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# MICROBIOLOGICAL DIAGNOSIS OF COMMUNITY ACQUIRED SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOTIC PATIENTS, ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND MOLECULAR CONCORDANCE WITH BACTEREMIA

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**ABSTRACT** Spontaneous Bacterial Peritonitis is a serious complication of cirrhosis of liver with portal hypertension ascites. Bacteria and rarely fungi can cause it usually by gut translocation of pathogens as well as bacteremia. Sepsis often is associated and may be potentially life threatening. It needs prompt and adequetly effective antibiotics followed by life-long prophylactic antibiotics. It is also an indication for liver transplant. Guidelines tell us that causative organisms are gram negative organisms, 3rd generation cephalosporin is the treatment of choice while quinolones are used to prevent a second attack, to reduce mortality. However, gram positive organisms are increasingly being reported as a cause, cephalosporin resistanece is increasing too and concordance of bacteremia are not uncommon. All need factors need a renewed attention now to refine our approach while treating this serious complication of decompensated cirrhosis.

**KEYWORDS :** SBP, Cirrhosis, Bacterial concordance, Antibiotic resistance, ascites

## INTRODUCTION

Management of patients with Cirrhosis of liver with superadded Spontaneous bacterial peritonitis (SBP) is a challenge for physicians. SBP among cirrhotic patients in community is a common complication.[1,2] Initial diagnosis in many of these patients are often missed due to absence of classical signs and symptoms.[3]Most cirrhotic patients admitted with bacterial peritonitis deteriorate quickly, develop multi organ failure and have high mortality, if not aggressively intervened.[1]These patients rapidly develop complications like encephalopathy, gastrointestinal bleeding, and renal failure.[2,4]Large multicentre studies, recorded prevalence of spontaneous bacterial peritonitis (SBP) to be as high as 27% in inpatients with cirrhosis.[1] One year survival after the first episode is about 40%[5] and in absence of prophylaxis with proper antibiotics, SBP recur in about 70%[4,6]; whereas proper prophylaxis improves survival and reduce the probability of recurrence to 20%.[7,8]

However, since isolation of bacteria from ascitic fluid is low to establish bacterial etiology which demands improvement in the existing methods of detection and prompt isolation of the causative organisms.

Further, increasing antibiotic resistance among bacteria causing SBP, complicates the selection of appropriate antibiotics in already deteriorating health of cirrhotic patients. INASL recommends Ceftriaxone as the empirical therapy for SBP. With increasing numbers of ESBL producers among SBP causing bacteria, this recommendation might need an urgent review.Therefore, the selection of the empirical antibiotic treatment should be guided by the severity and location where the infection was acquired,the risk factors for multidrug-resistant organisms,and the available information on the local expected bacteriology.

It is postulated that mostly, systemic infection precedes SBP. To establish the source of SBP, homogenicity of isolates are needed. An updated antibiogram of common isolates in a hospital will help physicians to choose the best option of empirical antibiotic in such cases.

### Aims and Objectives

- Direct smear demonstration and isolation of causative organisms of SBP in cases with cirrhosis.
- Comparision of detection rate between sample collection procedure and duration of collection and Direct smear examination.
- Determination of the antibiotic susceptibility pattern of the causative organisms .
- To determine the molecular concordance with the isolates of bacteremia and test for the strain homogeneity.

## MATERIALAND METHODS

This Cross sectional observational study was carried out in Assam Medical College Dibrugarh, Assam over a period of six months. All consecutive clinically suspected SBP cases without any history of antibiotc intake in the last 6 weeks attending Medicine OPD for six months were included.

Peritoneal fluid was collected from all the cases in sterile container, as well as in blood culture bottles in duplicate in aseptic conditions. Simultaneously, blood was collected for culture and subjected to VITEK-2 system for identification of organism.

Gram staining was done on smears prepared bedside as well as from the ascitic fluid samples collected in triplicate.Culture isolates were identified using conventional methods. Antimicrobial sensitivity of isolates was performed using the Kirby Bauer method and tests for detection of ESBL production were done using the CLSI guidelines, 2017. Similar organisms isolated from blood and ascitic fluid were confirmed by Polymerase Chain Reaction using16s rRNA primers. Sanger sequencing was done to confirm the strain homogeneity causing SBP and Bacteremia.

