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BACTERIOLOGICAL PROFILE AND ANTIBIOTIC RESISTANCE PATTERNS IN ADULT CIRRHOTIC PATIENTS WITH SPONTANEOUS BACTERIAL PERITONITIS IN SOUTHERN KERALA, INDIA

Gastroenterology

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ABSTRACT

Background And Objectives: Spontaneous Bacterial Peritonitis (SBP) is the most frequent infection occurring in liver cirrhosis. The aim of study was to describe the bacteriological profile and antibiotic resistance of SBP pathogens. **Materials And Methods:** A retrospective study was done for a period of 1 year in a tertiary care hospital in Southern Kerala, India. A total of 32 adult cirrhotic patients, meeting the inclusion and exclusion criteria were grouped into community onset (within 48 hours) and hospital onset (> 48 hours) based on timing of diagnosis of SBP and underwent ascitic fluid paracentesis and sample was sent for culture and sensitivity. Data thus obtained was grouped and studied using Chi Square test and analysis was done using IBM-SPSS. **Results:** Out of 32 cases, Gram negative bacteria were more common than gram positive bacteria and one third were classified as multi drug resistant (MDR) bacteria. Escherichia coli was the predominant bacterium of which one third were found to be MDR. MDR bacteria and Gram positive bacteria were more common in hospital onset group. Except for Third generation cephalosporins (45.5% vs.57.1%), resistance to quinolones (63.6% vs 61.9%), piperacillin-tazobactam (45.5% vs 19%) and carbapenems (36.4% vs 14.3%) were more frequently found in hospital onset group. **Conclusion:** One third of the SBP pathogens were found to be MDR. High antibiotic resistance were observed in third generation cephalosporins and quinolones which are used for treatment and prophylaxis of SBP. Hence judicious use of antibiotics according to culture and sensitivity is recommended.

KEYWORDS

INTRODUCTION

Cirrhosis is a condition which predisposes patients to various infections due to immune dysfunction. Spontaneous bacterial peritonitis (SBP) is seen in 10 – 30% [1,2] of cirrhotic hospitalized patients and is associated with a mortality rate of 41% [3]. Quick diagnosis and adequate treatment measures can definitely improve the prognosis of these patients.

Pre-existing hemodynamic derangement in cirrhosis along with SBP can lead to excessive release of pro-inflammatory cytokines. This will result in various complications such as Acute on Chronic Liver Failure(ACLF), multiple organ failure and death [4].

SBP is diagnosed by an absolute neutrophil count in ascitic fluid ≥250/cu mm in the absence of an intra-abdominal surgically treatable source of infection [2]. SBP can occur due to various pathological causes. Translocation of bacteria from intestinal lumen to mesenteric lymph nodes is suggested as one of the common mechanism [5]. Due to this reason, gram negative bacterium like *Enterobacteriaceae* were considered as the major causative organism for SBP thereby guiding empirical antibiotic treatment [1,5,6].

There has been a shift in SBP microbial patterns towards an increasing incidence of gram positive bacteria over the last few years [7-12]. In some recent studies, gram positive bacteria are isolated in half of culture positive SBP cases [3,13].

One of the major problem in SBP treatment is the emergence of multidrug resistant bacteria (MDR), especially in hospitalized patients [14-16]. MDR bacteria are defined as those with an acquired resistance to at least one agent in 3 or more antibiotic classes [17]. First line treatment in patients with SBP were third generation cephalosporins previously. In some studies, resistance rates of third generation cephalosporins in nosocomial SBP were around 30 – 40% [13]. According to studies done by Lutz et al[13], piperacillin/tazobactam for empirical treatment of healthcare related SBP and combination of carbapenems and glycopeptide for nosocomial SBP were suggested.

To tackle the problem, bacteriological profile and antibiotic resistance pattern of SBP should be studied and documented. There is no recent data available regarding antibiotic resistance patterns in cirrhotic patients with SBP in Kerala. Hence our aim was to evaluate the antibiotic resistance patterns in culture positive SBP patients for planning newer strategies in antibiotic policies.

