

Original Research Article

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Bacteriological Profile and Antibiotic Susceptibility Pattern of Isolates from Ascitic Fluid among Patients Diagnosed With Chronic Liver Disease in a Tertiary Care Hospital: An Observational Study

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ABSTRACT

Spontaneous bacterial peritonitis (SBP) is a condition where there is development of monomicrobial infection of the ascitic fluid in the absence of any intra-abdominal source of infection and raise in the polymorphonuclear leukocyte cells (PMN) more than 250cells/mm³. To describe the bacteriological profile and antibiotic susceptibility pattern of isolates in ascitic fluid from patients with SBP. Records of 500 patients with suspected diagnosis of SBP were analyzed for two years (January 2015- December 2016). All patients had undergone paracentesis after admission to the hospital. Ascitic fluid was cultured by automated blood culture (BacT/Alert) method. Antibiotic sensitivity testing was carried out by Kirby- Bauer disc diffusion method. Out of 500 cases of clinically suspected SBP, 483 (96.6%) were males and 12 (3.4%) were females. Study showed 25.6% (n=128) of subjects had SBP. Classical SBP was seen in 28 (21.8%), Bacterascites in 54 (42.1%) and Culture negative neutrocytic ascites (CNNA) in 46 (35.93%). *Escherichia coli* was isolated from 45(48.9%) cases, *Acinetobacter spp.* from 8 (8.88%), *Pseudomonas spp.* from 2 (2.22%), *Klebsiella spp.* was isolated from 7 (7.77%), *Enterobacter species* from 1(1.%), *Enterococcus spp.* from 17 (18.47%) and *Staphylococcus aureus* from 2(2.22%). Resistance to cephalosporins and quinolones 45.5% and 28.9% were observed respectively. *Escherichia coli* and *Enterococcus spp.* were the most common organisms isolated. More than 50% of the Gram negative bacteria are resistant to the cephalosporins which are the most frequently used antibiotic.

Keywords

Bacteriological profile, Ascitic fluid, Chronic liver disease, Antibiotic susceptibility

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Introduction

Spontaneous bacterial peritonitis (SBP) is the infection of the ascitic fluid that occurs in the absence of a visceral perforation and an intra-abdominal inflammatory focus. (Caruntu and Benea, 2006) Criteria for the diagnosis of SBP are fulfilled if the polymorphonuclear neutrophil cells (PMN) count from the ascitic fluid is > 250 cells/mm³ and culture yields

growth of a single organism. Three variants of SBP have been described. They are Classical SBP (culture positive with raise in PMN cells), Bacterascites (culture positive but no raise in PMN), Culture negative neutrocytic ascitis (culture negative with raise in PMN). (Harrisons textbook of internal medicine)

In cirrhotic patients, common pathogens causing SBP are enteric in origin which

account for 69%. *Escherichia coli* followed by *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and other Streptococcal species including *Enterococci* are the common organisms described.

Staphylococcus aureus is an unusual isolate in primary peritonitis accounting for 2- 4%. (Harrisons textbook of internal medicine)

Automated systems have increased the yield by 90% in ascitic fluid culture. (Runyon *et al.*, 1988) Complications like renal insufficiency, hypothermia, hyperbilirubinemia and hypoalbuminemia have been described if these patients (SBP) are not treated promptly. (Mandell, Douglas and Bennett's textbook of Principles and Practice of Infectious diseases)

The main objectives of this study includes to describe the bacteriological profile and the antibiotic susceptibility pattern of isolates obtained from ascitic fluid in patients, clinically diagnosed with spontaneous bacterial peritonitis.

Materials and Methods

The present study was a hospital based observational study, conducted on 500 clinically suspected cases of SBP from January 2015- December 2016, admitted in Gastroenterology and medical wards, in St. John's Medical College and Hospital, Bangalore.

