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Bacteriological profile and its drug sensitivity spectrum ofspontaneous bacterial peritonitis and its variants

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ABSTRACT
Background: - Ascitic fluid infection is important complication of cirrhosis. It can be classified into 5 categories based on ascitic culture results, PMN count and presence or absence of surgical source of infection. It includes spontaneous ascitic infection (SBP, MNB, CNNA), secondary bacterial peritonitis, polymicrobialbacterascites. Spontaneous ascitic infection is frequent among them.
Objective: - A study on bacteriological profile and drug sensitivity spectrum of spontaneous bacterial peritonitis and its variants in cirrhosis of liver.
Materials and Methods: - Prospective observational study wasconducted on 218 cases admitted in Jawaharlal Nehru Medical College, Aligarh, Uttar Pradesh, India. All the patient irrespective of age with cirrhosis and ascites (irrespective of aetiology) were included in study. Ascitic fluid samples were collected before initiation of antibiotics by bedside tapping in culture bottle and sterile plain vial aseptically using standard and universal precautions. Ascitic fluid was sent for biochemical analysis, culture and cytological analysis. Routine blood investigation CBC, RFT, LFT were also sent. Out of 218 cases, only 52 cases had ascitic fluid infection on basis of ascitic fluid analysis and was taken for further analysis.
Results: -Among 218 patients studied, only 52 cases had ascitic fluid infection on basis of ascitic fluid analysis and was taken for further analysis. Out of those infected 52 cases,38 (73%) cases had no growth in culture media and had PMN >250 per µl i.e.culture negative neutrophilic ascites (CNNA); 14(27%)cases had growth in culture media and had PMN >250 per µl i.e., classical spontaneous bacterial peritonitis (SBP). No cases of MNBnoted in our study. Out of 14 culture positive cases,9 (64%)cases had Escherichia coli growth, 3(21%) cases had klebsiella growth and 2(15%) cases had Staphylococcus Aureus growth in culture media. All culture (100%) had sensitivity to Ceftriaxone, Cefoperazone, Cefepime, Piperacillin + Tazobactam, Amikacin, Gentamicin, Tobramycin; 11(79%) culture positive cases were sensitive to Moxifloxacin; 10(71%) culture positive cases had sensitivity to Ofloxacin, Levofloxacin and Ampicillin + Clavulanic acid; 9(64%) culture positive cases were sensitive to Amoxicillin + clavulanic acid and 7(50%) culture positive cases had sensitivity to Cefotaxime. 2 out of 14 culture positive isolates had Staphylococcus Aureus growth which were resistant to Cefotaxime.
Conclusion: - Prevalence of SBP in cirrhotic patients was 23.85 %. Gram negative bacteria i.e., E. coli followed by klebsiella are predominant isolate. Gram positive isolate Staphylococcus Aureus were also noted. Higher rates of resistance against cefotaxime and fluoroquinolones was noted. Antibiotics such as Ceftriaxone, Cefoperazone, Cefepime, Piperacillin + Tazobactam, Amikacin, Gentamicin, Tobramycin canalso be used as an effective alternate antibiotic regime in SBP patients as empirical therapy.
Keywords: - Ascitic fluid infection, Culture negative neutrocytic ascites**,** Drug sensitivity, Paracentesis, Spontaneous bacterial peritonitis.

I. INTRODUCTION

Cirrhosis is a final destination of all chronic liver disease. It is defined as diffuse hepatic fibrosis with replacement of normal liver architecture by nodules [(1)]. Ascites (a pathological fluid collection in peritoneum) is a decompensated stage of cirrhosis.Ascitic fluid infection is an important complication of cirrhotic ascites. It can be classified into 5 categories based on ascitic culture results, PMN count and presence or absence of surgical source of infection. It includes spontaneous ascitic infection [spontaneous bacterial peritonitis (SBP), Monomicrobial non-neutrocyticbacterascites (MNA), Culture-negative neutrocytic ascites (CNNA)], secondary bacterial peritonitis, polymicrobialbacterascites.

SBP is prototype of spontaneous ascitic fluid infection. It is defined as PMN (polymorphic neutrophil) count >250/µL, culture positive and

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absence of surgical source of infection [2]. CNNAis a SBP variant associated with a similar mortality as seen in SBP [3]. It is defined as PMN count >250/µL, culture negative in the absence of antibiotic treatment and absence of surgical source ofinfection. MNA is defined as PMN count <250/µL, culture positive for single organism and absence of surgical source of infection [4].

