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MICROBIOLOGICAL PROFILE OF ASCITIC FLUID IN PATIENTS OF CIRRHOSIS IN A TERTIARY CARE HOSPITAL IN NORTHERN INDIA

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Abstract: **Background & objectives**: Patients with cirrhosis have increased risk of developing bacterial infections and ascitic fluid infection is its major complication. This study was planned to study the microbiological profile of ascites in patients of cirrhosis. **Material and Methods**: A total of 500 cases of ascites due to cirrhosis (diagnosed on the basis of clinical/ radiological/ endoscopic/ fibro-scan) admitted in gastroenterology ward and ICU were included. Ascitic fluid infections were classified on basis of ascitic fluid culture, PMN count and an evidence of an intra-abdominal source of infection. Samples were processed in automated Bactec or Bac-T /Alert. Further identification & antimicrobial susceptibility testing was done by VITEK-2 system. **Results**: Out of the 500 cases, ascitic fluid infections were seen in 216 (43%) cases, majority being culture negative neutrocytic bacterascites (CNNA)(48%) followed by spontaneous bacterial peritonitis (SBP)(36%) and monomicrobial non-neutrocytic bacterascites (MNB)(16%). 115 (23%) ascitic fluid samples were culture positive. The most common organism isolated were *Escherichia coli* (35%) followed by CONS (17%), *Klebsiella pneumoniae* (14%) and *Enterococcus faecium* (11%). Overall Gram negative organisms were most susceptible to tigecycline (83.33%) and colistin (76.9%) while least susceptible to amoxicillin/clavulanic acid (25.6%) and ciprofloxacin (20.5%). All the Gram positive organisms were sensitive to vancomycin and linezolid. **Interpretation & Conclusion:** Gram negative pathogens are the predominant cause of ascitic fluid infections in cirrhotic patients. Antimicrobial resistance is increasing therefore early detection and determination of antimicrobial susceptibility pattern is important to reduce the mortality and morbidity associated with ascitic fluid infections.

Keywords: Ascitic fluid, culture negative neutrocytic ascites (CNNA), monomicrobial non-neutrocytic bacterascites (MNB), polymicrobial bacteriascites, secondary bacterial peritonitis, spontaneous bacterial peritonitis (SBP)

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INTRODUCTION

Ascites is the most common complication of cirrhosis of liver and indicate the presence of liver degeneration & hepatic decompensation. [1] Patients with cirrhosis have increased 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). [2]

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. [2]

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. [3] Such delays in the initiation of therapy may result in 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 has 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 North India.

MATERIAL & METHODS:

A total of 500 cases of ascites due to cirrhosis admitted in the Gastro wards and ICUs were included 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 samples were processed in Bactec or Bac-T/Alert microbial detection system. Smears were prepared from the positive culture bottles and Gram staining of the smears was done. Simultaneously all the positive bottles were sub cultured on blood agar and MacConkey's agar plates. The plates were incubated at 37^0C for 18-24 hours. All negative bottles were kept for 7 days then discarded. Growth was identified by the colony characteristics, Gram staining and the final

identification and AST was done by the VITEK 2 system. [4] Various drug resistance mechanisms were studied (methicillin resistance, vancomycin resistance, ESBL, Amp-C, MBL/KPC) according to CLSI guidelines. [5] This study was approved by institutional ethical committee.

RESULTS

Among 500 cases of ascites due to cirrhosis majority were males (88%) and most common risk factor was alcohol consumption (57.8%) followed by viral hepatitis (18.6%). Mean age was 52.42 ± 11.6 years. Out of the total 500 cases ascitic fluid infections were seen in 216 (43%) cases, majority being culture negative neutrocytic bacterascites (CNNA)(48%) followed by spontaneous bacterial peritonitis (SBP)(36%) and monomicrobial non-neutrocytic bacterascites (MNB)(16%).

115 ascitic fluid samples were culture positive.Gram negative organisms were isolated in 78 (68%) while Gram positive organisms in 35 (30%) and yeast in 2 (2%) ascitic fluid samples. The most common organisms isolated were *Escherichia coli* (35%) followed by CONS (17%), *Klebsiella pneumoniae* (14%), *Enterococcus faeium* (11%) and *Acinetobacter baumannii* (9%) (Figure 1). Among all Gram negative isolates 83.3% were sensitive to tigecycline, 76.9% sensitive to colistin. It also showed high susceptibility to carbapenems (53.5-56.4%), while low susceptibility was seen to cefoperazone/sulbactam (34.6%), piperacillin/tazobactam (26.9%), cefepime (25.6%) and amoxicillin/clavulanic acid (25.6%) (Figure 2).

