ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 49. 23
PubMed - National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 4, Issue – 1, February - 2019, Page No. : 12 - 17

Bacteriological Profile and their Antibiotics Sensitivity of Ascitic Fluid in Patients of Cirrhosis in a Tertiary Care Hospital Patna, Bihar

[1]Dr. Wasim Ahmad, Senior Resident, Department of Microbiology, IGIMS, Patna, Bihar
[2]Dr. Vidyut Prakash, Senior Resident, Department of Microbiology, IGIMS, Patna, Bihar
[3]Dr. Namrata Kumari, Professor, Department of Microbiology, IGIMS, Patna, Bihar
[4]Dr. Ashish Kumar Jha, Associate Professor, Department of Gastroenterology, IGIMS, Patna, Bihar
Corresponding Author: Dr. Vidyut Prakash, Senior Resident, Department of Microbiology, IGIMS, Patna, Bihar

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

It was a hospital based cross sectional study, in which we identified the aerobic bacterial pathogens and their antimicrobial sensitivity pattern from the suspected ascitic fluid infection of the liver cirrhosis patients admitted to IGIMS Patna. On the basis of culture and common laboratory identification technique, 15.6 % of ascitic fluid showed positive culture result. Among these isolates, commonest was *Escherichia coli* (29.03%) followed by *Pseudomonas aeruginosa*(24.19%), *Acinetobacter spps* (1 1.29 %), *Citrobacter spps* (8.10 %), Staphylococcus aureus (9.67 %), *Klebsiella spps* (6.45 %), *Enterococcus spps* (4.83%) ,CONS (4.83 %) and *Enterobacter aerogenes* (1.61%). Gram negative isolates showed higher sensitivity to colistin,tigecyclin and were moderately sensitive to carbapenems. Majority of the gram positive organisms were sensitive to vancomycin, linezolid and moderate susceptibility was seen to aminoglycosides. Low sensitivity was seen to cotrimoxazole, ampicillin and Fluoroquinolones. Early diagnosis and treatment of ascitic fluid infection in cirrhotic patient leads to decreased mortality/morbidity and economic burden of the patient.

Keywords: Ascitic fluid, Cirrhosis, Antimicrobial sensitivity, aerobic bacteria.

Introduction

Chronic liver disease (CLD) occurs irrespective of age, sex, region or race throughout the world. Chronic liver diseases cause significant morbidity and mortality worldwide. There are many etiological factors lead to a similar clinicopathological syndrome in CLDs, although the rates of progression and clinical course may be different [1]. Mortality data is often used to assess the disease burden and there had been a 46% increase in CLD mortality in the world between 1980 to 2013, underscoring the emerging public health importance of CLD. Most of this increase in CLD mortality has been reported from the low and low-middle income (LMIC) countries of Asia and Africa [2].

Cirrhosis is the end result of a variety of liver diseases, which is characterized by fibrosis and distortion of the architecture of the liver with the formation of regenerative nodules. Cirrhosis can have varied clinical manifestations and complications. According to WHO, about 46% of global diseases and 59% of the mortality is because of chronic diseases and almost 35 million people in the world

die of chronic diseases [3]. Rates of incidence of liver disease are steadily increasing over the years. According to National statistics in the UK, liver diseases have been ranked as the fifth most common cause of death [4]. Liver diseases are recognized as the second leading cause of mortality amongst all digestive diseases in the US [5].

Ascites is the most common complication of cirrhosis of the liver and indicate the presence of liver degeneration & hepatic decompensation [6]. Patients with cirrhosis have increased the risk of developing bacterial infections. Ascitic fluid infection can be classified into five categories based on PMN count, ascitic fluid culture and a surgical cause of infection.

They are culture negative neutrocytic ascites (CNNA), monomicrobial non-neutrocytic bacterascites (MNB), polymicrobial bacteriascites, secondary bacterial peritonitis and spontaneous bacterial peritonitis (SBP) [7]. Bacterascites (BA) is defined as ascitic fluid leukocyte count <250/mm3 with positive blood culture and Culture-negative neutrocytic ascites (CNNA) is defined as ascitic fluid leukocyte count ≥500/mm^3 or neutrophil count ≥250/ mm^3 with negative culture.