Most common pathogenic organisms isolated inSBP were gram negative bacteria (Escherichia coli followed by Klebsiella) [5]. In recent study, gram positive isolate and resistant gram-negative bacteria are more common isolate [6]. It may be due to overzealous use of SBP prophylaxis, intestinal decontamination, irrational use of antibiotics, and repeated hospital admission. Empirical treatment with cefotaxime is preferred till culture and sensitivity reports become available [7]. This study attempts to reveal the changing bacteriological spectrum of spontaneous bacterial peritonitis and to unfold the changing spectrum of antibiotics that might be helpful in eradicating ascitic fluid infection.

II. MATERIALS AND METHODS

Study population:-This study was carried out on 218 patient admitted in department of medicine. Jawaharlal Nehru Medical College, AMU, Aligarh.

Study Design: Prospective, Observational, hospital based study**.**
Subject: 218
Inclusion criteria: All patients of cirrhosis of liver with ascites of varied aetiology and irrespective of gender of above 14 years.

Exclusion Criteria: -

- Ascites due to renal, cardiac, tubercular, malignant pathology.
- Secondary bacterial peritonitis and polymicrobialbacterascites.
- Patients who were not willing to participate in the study.
- Pregnant women.

Ethics Approval: -
The study protocol was approved by board of studies in September, 2018 and passed by ethical committee of the institution in December, 2018.

Methodology

This study was a prospective, observational cohort study where 218 patients of either gender ≥ 14 years, presenting with cirrhosis with ascitis were included after written informed consent. A detailed history and physical examination were carried out for every subject enrolled in the study as per a predesigned proforma. Routine blood investigations such as complete blood count (CBC), renal function tests (RFT), liver function tests (LFT), random blood sugar, serum electrolytes, lipid profile, were performed at central laboratory of the hospital. Upper GI endoscopy and liver biopsy were performed in Medicine department. Paracentesis (only diagnostic tap) was performed using all standard precautions for all study participants. Eight to ten ml of ascitic fluid was inoculated in brain heart infusion (BHI) broth bottles (for aerobic and anaerobic culture) at the bed side using aseptic technique. BHI culture bottles were incubated for 05 days at 37°C and monitored daily for any signs of positive culture (turbidity, gas production). Bottles showing any signs of positivity were sub-cultured on blood agar, chocolate agar and MacConkey's agar. Sensitivity testing was performed on Mueller Hinton agar using Kirby Bauer disk diffusion method.

Study Design-

This prospective observational study was conducted on 218 cirrhotic patients with ascites admitted to Jawaharlal Nehru Medical College and Hospital Aligarh, Uttar Pradesh, India, over a period of 23 months (November 2018 to August 2020). Patients of age >14 years with cirrhosis and ascites diagnosed based on clinical examination, endoscopy, liver biopsy, or ultrasound were included in the study after taking written informed consent. Those having ascites due to aetiology other than cirrhosis were excluded from the study. Out of 218 cases, only 56cases had ascitic fluid infection on basis of ascitic fluid analysis. Out of 56 cases with ascitic fluid infection, 4 cases which showed secondary bacterial peritonitis in ascitic fluid were excluded from the study and thus, remaining 52 cases were taken for further analysis.

Statistical Analysis-

All statistical data were analyzed using SPSS software version 26. Continuous variables were expressed as mean ± standard deviation while proportions were expressed as percentage.

III. RESULTS

In our study,38(73%) cases had no growth in culture media and had PMN>250 per µL i.e., CNNA, 14(27%) cases had growth in culture media and had PMN >250 per µLi.e., classical SBP.No cases of MNB notedin our study.

Out of 14 culture positive cases, 9 (64%) cases had Escherichia coli growth, 3 (21%) cases

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had klebsiella growth and2(15%)cases had Staphylococcus Aureusgrowth in culture media as evidenced in **Figure 1**