Statistical analysis was done using Fisher's exact test and p- value of ≤0.05 was considered significant. All categorical variables were presented in the form of percentages, tables and graphs.

## RESULTS

A total of 56 numbers of cases were received. Ascitic fluid collected in blood culture bottle showed isolation rate of 46.42% (n=26) as compared to significantly (p<0.001) lower [3.57% (n=2)] isolation from samples collected in sterile container. (Fig.1)

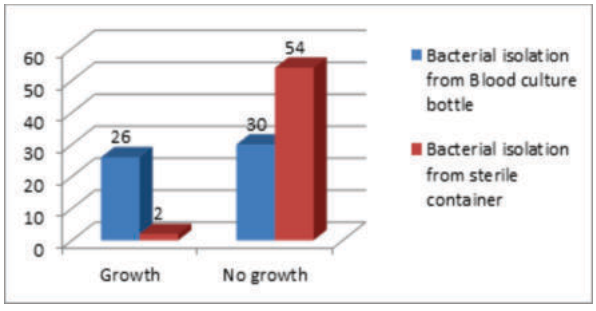


**Fig.1- Showing No. Of Isolates From Ascitic Fluid Collected In Sterile Container And Blood Culture Bottle.**

Direct smear examination by Gram staining showed 84.6% positivity when examined within half an hour of sample collection and only 30.7% positivity when examined after half an hour. On the other hand there was significantly lower (0%) positivity in Gram staining done

from samples collected in sterile containers (p<0.001). Among the organisms isolated, Escherichia coli was highest (30.70%) followed by Klebsiellapneumoniae(19.23%)and Staphylococcus aureus(15.38%). [Fig.2]

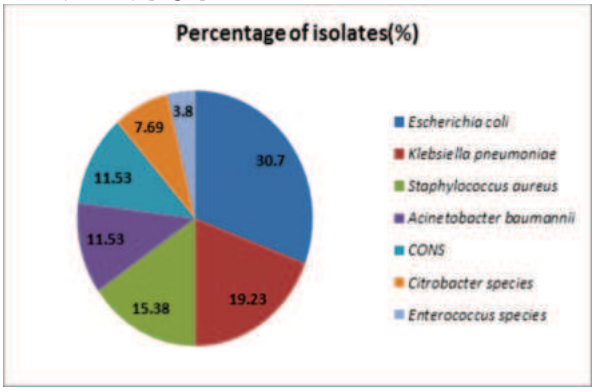


**Fig.2- Showing Percentage Isolation Of Bacteria From Ascitic Fluid**

On examining the antibiotic sensitivity pattern, it was seen that Gram negative isolates were mostly resistant to the third generation Cephalosporins ,Amikacin and Ampicillin-Sulbactam; whereas they were mostly sensitive to Carbapenems. [Fig.3] On the other hand, CONS was comparatively more resistant to almost all the antibiotics compared to the other Gram positive organisms. All the Gram positive organisms were sensitive to Linezolid ,Teicoplanin and Vancomycin. [Fig.4]