Objectives

1. To determine the bacteriological profile in culture positive SBP patients.
2. To describe the drug resistance patterns of these SBP patients.
3. To compare the bacteriological profile and drug resistance patterns in community onset and hospital onset groups

MATERIALS AND METHODS

A retrospective cross- sectional study was done for a period of 1 year (June 2021 – June 2022) at Department of Gastroenterology, Pushpagiri institute of medical science and research center, a tertiary care hospital in Southern Kerala. A total of 32 adult patients with liver cirrhosis diagnosed as SBP and who are culture positive were selected after taking informed consent from them. The data was further collected based on electronic medical records. As it was a retrospective data analysis, ethical committee clearance was not needed according to the institution protocol. Patients with secondary intra-abdominal infections, culture-negative SBP, a history of peritoneal dialysis and patients who are HIV positive were excluded. SBP diagnosis was based on a neutrophil count in ascitic fluid ≥250/mm^3.The patients were grouped into community-onset and hospital-onset based on timing of diagnosis of SBP (within 48 hours and >48 hours) and underwent ascitic fluid paracentesis. 5ml of ascitic fluid was inoculated into automated blood culture bottle (BacT/ALERT) containing Tripticase soy broth and was sent for culture and sensitivity

immediately. In the microbiology lab it is loaded into the BacT/ALERT machine. Whenever positive signal for the presence of organism is indicated, the blood culture bottle is subcultured and the organism is identified in a fully automated system Vitek-2. The antibiotic sensitivity testing of the organism is done is also done in Vitek-2 using CLSI-2020 guidelines. Bacteriological profile and antibiotic resistance pattern were compared between the two groups. The study was approved by the institutional ethical committee in the tertiary care hospital.

Statistical Analysis

Categorical variables were presented as proportions and continuous variables as means (±SD) if normally distributed or as median (IQR) if they had a non-normal distribution. Data obtained was grouped and studied using Chi Square test and analysis was done using IBM-SPSS, $p<0.05$ was taken as significant level.

RESULTS

The study cohort comprised 27 males and 5 females. The mean age of the patients was 57.3 years. Alcoholic liver disease was found to be most frequent etiology of cirrhosis in 71.9% of patients. Most of the patients were in advanced liver disease, Child-Pugh class C (81%) and Child-Pugh class B (18.8%). The median Model for end stage liver disease score was 22. Most common symptoms were abdominal pain (40.6%), altered sensorium (31.3%) and fever (28.1%). Among this 32 patients, 8 patients (25%) were receiving antibiotic prophylaxis for SBP at the time of admission. Among this 32 patients, 8 patients (25%) had previous episodes of SBP and 24 patients (75%) had history of hospitalization and taken antibiotics treatment within preceding 3 months.

Figure 1 shows the overall bacteriological profile in SBP patients obtained in this study. Table 1 shows the comparison of bacterial profile between community onset and hospital onset groups. Out of 32 ascitic fluid samples, Gram negative bacteria constitutes 78.1% of cases and Gram positive bacteria constitutes 21.8%. Multi drug resistant (MDR) bacteria constitutes about 28.1% of the total bacterial isolates obtained in this study. All the MDR bacterial isolates obtained were Gram Negative. Most common pathogen was *E. coli* (56.2%) followed by *Klebsiella pneumoniae* (15.6%) and *Burkholderia cepacia* (3.1%). Five patients (27.7%) had MDR *E. coli* (4 ESBL producing and 1 Carbapenemase-producing *E. coli*). Among Gram positive bacteria, *Streptococcus spp* (9.4%) and *Enterococcus spp* (9.4%) were found in equal numbers. Out of the 3 *Streptococcus spp* isolated 2 were *Streptococcus agalactiae*. MDR bacteria and Gram positive bacteria were more common in Hospital onset group (45.5%, 36.4%, p=0.03).