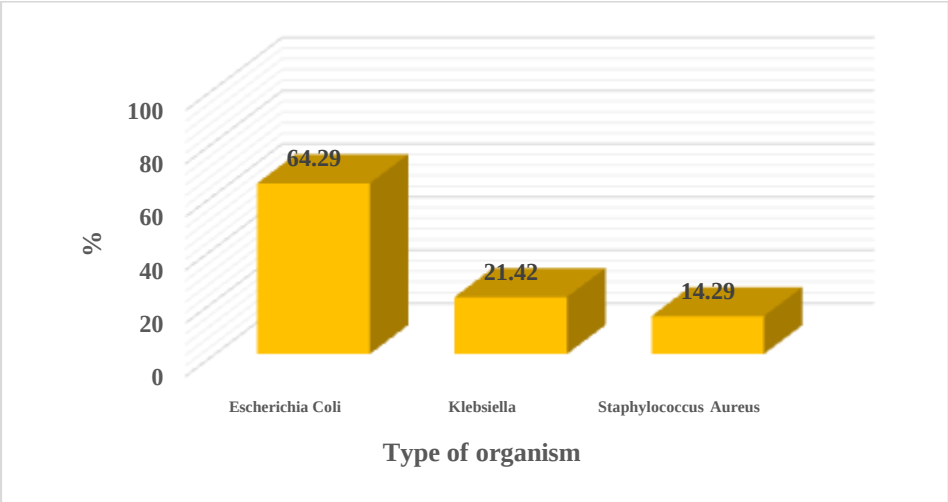

Figure 1: Distribution of type of organism in Culture positive

2 out of 14 culture positive isolates had Staphylococcus Aureus growth which were resistant to cefotaxime. Drug susceptibilities of various culture positive cases are depicted in **Table 1.**

Table 1.

S.No.	Drug	Sensitive	Resistant
1	Ceftriaxone	14 100 %)	0 (0 %)
2	Ceftriaxone + Sulbactam	14 (100 %)	0 (0 %)
3	Cefotaxime	7 (50%)	(50 %)
4	Cefoperazone	14 (100 %)	0 (0 %)
5	Cefepime	14(100 %)	0 (0 %)
6	Piperacillin + Tazobactam	14 (100 %)	0 (0 %)
7	Ofloxacin	10 (71 %)	4 (29%)
8	Levofloxacin	10 (71%)	4 (29%)
9	Moxifloxacin	11 (79%)	3 (21%)
10	Amikacin	14 (100 %)	0 (0 %)
11	Gentamicin	14 (100 %)	0 (0 %)
12	Tobramycin	14 (100 %)	0 (0 %)
13	Ampicillin + Clavulanic acid	10 (71 %)	4 (29%)
14	Amoxicillin + Clavulanic acid	9 (64%)	5 (36%)

IV. DISCUSSION

In our study,73% (38) cases had no growth in culture media and had PMN >250 per µL i.e., CNNA, 27% (14) cases had growth in culture media and had PMN >250 per µL i.e., classical SBP. No cases of MNB noted in our study. CNNA is rarely diagnosed when sensitive culture methods are used,CNNAoccurred probably due to previous antibiotic treatment (even 1 dose), Inadequate volume of fluid inoculated, or spontaneously resolving SBP (ascitic paracentesis is performed

after bacteria are killed by host defence system before neutrocytic count normalises) [8].

Out of 14 culture positive cases, E.Coli was isolated in 64% cases, whereas 21% cases had klebsiella growth and 15 % cases had Staphylococcus Aureus growth in culture media.

Escherichia Coli, followed by Klebsiella pneumoniae causesmost of the episodes of SBP. Bacterial translocation from the intestinal lumen is mainly considered the preceding factor for thedevelopment of SBP that is why gram-negative aerobic bacteria from the family of Enterobacteriaceae (60%) are reported as the predominant cause of SBP. Recently SBP episodes caused by gram positive bacteria are being increasingly reported. These changes in bacteriological spectrum are proposed to be due to indiscriminate use of antibiotics, increasing number of invasive procedures and hospitalization in intensive care units. This suggests a need for the constant assessment of common bacterial pathogens and their antibiogram to guide empirical treatment of SBP patients.

In our study, all cultures (100%) were sensitive to Ceftriaxone, Ceftriaxone + Sulbactam, Cefoperazone, Cefepime, Piperacillin + Tazobactam, Amikacin, Gentamicin, Tobramycin; 79% culture positive cases were sensitive to moxifloxacin; 71%culture positive cases were sensitive to ofloxacin, levofloxacin and Ampicillin + Clavulanic acid; 64%culture positive cases were sensitive to amoxicillin + clavulanic acid and 50% culture positive cases were sensitive to cefotaxime. 2 out of 14 culture positive isolates had Staphylococcus Aureus growth which were resistant to cefotaxime.

Cefotaxime is preferable antibiotic as empirical therapy in various previous studies [9]. But in our study cultures were resistant to cefotaxime in 50% cases. It may be due to irrational use of antibiotics, increase frequency of gram-positive isolates, nosocomial infection due to repeated hospital admission. Higher rates of resistance against fluoroquinolones were also noted in our study. It may be due to indiscriminate use of fluoroquinolones.

Our study findings support the possibility that the microbial aetiology of SBP changes over time. Consequently, empirical therapy for SBP might need to be determined by patterns of local bacterial resistance. Therefore, the initial use of cefotaxime might no longer be optimal. The present study suggests that ceftriaxone, cefoperazone, cefepime, Piperacillin + tazobactam, amikacin, gentamicin, tobramycin could be used as an effective alternate antibiotic in SBP patients.

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