Among ascitic fluid infections, SBP is the most common & one of the leading cause of morbidity & mortality. Escherichia coli, Streptococcus spp. and Klebsiella pneumoniae cause most episodes of spontaneous bacterial peritonitis. MNB is seen in patients who are not receiving selective intestinal decontamination. CNNA is culture negative while polymicrobial bacterascites by definition, polymicrobial. Anaerobes are seen in 1% of the infections. The most apparent difference between spontaneous varieties and secondary are that the former are always monomicrobial while the latter are usually polymicrobial [7]. Because of the tremendous variability in presentations and also because such presentations may overlap with other conditions often seen in cirrhosis, prompt diagnosis, and treatment of the ascitic fluid infection may be delayed [8]. Such delays in the initiation of therapy may result in a fatal outcome. Early detection and identification of clinically relevant microorganisms in ascitic fluid culture and determination of antimicrobial susceptibility pattern for appropriate administration of antimicrobial therapy have been shown to reduce mortality and morbidity associated with ascitic fluid infection. Therefore, the present study was undertaken to know the microbiological profile of ascitic fluid infections and assess the current antibiotic susceptibility patterns of isolates from ascitic fluid cultures of patients admitted in Gastroenterology ward and ICUs in a teaching hospital in Patna, Bihar.

Material & Methods

The present study was hospital based cross sectional study carried out in the Department of Microbiology in association with Gastroenterology Department of Indira Gandhi Institute of Medical Science (IGIMS) Patna, Bihar. The total duration of study was 6 months; period extends from January 2018 to June 2018. A total of 398 cases of ascites due to cirrhosis admitted in the medicine wards and ICUs of IGIMS Patna were included in the study. The inclusion criterion was adult patients of both genders with clinical cirrhosis of the liver with ascites. Exclusion criteria were ascites due to renal, cardiac, tubercular, malignant pathology, secondary peritonitis, pregnant women, age less than 15 years and patients who were not willing to participate in the study. Ascitic fluid infections were classified on basis of ascitic fluid culture, PMN count and an evidence of an intra-abdominal source of infection.

Ascitic fluid was collected by bedside tapping in Brain heart infusion (BHI) culture bottle aseptically using standard and universal precautions to ensure that a sterile sample was collected before delivery to the microbiology

laboratory. About 5ml of ascitic fluid samples were subjected to Brain heart infusion (BHI) broth culture bottle and incubated at 37 ^{o}C . BHI broths are checked daily for the presence of growth, which was reflected by the presence of turbidity in broth. Smears were prepared from the positive culture bottles and Gram staining of the smears was done. Simultaneously all the positive bottles were subcultured on blood agar and MacConkey's agar plates. The plates were incubated at 37 ^{o}C for 18-24 hours. Growth was identified by the colony characteristics, Gram staining and the battery of biochemical test. All negative BHI broth bottles were kept for 7 days then discarded.

Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. Antimicrobial susceptibility tests were done on Mueller-Hinton agar (Himedia). The Gram-negative organisms were tested against Gentamicin, Amikacin, Tobramycin, Ciprofloxacin, Amoxycillin+Clavulinic acid, Ceftazidime, Ceftriaxone, Piperacillin + Tazobactam, Imipenem, Tigecycline, and Colistin. The gram-positive organism was tested against penicillin, cefoxitin, Erythromycin Tetracyclin Ciprofloxacin, Cotrimoxazole, Gentamicin, High-level Gentamicin, Clindamycin, Doxycyclin, Amikacin, Vancomycin, and Linezolid. Growth inhibition zone diameters were measured in milliliters and results interpreted as recommended by the Clinical laboratory standards institute (CLSI) guidelines 2018.

Result

Among 398 cases of ascitis due to cirrhosis majority were males (71.6%) (Table 1). Out of the total 398 cases, ascitic fluid infections were seen in 143 (36 %) cases, majority being culture negative neutrocytic bacterascites (CNNA)(57%) followed by spontaneous bacterial peritonitis (SBP)(31%) and monomicrobial nonneutrocytic bacterascites (MNB)(12%) . Out of 398 samples 62(15.6%) ascitic fluid samples were culture positive. Gram-negative organisms were isolated in 50 (81%) while Gram positive organisms in 12 (19%) in ascitic fluid samples. The most common organisms isolated were *Escherichia coli* (29.03%) followed by Pseudomonas spps (24.19%), *Acinetobacter spps* (11.29 %) ,Citrobacter spps (8.10 %), Staphylococcus aureus (9.67 %), *Klebsiella spps* (6.45 %), *Enterococcus spps* (4.83%) ,CONS (4.83 %) and Enterobacter aerogenes (1.61%) [Fig. 1]. Among Gram-negative, all isolates are sensitive to colistin, >90 % were sensitive to tigecycline. They also showed moderate susceptibility to carbapenems, while low susceptibility was seen to amoxicillin/clavulanic acid, piperacillin/tazobactam, cephalosporins, aminoglycosides and fluoroquinolones [Table-2]. Majority of Gram-positive organisms were sensitive to vancomycin and linezolid; moderate susceptibility was seen to aminoglycosides. Low sensitivity was seen to cotrimoxazole, ampicillin and Fluoroquinolones [Table 3]. Out of the Gram-positive isolates, 33% of *S.aureus* and 50% of CONS were cefoxitin resistant, so these isolates considered as methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant CONS (MRCONS) respectively.