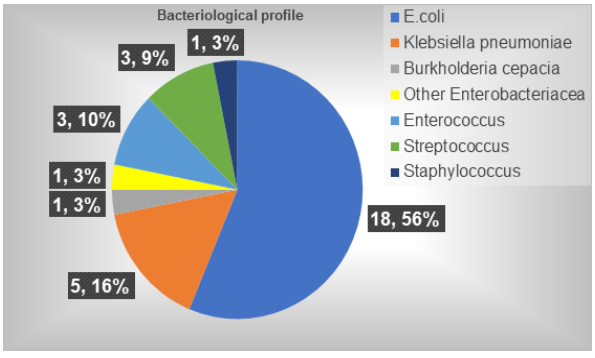

Figure I: Bacteriological Profile In SBP Patients

Table I: Bacteriological Profile In Community Onset And Hospital-onset SBP

Bacteria	Community onset (n=21)	Hospital onset (n=11)	Total (n=32)
E. coli	14(66.7%)	4(36.4%)	18(56.3%)
Klebsiella pneumoniae	3(14.3%)	2(18.2%)	5(15.6%)
Burkholderia cepacia	-	1(9.1%)	1(3.1%)
Other Enterobacteriacea (Enterobacter cloacae)	1(4.8%)	-	1(3.1%)
Enterococcus faecium	1(4.8%)	2(18.2%)	3(9.4%)
Streptococcus spp	1(4.8%)	2(18.2%)	3(9.4%)
Staphylococcus aureus	1(4.8%)	-	1(3.1%)

Table II shows the antibiotic resistance pattern in community onset and hospital onset SBP. Resistance to third generation cephalosporins, quinolones, piperacillin/ tazobactam and carbapenems were found in 53%, 62.5%, 28.1% and 21.8% of cases respectively. Except for third generation cephalosporins (45.5% vs.57.1%), resistance to quinolones (63.6% vs 61.9%), piperacillin/tazobactam (45.5% vs 19%) and carbapenems (36.4% vs 14.3%) were more frequently found in Hospital onset group. The mean length of stay in hospital was 8.2 days and 12 patients (37.5%) died during the period.

Table II: Antibiotic Resistance Pattern In Community Onset And Hospital-onset SBP

Antibiotic Resistance	Total(n=32)	Community acquired(n=21)	Nosocomial (n=11)	P value
Third generation cephalosporins	17(53.1%)	12(57.1%)	5(45.5%)	0.529
Quinolones	20(62.5%)	13(61.9%)	7(63.6%)	0.923
Piperacillin/ tazobactam	9(28.1%)	4(19%)	5(45.5%)	0.115
Carbapenems	7(21.9%)	3(14.3%)	4(36.4%)	0.151
Penicillin	3(9.4%)	1(4.8%)	2(18.2%)	0.216
Vancomycin	No resistance	No resistance	No resistance	Nil
Linezolid	1(3.1%)	1(4.8%)	No resistance	0.462
MDR	9(28.1%)	4(19%)	5(45.5%)	0.115

DISCUSSION

In this study, we evaluated the bacteriological profile and antibiotic resistance patterns in a cohort of liver cirrhotic patients presenting with culture-positive SBP in a tertiary care hospital. Majority of these patients were males and Alcoholic Liver disease was found to be the major etiology for cirrhosis. Most of our patients presented with advanced liver disease (Child-Pugh class C) which was comparable to other studies[18,19].

In our study, gram-positive bacteria constitutes 21.9% in ascitic fluid cultures. However, our study proved that *E.coli* still represents the most common microorganism in culture-positive SBP (56.2%). *E. coli* was isolated in ascitic fluid cultures varying between 22.9 and 40.7% in some recent studies[19,20] which is similar to our study. MDR bacteria were present in 28.1% of patients of which all were gram negative. A high thirty day mortality rate[20] were usually observed in SBP patients infected with MDR bacteria. In our study there were five patients with MDR *E.coli* of which four were ESBL producing and one was Carbapenemase producing bacteria. Rate of ESBL *E. coli* detection varied from 10.5 to 61.5 % in some studies[19,21].

