects and treatment of SBP,³⁻⁸ but the microbiological investigations for diagnosis

of SBP are lacking in these reports. So this study was carried out.

Materials and Methods

This was a prospective study of one year duration from January to December 2010. One hundred consecutive adult patients admitted in this tertiary care hospital with cirrhosis were studied after clearance from Institutional Ethics Committee. Ascites due to renal, cardiac, tubercular and malignant

pathology, secondary peritonitis and HIV positive patients were excluded from the study.

Seven ml of ascitic fluid was tapped from the patient under proper aseptic precautions. Five ml of it was inoculated directly into 25 ml of tryptic soy broth at the bedside³. Tryptic soy broth was incubated at 37⁰C and 3 subcultures were done on Blood Agar (BA) and MacConkey Agar (MA) after 24 hours (2nd day), 72 hrs (4th day) and on the 7th day and the plates were incubated at 37⁰C.

Remaining 2 ml of the fluid was centrifuged at 1500 rpm for 10 minutes. Supernatant was discarded. Deposit obtained was utilized for Gram staining and then divided into 2 parts⁹. One part was processed by conventional method on BA and MA. BA was incubated at 37⁰C at 5% CO₂ atmosphere for 48 hours. MA was incubated at 37⁰C for 48 hours. The other part was plated on Blood Agar incorporated with 2% Tween 80 and incubated at 37⁰C at 5% CO₂ atmosphere for 48 hours. Any growth in BA and/or MA plates was identified by standard biochemical tests.

The three methods of culture were statistically analysed by Chi square test (Open Epi software version 2.3).

Results and Discussion

One hundred patients of hepatic cirrhosis with ascites were studied, of which 93 were male and 7 were female. Out of total 100 cases, number of SBP cases was 58 (58 %). All culture positive cases of SBP (52) showed growth in tryptic soy broth (TSB) by direct bedside inoculation (100%). Bacteria grown by only direct bedside inoculation in TSB were 55.77 %. By conventional method, growth was seen in

only 36.54 % and by Blood agar incorporated with 2% Tween 80, growth was seen in 44.23% cases (Figure 1).

By Chi square test, growth by direct bedside inoculation in TSB was statistically significant in comparison to conventional method in 100 cases ($p < 0.00001$) as well as in 58 SBP cases ($p < 0.000001$). The same was true between direct bedside inoculation in TSB and Blood agar incorporated with 2% Tween 80. However, growth by conventional method was not statistically significant in comparison to Blood agar incorporated with 2% Tween 80 in overall 100 cases ($p = 0.4942$), as well as in 58 SBP cases ($p = 0.4397$).

Gram negative bacteria predominantly grew by all the three culture methods – 41/52 from direct inoculation in TSB, 18/19 from conventional method and 21/23 from BA incorporated with Tween 80. *Escherichia coli* was the commonest bacteria, followed by *Pseudomonas aeruginosa* and *Acinetobacter* species. Amongst the Gram positive cocci, *Enterococcus* species and *Streptococcus* species were recovered, followed by Methicillin Sensitive *Staphylococcus aureus* (MSSA). (Table 1).

In the present study, spontaneous bacterial peritonitis (SBP) was present in 58% of cases and the remaining 42% were non-SBP cases. This is similar to the studies reported from Peshawar and Larkana in 2010 (58% and 54% respectively).^{6,9} The probable reasons for higher prevalence in the present study and latter studies may be late referral to the tertiary care hospital, low socio economic conditions and malnutrition.⁹

In the present study, all the 52% culture positive cases grew in TSB after direct bedside inoculation (100%), and the conventional method showed growth only in 36.54% (Figure 1).

Table.1 Different bacteria grown by the three methods

Bacteria	Conventional Method		Direct bedside inoculation in TSB		Blood agar incorporated with 2% Tween 80	
	No.	%	No.	%	No.	%
Gram negative bacilli						
<i>Escherichia coli</i>	11	57.89	21	40.38	13	56.52
<i>Pseudomonas aeruginosa</i>	4	21.07	6	11.54	4	17.38
<i>Acinetobacter</i> species	1	05.26	6	11.54	1	04.35
<i>Enterobacter</i> species	1	05.26	5	09.62	2	08.70
<i>Proteus mirabilis</i>	1	05.26	2	03.85	1	04.35
<i>Klebsiella pneumoniae</i>	0	00.00	1	01.92	0	00.00
Gram positive cocci						
<i>Enterococcus</i> species	0	00.00	4	07.69	0	00.00
<i>Streptococcus</i> species	1	05.26	4	07.69	1	04.35
Methicillin Sensitive <i>Staphylococcus aureus</i>	0	00.00	3	05.77	1	04.35
Total	19	100	52	100	23	100

Studies from Asian as well as western countries conducted till date have shown superiority of direct bedside inoculation in TSB over the conventional method^{3,4,9}. The earliest documented evidence of the comparison of these two methods by Runyon et al was in 1988, who reported 93% growth by the bedside inoculation in TSB and only 43% by the conventional method. The difference was also statistically significant ($p < 0.0001$).¹⁰

Low concentration of bacteria in infected ascites and the time lag between ascitic fluid collection and its inoculation on conventional media in the laboratory, makes the conventional method insensitive.¹⁰ In the present study also by Chi square test, the former method was

statistically significant ($p < 0.00001$), as compared to the latter method. The study by Castolette et al from Spain have also shown a significantly higher sensitivity of the direct bedside inoculation in TSB ($p = 0.0001$).¹¹ An Indian study in 1994 also brought out superiority of direct inoculation into blood culture bottle than the conventional method ($p < 0.001$)¹² and a recent study in 2009 from Egypt also reported significant detection by bedside inoculation than the conventional method ($p < 0.001$).¹³

A case was reported in 2009 from Hyderabad of an adult alcoholic male, whose ascitic fluid was cultured on blood agar with 2% Tween 80, apart from direct plating on BA and MA.¹⁴ *Klebsiella*

pneumoniae grew by the former method and not by the latter. By Chi square test, direct bedside inoculation in TSB turned out to be statistically significant in comparison to BA incorporated with 2% Tween 80 ($p = 0.00002332$) in this study. Comparing the growth of bacteria by conventional method and BA incorporated with 2% Tween 80, however, did not turn out to be statistically significant.

By all the three methods of ascitic fluid culture, Gram negative bacilli (GNB) predominated (>78%). Almost all the studies of ascitic fluid cultures have shown predominance of Gram negative bacilli ranging from 28.17% to 100%.^{3-6,13,15,16} Amongst the Gram negative bacilli, *Escherichia coli* was the commonest bacteria (>40%) by all the three methods, followed by *Pseudomonas aeruginosa* (<21%) (Table 1). Various studies have isolated *Escherichia coli* from 22.22 to 75% from ascitic fluid.^{3,4,13,15} *Pseudomonas aeruginosa* and *Acinetobacter species* have also been isolated from ascitic fluid cultures from other studies.^{3-6,15-17} Amongst the Gram positive cocci, *Enterococcus species* and *Streptococcus species* were more. One *Streptococcus species* isolated from TSB was Bacitracin sensitive. Therefore, it was *Streptococcus pyogenes*. All *Enterococcus species* were isolated by direct inoculation in TSB. No MRSA.

Spontaneous Bacterial Peritonitis (SBP) is a frequent, yet serious complication in patients of advanced cirrhosis with ascites. Culture on blood agar incorporated with 2% Tween 80 was a novel method of ascitic fluid culture, however the yield of positive culture was not satisfactory by this method. This study shows that direct bedside inoculation in TSB is a superior method of ascitic fluid culture than the

conventional method for the diagnosis of SBP.

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