Table 1 – Agewise and sexwise distribution of patients

	16-30 years	31-45 years	46-60 years	61-75 years	>75 years	Total
Male	41	71	98	66	9	285
Female	20	31	35	24	3	113
Total	61	102	133	90	12	398

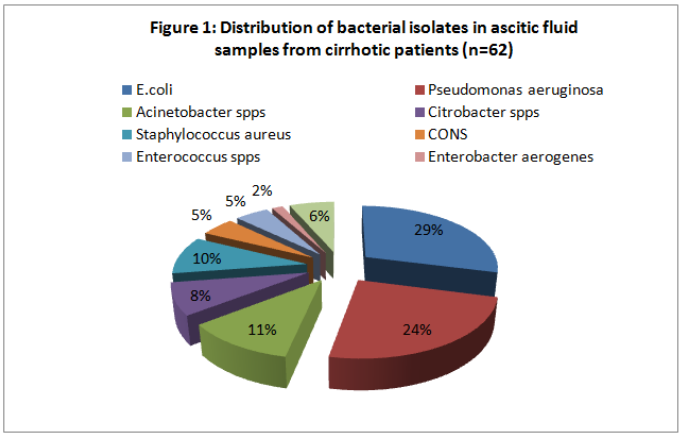

Table 2 – Antibiotics sensitivity percentage of different Gram Negative Bacilli

Antibiotics	*Escherichia coli*	*Pseudomonas spps*	*Acinetobacter spps*	*Citrobacter spps*	*Enterobacter aerogenes*	*Klebsiella spps*
Amikacin	44	60	43	00	00	50
Gentamycin	11	40	28	20	00	25
Tobramycin	33	47	43	40	00	50
Ciprofloxacin	5	20	14	20	00	25
Amoxicillin –clavulinic acid	50	33	43	40	00	50
Ceftriaxone	17	33	28	00	00	25
Ceftazidime	17	40	57	00	00	50
Piperacillin- tazobactum	67	53	42	40	100	50
Imipenem	83	67	43	40	100	75
Tigecyclin	95	NA	86	100	100	100
Colistin	100	100	100	100	100	100

Table 3 - Antibiotics sensitivity percentage of different Gram Positive Cocci

Antibiotics	*Staphylococcus aureus*	*Coagulase negative staphylococcus (CONS)*	*Enterococcus spps*
Ampicillin	50%	67%	33
Cefoxitin	67	50	NA
Erythromycin	50	00	NA
Cotrimoxazole	50	33	NA
Ciprofloxacin	67	67	33
Amikacin	100	100	NA
Gentamycin	66	50	NA
High level gentamycin	NA	NA	67
Vancomycin	100	100	100
Linezolid	100	100	100

NA- Not Applied

Discussion

Bacterial infections are very common in patients with cirrhosis and currently represent one of the most important reasons for the progression of liver failure. In recent years, infections caused by multi-drug resistant bacteria are increasing at an alarming rate thus early identification and management of infection with appropriate antibiotics is very important to reduce the disease burden. In the present study, the majority of the cases were males (81%). High male to female ratio was seen in this study (4.3:1), as alcohol consumption is more common among males in our country which is the most common cause of cirrhosis. A similar but relatively high male to female ratio (8:1) has also been reported in the literature [9]. In our study most of the patient suffering from cirrhosis comes under 46-60 years of age group followed by 31-45 years and 61-75 years, so mean age of patients in our study is about 54 years, which is nearly correlating with the mean age in various studies (51-54 years) [10]. We also concluded that, majority of patients had history of alcoholism as the underlying cause of cirrhosis followed by viral hepatitis. This is consistent with other study [9]. In this study, Gram-negative organisms were more common than Gram-positive organisms. Among the Gram-negative isolates, *E.coli* was most common isolate (29.03%) followed by Pseudomonas spps (24.19%), *Acinetobacter spps* (11.29 %). Similarly, in several other studies, *E.coli* was found the most common cause of ascitic fluid infection [11].