All cases suspected to have known to have cirrhosis with ascites were included in this study. All patients with cirrhosis irrespective of aetiology of cirrhosis (alcohol, HCV, HBV, autoimmune, cryptogenic etc) were included in the study. Patients having secondary peritonitis due to appendicitis, gastrointestinal perforation, abdominal tuberculosis, septicemia, intestinal obstruction, trauma and

malignancy and with history of antibiotic therapy were excluded from the study.

Patient's demographic details were collected and various presenting complaints such as abdominal distention, abdominal pain, pedal edema, fever, jaundice, decreased urine output, malena or loose stools were collected. Laboratory information system was used to collect data of patients and their detailed chart review was done.

The study was approved by the ethical committee of our institute (IEC Study Ref.No.13/2016)

Methodology

Ascitic fluid was collected with aseptic precautions, prior to administration of antibiotics and 10ml was directly inoculated into the blood culture blood in the wards. Ascitic fluid cultures were done by BacT/Alert Microbial Detection System and isolate identification done by standard phenotypic methods. Antimicrobial susceptibility testing was performed for all isolates by Kirby-Bauer disc diffusion method as recommended in the Clinical and Laboratory Standards Institute guidelines 2015. (CLSI, 2015)

The following antibiotics tested by disc diffusion: Ampicillin (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Amikacin (30 µg), Netilmicin (30 µg), Co-trimoxazole (1.25 µg Trimethoprim /23.75µg Sulfamethoxazole), Meropenem (10 µg), Piperacillin/Tazobactam (100/10 µg) for Gram negative bacilli.

Penicillin, Ampicillin (15µg), Cefoxitin (30µg), Chloramphenicol (30µg), Erythromycin (15µg), Vancomycin (30µg), Teicoplanin (30µg)), Ciprofloxacin, and Tetracycline (30µg) were used to determine the susceptibility patterns of the Gram positive cocci.

Suspected patients with SBP underwent paracentesis aseptically before starting antibiotics



10 ml of ascitic fluid was collected in blood culture bottle and sent to laboratory (Runyon *et al.*, 1988)



Ascitic fluid filled bottle was incubated in BacT Alert automated system



BacT Alert machine signals when there is growth of microorganism



Microorganism were identified by Gram staining, culture and biochemical reactions



Antibiotic susceptibility testing reported using by Kirby Bauer method (CLSI)

Statistical analysis

The results of the study were analysed using descriptive statistics. Continuous data was analysed using student t test and categorical variables using chi square test. Fisher -exact test was done to find the association between the variables if the number of data was less than 5. P value <0.05 is considered as significant.

Results and Discussion

Out of the total 500 cases of clinically suspected SBP, 483(96.6%) were males and 17 (3.4%) were females. This high male to female ratio was probably due to the fact that most of the men 445(92.1%) had history of alcohol intake. Maximum numbers of cases were in age group of 30-50 years. The youngest case included in this study was 22 years and oldest case was 80 years. Mean age was 46.72±10.95 years. (Table 1)

The common presenting complaints of cases were abdominal distention followed by

jaundice, fever, altered sensorium, oedema feet, pain abdomen and Upper gastrointestinal bleed. (Figure 1)

Of the 500 clinically suspected cases of SBP, the ascitic fluid demonstrated, polymorphonuclear cells > 250 cells/mm³ was seen among 73 (14.6%) and cells < 250 cells/mm³ were seen in 427 (85.4%) patients. Study showed 25.6% (n=128) of subjects had SBP. 82/500 (16.4%) yielded growth.

Among the culture positive patients, 28/82(21.8%) had raise in the polymorphonuclear cell count (Classical SBP) and 54/82(42.1%) had PMN<250 cells/mm³ (Bacterascites). Of the 408 that where culture negative, 46/408(35.93%) samples had raised PMN, labelled as culture negative neutrocytic ascites (CNNA).

Table 2 describes the variants of SBP, the common variant noticed was Bacterascites. Among the culture positive patients, 28/82(21.8%) had raise in the polymorphonuclear cell count (Classical SBP) and 54/92(42.1%) had PMN<250 cells/mm³ (Bacterascites).