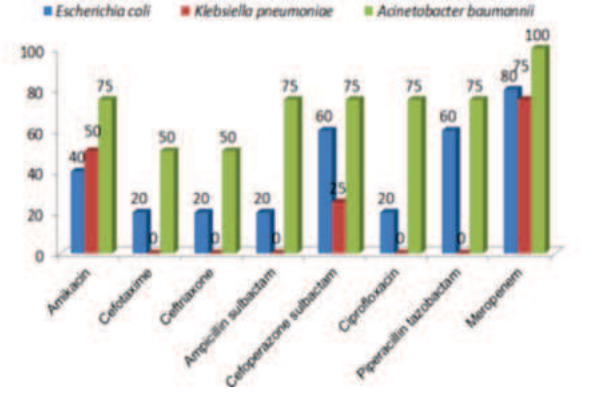


**Fig.3- Percentage Antibiotic Sensitivity Pattern Of The Gram Negative Isolates**

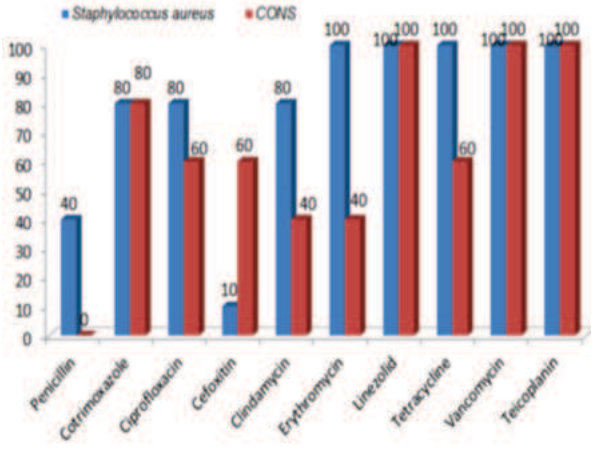


**Fig.4- Percentage Antibiotic Sensitivity Pattern Of The Gram Positive Isolates**

On doing blood culture, concomitant bacteremia was seen in 38.5% cases. Similar growth from both ascitic fluid and blood culture was seen in 23.07% cases. On subjecting these similar isolates to Sanger sequencing, it was found that 15.38% showed strain homogenecity. [Fig.5][Fig.6]

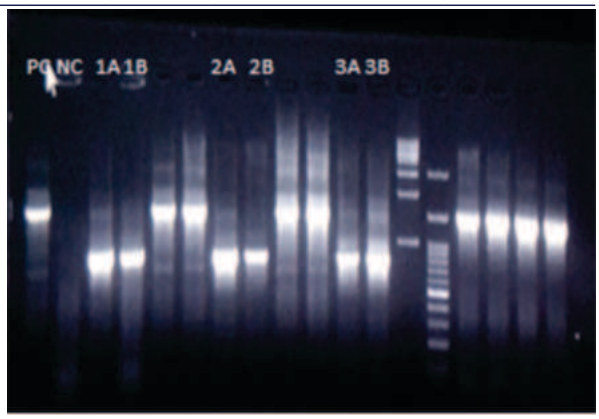


**Fig.5- Showing 16srRNA PCR for Multi Locus Sequencing of Isolates to Show Homogeneity (B=Blood, A=Ascitic fluid)**

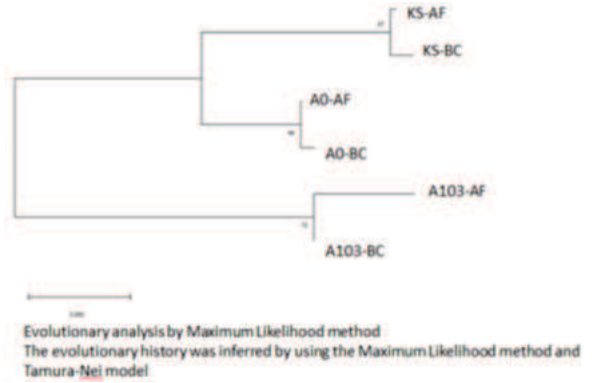


**Fig.6- Showing Phylogenetic Tree Analysis For Concordance**

## DISCUSSION

While hospital acquired SBP is a major complication in cirrhosis, Community acquired SBP is also now emerging as a major threat to the public health. Prompt baseline paracentesis and a smear for Gram stain prepared at bedside can correctly determine the Gram stain character of infecting organisms. This helps in improving the diagnosis, early initiation of antibiotic therapy and prognosis.

In our study, it is seen that sterile containers used for collection of ascitic fluid for microbiological diagnosis of SBP, lowered the probability of isolation to as low as 3.6% compared to isolation from blood culture bottles. According to study conducted by Runyon BA et al, in 29 episodes in which the bedside bottles were culture positive, only 22 (75.9%) of the laboratory inoculated sets demonstrated growth; this difference was statistically significant ($P < 0.02$).[9]Similarly, a study carried out by Girish et al showed that In 25 episodes in which bedside bottles used were culture positive only in 8 episodes by the delayed culture method demonstrated growth; this difference was statistically significant ($P < 0.001$).[10] Perhaps during transportation organisms become non-viable. Hence sample collection in suitable culture media improves the bottle isolation rate, and further it can be monitored for any growth and if turbidity is noticed, a Gram stained smear can be prepared immediately and presumptive report can be sent to the treating physician early. This will also help in choosing antibiotics.