In present study, the gram-negative organisms showed high susceptibility to colistin and tigecycline whereas moderate susceptibility to carbapenems and low susceptibility was seen to amoxicillin/clavulanic acid, piperacillin/tazobactam, cephalosporins, aminoglycosides, and fluoroquinolones. which had contrasting results as compared to another study where susceptibility to aminoglycosides (amikacin) was fairly high (92.3%) [12]. However low susceptibility was seen to cephalosporin, amoxicillin/clavulanic acid and quinolones, which was

comparable to results in other studies done in Mumbai and Egypt [12,13].

Gram-positive organisms in the current study showed the highest susceptibility to linezolid,

Vancomycin, moderate sensitivity to aminoglycosides and low sensitivity to ampicillin and fluoroquinolones. These results correlated with a study done in Tehran where high sensitivity was seen to vancomycin low to trimethoprim/ sulfamethoxazole (Cotrimoxazole) and ampicillin was reported [14]. Implementation of infection control practice and continuous monitoring of ICU/ward acts as preventive measures for development of antibiotics resistance in health care settings.

Conclusion

Infection of ascitic fluid is a fatal complication of patients with chronic liver disease, if not treated it can lead to death. Infection of Gram-negative organisms dominated in ascitic fluid cultures of CLD patients. However, culture-positive cases were low, which may be due to the prior intake of antibiotics and use of conventional culture methods. Therefore, patients should be treated on the basis of PMN ≥250/mm3 in ascitic fluid, which is considered as standard criteria. Most of the isolates were susceptible to Tigecycline & colistin. Early diagnosis and treatment of ascitic fluid infection may result in a high success rate. Thus, laboratory analysis including the culture of ascitic fluid in all suspected patients will help in improving prognosis of the patients.

References

1. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet 2008;371:838–851. pmid:18328931
2. Mokdad AA, Lopez AD, Shahraz S, Lozano R, Mokdad AH, Stanaway J et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. BMC Med 2014;12:145 pmid:25242656
3. Murray CJ, Lopez AD. Evidence-based health policy – lessons from the Burden of Disease Study. *Science* 1996; 274: 740-743. UK national statistics, http://www.statistics.gov.uk
4. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. *Gastroenterology* 2009; 136: 1134 –1144.
5. Bacon BR. Cirrhosis and its complications. In: Longo DL, Fauci AS, Karper DL, Hausen SL, Jameson JL, Loscalzo J editors. Harrison's principles of Internal medicine. 18th ed McGraw Hill New York; 2012:2592-3.
6. Runyon BA. Ascites and spontaneous bacterial peritonitis. In: Feldman M, Friedman LS, Brandt LJ editors. Sleisenger, Fordtran's Gastrointestinal and liver disease Pathophysiology/Diagnosis/Management. 9th ed Saunders Elsevier Philadelphia;2012:1517-25.
7. Hoefs JC, Canawati HN, Sapico FC, Hopkins RR, Weiner J, Montgomerie JZ. Spontaneous bacterial peritonitis. Hepatology. 1982; 2:399-407.
8. Nadagouda SB, Mahesh B, S.V. Kashinakunti, M S Birader. Spontaneous bacterial peritonitis in cirrhosis of liver with ascites-a cross sectional study Int J Biol med Res. 2013;4(2):3143-7.
9. Syed VA, Ansari Ja, Karki P, Regmi M, Khanal B. Spontaneous Bacterial Peritonitis in cirrhotic ascites: A prospective study in a tertiary care hospital, Nepal. Kathmandu Univ Med J.2007;5:48-59.
10. Reginato TJ, Oliveira MJ, Moreira LC, Lamanna A, Acencio MM, Antonangelo L. Characteristics of ascitic fluid from patients with suspected spontaneous bacterial peritonitis in emergency units at a tertiary hospital. Sao Paulo Med J. 2011;129(5):315-9.

11. S Bankar, A De, S Baveja. Study of ascitic fluid for diagnosis of spontaneous bacterial peritonitis (SBP) in adult patients with cirrhosis. IJMAS. 2014;3(1): 1-9.
12. El-Bendary MM, Abdel-Aiz M, EL-Sherbiny Walid A, Farag R, El-Gilany AH, Zaghloul Mohd HE. Spontaneous Bacterial Peritonitis: Clinico-epidemiological and microbiological study. Benha Med J. 2009;26:287-9.
13. Sheikhbahaei S, Abdollahi A, Hafezi-Nejad N, Zare E. Patterns of antimicrobial resistance in the causative organisms of spontaneous bacterial peritonitis: A single centre, six-year experience of 1981 samples. International Journal of Heptology. 2014;2014:917856.