The Neutrophil count > 250 cells/mm³ commonly seen among the age group of 30-50 years which was statistically significant (p<0.003). There was also statistical significance (p<0.0001) in Neutrophil count > 250 cells/mm³ and Gram negative bacterial infection as a causative agent of SBP as shown in table 5 and table 6.

One isolate of *Pseudomonas* species was carbapenem resistant and colistin sensitive and other *Pseudomonas* isolate was sensitive to Cephalosporins.

One isolate of *Enterobacter* species was sensitive to Aminoglycosides and Cephalosporins.

Table.1 Characteristics of SBP patients

Parameters	
No. Of patients(n)	500
Age	22-80years
Mean age	46.72± 10.95 years
Male	483(96.6%)
Female	17(3.4%)
Alcohol induced Liver cirrhosis	446(89.2%)
HBV induced Liver cirrhosis	35(7%)
HCV induced Liver cirrhosis	11(2.2%)
Cryptogenic Liver cirrhosis	8(1.6%)

Table.2 Variants of Spontaneous bacterial peritonitis: n=128

Variants	Prevalence
Classical SBP	28 (21.8%)
Bacterascites	54(42.1%)
Culture negative neutrocytic ascitis	46 (35.93%)

Table.3 Organisms from samples which yielded culture positive: n=82

Bacteria	No. of cases n=82	Percentage
<i>Escherichia coli</i>	45	48.9%
<i>Enterococcus species</i>	17	18.47%
<i>Acinetobacter spp.</i>	8	8.88%
<i>Klebsiella spp.</i>	7	7.77%
<i>Pseudomonas species</i>	2	2.22%
<i>Staphylococcus aureus</i>	2	2.22%
<i>Enterobacter species</i>	1	1.11%

Table.4 Association between sex and immune status

Sex	Normal immune status	HBs Ag positive	HCV positive
Males	445(98.9%)	26 (74.3%)	10(90.9%)
Females	5(1.1%)	9 (25.7)	1(9.1%)

^bp value calculated by Fishers exact test p value<0.05 is significant

Table.5 Association between age in years and neutrophil count

Age in years	Neutrophil count <250 cells	Neutrophil count >250 cells	Test value	P value
< 30 years	33 (86.8%)	5 (13.2%)	15.18 ^a	< 0.001
30 -50 years	217 (81.6%)	49 (18.4%)		
>50 years	131 (95.6%)	6 (4.4%)		

^ap value calculated by Chi square test p value<0.05 is significant

Table.6 Association between age in years and common organism causing infection

Age in years	Gram negative bacteria	Gram positive bacteria	Test value	P value
< 30 years	2(40%)	3 (60%)	18.78 ^b	0.001
30 -50 years	32 (86.4%)	7 (17.94%)		
>50 years	19 (51.35%)	18 (48.6%)		

^bp value calculated by Fishers exact test p value<0.05 is significant

Table.7 Antibiotic susceptibility pattern of Gram negative bacteria

Antibiotics	E.coli (n=45)	Acinetobacter spp.(n=8)	Klebsiella spp.(n=7)
Aminoglycosides (gentamicin, amikacin, netilmycin)	33(73.33%)	34%	4(57.1%)
Cephalosporins (cefotaxime, ceftazidime)	25(55.5%)	0%	4(57.1%)
Fluoroquinolones (ciprofloxacin)	32(71.1%)	33.8%	4(57.1%)
Carbapenemes (Meropenem)*	41(91.1%)	57.1%	50%
Piptaz (aminopenicillin + Beta lactamase inhibitor)	35(77.7%)	0%	50%

*Isolates that were resistant to Meropenem, were sensitive to Colistin and Tigecyclin.