In this study, it was found that Gram negative bacterial (GNB) infection was predominant. Among the GNB, Escherichia coli was highest followed by Klebsiellapneumoniae which is consistent with most other studies conducted across the globe.[1, 11, 12, 13, 14,15] Arising prevalence of gram-positivebacteria was reported over the past years in North America, SouthAmerica, and Europe representing at present48%–62%of the isolated organisms;[16,17,18] which is contrary to our findings, where GNB was more predominant. Among the Gram positive cocci (GPC) isolated, Staphylococcus aureus was highest followed by CONS and Enterococci whereas most other studies carried out elsewhere showed growth of Streptococcus and Enterococcus more than Staphylococcus.[11,12,13,14] But GPC is showing a gradual increase and is ominous

The current recommendation for empirical antibiotic therapy in clinically suspected SBP cases is the Broad spectrum- 3rd generation Cephalosporin group.Though GPC were sensitive to almost all the classes of antibiotics, this study however showed that >50% Gram negative isolates were resistant to this group of antibiotic.While 20% of Staphylococcusaureus isolates were resistant to fluoroquinolone group of antibiotics, all Klebsiellapneumoniae isolates and 80% of E.coli isolates were resistant to Ciprofloxacin and were phenotypically tested as presumptive Extended Spectrum Beta Lactamase (ESBL) producers; which implies that they have acquired resistance against the Cephalosporin, Penicillin, quinolones and Monobactam groups of antibiotics.This finding is contrary to studies carried out elsewhere which showed higher rate of sensitivity.[19]Our study showed an alarming rate of multidrug resistance in community acquired SBP cases and may be a result of indiscriminate antibiotic use for other reasons. While multidrug resistance has been reportedly high as seen from other studies[1], 4%–16%ofcommunity-acquired spontaneous bacterial peritonitis is also caused by multidrug-resistant organisms as reported elsewhere.[1, 2, 16,20,21,22] On the other hand, Carbapenems were highly sensitive to most of the isolates. However, these being the last resort left for us, Cephalosporins or Ampicillin with Beta lactamase inhibitor combination (60-70% sensitive) can be used as a better and economic option for empirical therapy, sparing the penems for serious and a selective subgroup.

From the study, it is evident that concomitant bacteremia is too not uncommon; which is similar to findings from various other studies. Sequence typing is the gold standard test for showing bacterial homogeneity and this study is a novel one of this kind reported from this part of country.Our study could establish molecular concordance in 15.38% of total cases (66% of similar isolates) with strains causing SBP and bateremia which is quite serious as this leads to a higher degree of decompensation of the patients. However, whether sepsis following infection from other source of infection elsewhere in the body is a cause of SBP or SBP led to sepsis needs more research, but both may coexist.

An important corollary finding was that all the molecular confirmed strains showed exactly similar antibiotic susceptibilty pattern. Thus, antibiogram typing can correctly predict strain similarity where DNA sequencing or PCR with advanced laboratory facilities are not available and thus highly economical too. A local antibiogram is invaluable for each centre dealing with a large number of cirrhotics in different parts of our country.

## CONCLUSION

Prompt diagnosis can be life-saving in SBP in cirrhosis with proper antibiotic treatment. All samples should be sent in a suitable enrichment broth. Bedside smear preparation for Gram staining aids in early diagnosis and help starting empirical antibiotic use. Almost all Gram negative bacteria causing SBP were ESBL producers in the our centre inspite of the small number of patients.Therefore, this calls for a locally specific standard/protocol treatment guideline for initiation of empiric therapy. This can also contribute to stimulating further validation studies of national guidelines.

DNA sequencing confirmed concordant isolation from blood as well as peritoneal fluid in 66% of the isolates showing similar growth pattern.However, the more cost effective and easier technique of antibiogram typing can be a good presumptive test to detect concomitant sepsis with the same infecting strain. But, whetherSBP leads to sepsis or the other way round happens, opens up a new question as sepsis in SBP leads to >80% mortality and each hour of delay in appropriate antimicrobial therapy is associated with 1.86 fold increased hospital mortality.[22]

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