Klebsiella pneumoniae was the second most common bacteria in our study (15.6% of total bacterial isolates obtained). Out of the five *Klebsiella pneumoniae* isolated here three were MDR. Four *Klebsiella pneumoniae* isolates obtained here were ESBL producers and one was Carbapenemase producer. The detection rate of ESBL *Klebsiella pneumoniae* was 20.7% in studies by Li et al[19].In contrast to our study, all *Klebsiella pneumoniae* strains were ESBL-positive as reported by Guo et al in their study[21]. In our study, MDR bacteria (45.5%, p=0.03) and Gram positive bacteria (36.4%, p=0.02) were more common in Hospital onset group.

Drug resistance rates to third generation cephalosporins for SBP pathogens were around 30-40%[13,22] over the years. This is very alarming since the first-line empirical antibiotic therapy for SBP were third generation cephalosporins. Another important finding in our study was the resistance rate of Piperacillin/tazobactam (28.1%) and Carbapenems (21.8%) which was higher than the values reported in other series. The carbapenem resistance rate was 8% in study done by Lutz et al[13]. In contrast to our study, carbapenem resistance were not seen among gram negative microorganisms in studies conducted by Al-Ghamdi et al[18] and Guo et al[21]. Carbapenem resistance can be an alarming feature for the prognosis of SBP patients with a reduced 30-day survival rate[20].

In our study, drug resistance was not seen with vancomycin. Hence vancomycin can be used for gram positive infections due to its low resistance as seen in other studies[20,21].

When you compare the microbes of hospital onset and community onset SBP episodes, there was a significant difference between these 2 groups regarding *Streptococcus spp.* and *Staphylococcus spp.* While *Streptococcus spp*. were significantly more common in hospital onset

SBP (18.2 vs 4.8%), *Staphylococcus aureus* was isolated in community onset SBP (4.8%). *Streptococcus spp*. occurred significantly more often in community onset SBP according to a study by Friedrich et al[22] which was different from our study. This disparity may be due to the small sample size in our study. *Enterococcus faecium* was seen more in hospital onset infection (18.2 vs 4.8 %) in our study. According to Li et al[16], *Enterobacteriaceae* were more common in community onset SBP patients while *Enterococcus spp* were more common in hospital onset SBP patients .

In our study, 25% of patients were receiving antibiotic prophylaxis with Norfloxacin for SBP at the time of admission which was almost similar to other studies [3]. MDR bacteria were more common in nosocomial onset group and patient receiving antibiotic prophylaxis. Among the 32 patients, 25% of them had previous episodes of SBP and 75% patients had history of hospitalization and taken antibiotics treatment within preceding 3 months. The in hospital mortality rate was found to be 37.5%. Since these patients were having other infections like urinary tract infections, skin and soft tissue infections and pneumonia, we have not done the analysis linking mortality to infection with MDR organisms.

There were some limitations for the current study. Firstly, our study was a single center analysis, so the results could not be generalised. Secondly, there is a chance for selection bias as this is a retrospective study so there may be some missing data. Another limitation of our study is the small sample size which can lead to disparity in results while comparing with other studies. Hence further studies are needed to determine the epidemiology and bacterial resistance in SBP cases so that more efficacious antibiotic therapy can be provided.

CONCLUSION

Although most of the pathogens causing SBP were Gram negative, our results showed gram positive organisms in 22% of cases. One third of the bacteria isolated were MDR. MDR organisms were more common among nosocomial onset groups and those patients receiveing antibiotic prophylaxis. Resistance to third generation cephalosporins and quinolones, which are used for empirical antibiotic treatment and prophylaxis of SBP were high. Hence new treatment protocols for SBP should be implemented and antibiotic use according to local antibiogram should be strictly followed.

Statement Of Ethics

The authors have no ethical conflicts to disclose. The research was conducted ethically in accordance with the World medical association declaration of Helsinki.

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Conflict Of Interest

The authors have no conflict of interests to declare.

Credit Authorship Contribution Statement

All of the authors made significant contributions to this paper regarding the design of research protocol, data analysis, writing and critical review in such a way that they participated sufficiently in the work to assume responsibility for the content

List Of Abbreviations

SBP – Spontaneous bacterial peritonitis
MDR – Multidrug resistant
HIV – Human immunodeficiency virus
ESBL – Extended spectrum betalactamase
TGC – Third generation cephalosporins

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