Table.8 Distribution of variants of SBP documented in present as compared to other studies

Variant SBP	Present study N=500	Jeevan <i>et al.</i> , ⁽⁸⁾ N= 300	Kavita <i>et al.</i> , ⁽¹⁰⁾ N=122	Purohit <i>et al.</i> , ⁽⁷⁾ N=217	Vandana <i>et al.</i> , ⁽⁶⁾ N=600
Study period	Jan2015-Dec 2016	Jan2014-dec 2014	March2012-May 2013	Dec2011-Nov 2012	Jan2007-Dec 2011
CNNA	35.93%	47%	80%	56.4%	57.1%
Classical SBP	21.8%	51%	12%	43.6%	35.8%
Bacterascites	42.1%	2%	8%	Did not comment	7%

Table.9 Comparison of distribution of the isolates between our study and other studies

Isolate	Present study %	Jeevan <i>et al.</i> , ⁽⁸⁾ %	Reuken <i>et al.</i> , ⁽¹¹⁾ %	Purohit <i>et al.</i> , ⁽⁷⁾ %	Vandana <i>et al.</i> , ⁽⁶⁾ %	Tsung <i>et al.</i> , ⁽¹¹⁾ %
<i>Escherichia coli</i>	48.9% (n=45)	37.5%	64.5%	54.9% (n=17)	50% (n=15)	25%
<i>Klebsiella spp.</i>	7.77% (n=7)	16.6%	10.41%	16.2% (n=5)	16.66% (n=5)	19.1%
<i>Acinetobacter spp.</i>	8.88% (n=8)	-	2.08%	-	3.33% (n=1)	2.1%
<i>Pseudomonas spp.</i>	2.22% (n=2)	-	6.25%	9.6% (n=3)	6.66% (n=2)	4.3%
<i>Enterobacter spp.</i>	1.11% (n=1)	-	4.16%	-	6.66% (n=2)	4.3%
<i>Enterococcus spp.</i>	18.47%* (n=17)	16.66%	39.5%	-	-	12.8%
<i>Staphylococcus aureus</i>	2.22%** (n=2)	-	16.66%	19.3% (n=6)	-	6.4%

**All the *Staphylococcus aureus* isolates in our study were sensitive to Methicillin.

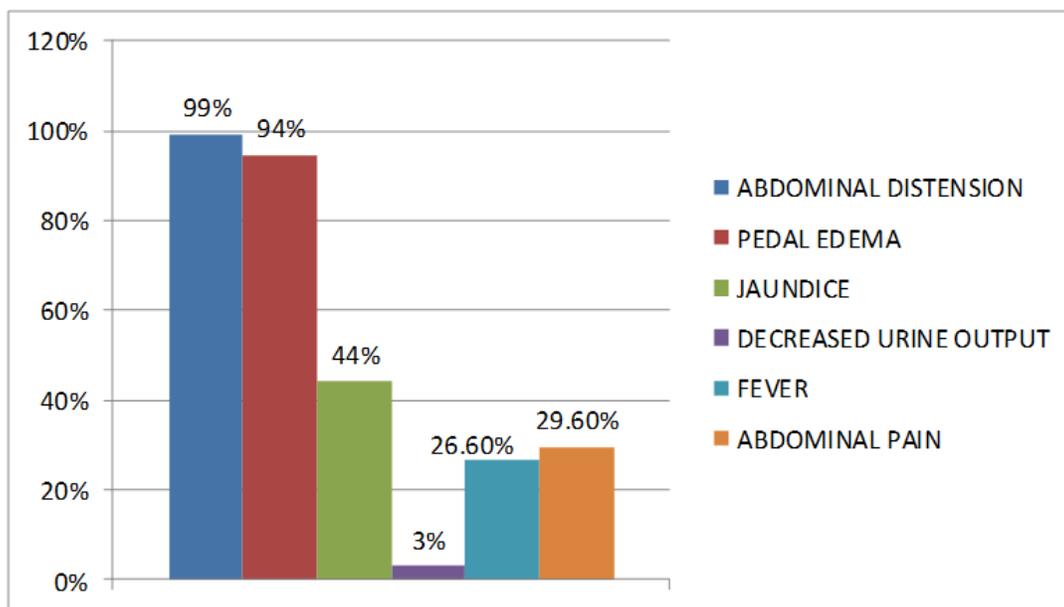
*All the *Enterococcus* species except for one isolate were sensitive to Glycopeptides

Table.10 Sensitivity pattern of the Gram negative bacilli from our study and related studies

Antibiotics	Present study %	Jeevan <i>et al.</i> , ^{(8)*}	Vandana <i>et al.</i> , ^{(6)*}
Third generation Cephalosporins	55.5%	50%	50%
Quinolones	71.1%	53.3%	53.3%
Aminoglycosides	73.3%	-	-
Pipercillin-Tazobactam	77.7%	70%	70%
Meropenem	91.1%	-	96.6%

*Studies have reported as overall sensitivity of the isolates.

Fig.1 Describing various clinical features of the participants in the study population



Antibiotic sensitivity of Gram positive bacteria was out of 17 isolates of *Enterococcus species*, 11 (69.2%) were sensitive to penicillin, 11 (69.2%) were sensitive to aminoglycosides, 16(94.11%) were sensitive to ciprofloxacin and 16 (94.11%) were sensitive to glycopeptides. One strain of *Enterococcus spp.* was resistant to Vancomycin.

Two strains of *Staphylococcus aureus* were sensitive to Penicillin, Methicillin, Erythromycin, Aminoglycosides, Fluoroquinolones.

Our study comprised subjects between 22-80 years (46.5 ± 10.8 years), 483(96.6%) were males and 17 (3.4%) were females.

Alcohol intake was the commonest reason for liver cirrhosis and SBP, accounting to 89.2% which is similar to the study conducted by Jeevan *et al.*, [2015] followed by Hepatitis B induced liver cirrhosis (7%), Hepatitis C induced liver diseases (2.2%) and Cryptogenic causes of liver cirrhosis, contributing to 1.6%.

The clinical manifestations of SBP have a varied presentation. Most patients of SBP in our study had symptoms and or signs clearly suggestive of peritoneal infection, especially pain abdomen, fever and altered gastrointestinal motility, similar presentation has been reported by various other studies.

Mihas *et al.*, reported varied manifestations ranging from fever to gastrointestinal bleeding and hepatic encephalopathy (Mihas *et al.*, 1992).

Other studies report that patients may present with development of hepatic encephalopathy or renal failure which may be the predominant or only feature at the time of presentation to the hospital. (Payal H. Purohit *et al.*, 2015)

The clinical presentations found in this study were fever (26.6%), abdominal distention (99%), pedal edema (94%), encephalopathy (38%) jaundice (44%) and decreased urine output (3%). Subjects commonly presented with abdominal distension in combination with pedal edema and jaundice.

All cirrhotic patients with ascites are prone to develop SBP. The prevalence of SBP in hospitalized patients with cirrhosis and ascites is between 10% and 30%. (Bhat *et al.*, 2013) Prevalence of SBP and its variants in our study is 27.8% that is similar to the findings by Puri *et al.*, [1996]. SBP was commonly seen among males in the age group of 30-50 years that is comparable to other studies [Payal H. Purohit *et al.*, 2015].

Common variant of SBP was Bacterascites 42.1% followed by CNNA- 35.93% and classical SBP- 21.8%.

Neutrophil count > 250 cells/mm³ was commonly seen in age group of 30-50 years which was statistically significant. Gram negative bacterial infection and neutrophilic counts >250 cells/mm³ were commonly seen among age group of 30-50 years which was statistically significant.

SBP was detected in 27.8% of the patients in our study group. The most common causative organism of SBP was enteric gram negative bacilli (*Escherichia coli*) followed by *Enterococcus species*. Resistance to cephalosporins and quinolones was 45.5% and 28.9% and alcoholic liver disease was the commonest etiology of liver cirrhosis.

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