All the gram positive organisms were sensitive to linezolid. Majority of Gram positive organisms showed susceptibility to vancomycin (94.2%) and teicoplanin (91.4%). High susceptiblity was seen to daptomycin (86.3%) and rifampicin (72.7%). Low sensitivity was seen to cotrimoxazole (45.5%), clindamycin (42.8%), ciprofloxacin (34.2%) and penicillin(28.5%). Out of the 78 Gram negative isolates, 11 (14%) were confirmed ESBL producers out of which 85% were *E.coli* and 15% were *K.pneumoniae*. Out of the 11 (14%) confirmed AmpC producers 12.5% were *E.coli* and 37.5% were *K.pneumoniae* isolates. However 6.4% were confirmed MBL/KPC producers out of which 40% was *E.coli* and *K.pneumoniae* each and 10% of *A.baumanii* isolates. Out of the Gram positive isolates, 50% of *S.aureus* and 70% of CONS were methicillin resistant. While 15% *Enterococcus faecium* were found resistant to vancomycin. Out of 115 patients who were culture positive, 18 (15.6%) expired and 19 (16.5 %) discharged against medical advice (DAMA). Most common factors leading to mortality were presence of both alcohol consumption and viral hepatitis (14.7%) followed by viral hepatitis (11%).

DISCUSSION

Bacterial infections are very common in patients with cirrhosis and currently represent one of the most important reasons for 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 majority of the cases were males (88%). High male to female ratio was seen in this study (7.3:1) as alcohol consumption is more common among males in our country which is the most common cause of cirrhosis. A similar male to female ratio (8:1) has also been reported in literature. [6] In our study the mean age of the patient suffering from cirrhosis is 52.7 years which is nearly correlating with the mean age in various studies (51-54 years). [7,8] We found that, majority of patients had history of alcoholism (57.8%) as the underlying cause of cirrhosis followed by viral hepatitis (18.6%). This is consistent with other study. [6]

Results of our study depict that SBP was diagnosed in 38% of patients where as its variants, CNNA and MNNB were diagnosed in 46% and 16% of the cases respectively. In a study conducted at Chandka Medical college Larkana, authors found SBP in 34.5% of their patients whereas CNNA and MNB in 62.1% and 3.4% respectively. [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 (35%) followed by *K.pneumoniae* (14%) and *A.baumanii* (9%). Similarly in several other studies *E.coli* was found the most common cause of ascitic fluid infection. [10,11]

In the current study, the gram negative organisms showed high susceptibility to tigecycline (83.33%) and colistin (80%) where as moderate susceptibility to amikacin (62.8%) and carbapenems (49-57%) which had contrasting results as compared to another study where susceptibility to amikacin was fairly high (92.3%) [12]. However low susceptibility was seen to cefepime (25.6%), amoxicillin/clavulanic acid (25%) which was comparable to results in other studies done in Mumbai and Egypt. [12,13]

Gram positive organisms in the current study showed highest susceptibility to linezolid, vancomycin, teicoplanin and moderate to aminoglycosides and tetracyclines and low sensitivity to penicillin and fluoroquinolones. These results correlated with a study done in Tehran where high sensitivity was seen to vancomycin, imipenem and low to cephalosporins, trimethoprim/ sulfamethoxazole and penicillin was reported. [14]

Micro-organisms that are resistant to antibiotics, such as those possessing extended-spectrum beta-lactam (ESBL) are becoming a clinical problem. In our study, the prevalence rate of ESBL producing organisms among *E.coli* and *K.pneumoniae* isolated from ascitic fluid was 22.5% and 12.5% respectively. A prospective study done in Egypt from 2006-07, showed prevalence of ESBL producing organisms among *E.coli* and *K.pneumoniae* to be 18.2% and 16.7% respectively

while in the retrospective period of the same study done (2004-05) it was observed that 8.3% *E.coli* and 12.5% *K.pneumoniae* were ESBL producers. [13]

Amongst the 35 (30%) Gram positive isolates majority were CONS (57%) followed by *E.faecium* (37%) and *S.aureus* (6%). All *Staphylococcus spp*. isolates were susceptible to vancomycin while 15% of *Entercoccus faecium* showed resistance towards vancomycin. Methicillin resistance in CONS and *S.aureus* was 70% and 50% respectively, which was quite high compared to other studies where prevalence of MRSA and VRE was 28% and 31% amongst the isolates. [15]

In our study the prevalence of AmpC & MBL producing *E.coli* and *K.pneumoniae* was 12.5% & 5% and 37.5% & 12.5% respectively.

Outcome was variable. Out of the 500 cases included, almost 9% patients expired which was similar to mortality rate seen in another study (8.5%) [16] while higher mortality rate was reported by Kamani L (12.2%). [17] Preventive measures like continuous surveillance of the wards and ICUs and a strict implementation of infection control practice can go a long way in containing the menace of drug resistance in the heath care setting.

CONCLUSION

Cirrhosis is the end result of many types of chronic liver diseases. Because of the tremendous variability in presentations often seen in cirrhosis, prompt diagnosis and treatment of the ascitic fluid infection may be delayed. Such delays in the initiation of therapy may result in fatal outcome. The magnitude of the problem with respect to antimicrobial-resistant pathogens causing infection is challenging, because the levels of antimicrobial resistance vary for different types of healthcare facilities and from different geographic areas. Thus this study was undertaken, aiming for early detection and identification of clinically relevant micro organisms in ascitic fluid culture and determination of antimicrobial susceptibility pattern for appropriate administration of antimicrobial therapy to reduce mortality and morbidity associated with ascitic fluid infection.

Figure 1: Distribution of isolates in ascitic fluid samples from cirrhotic patients (n=115)

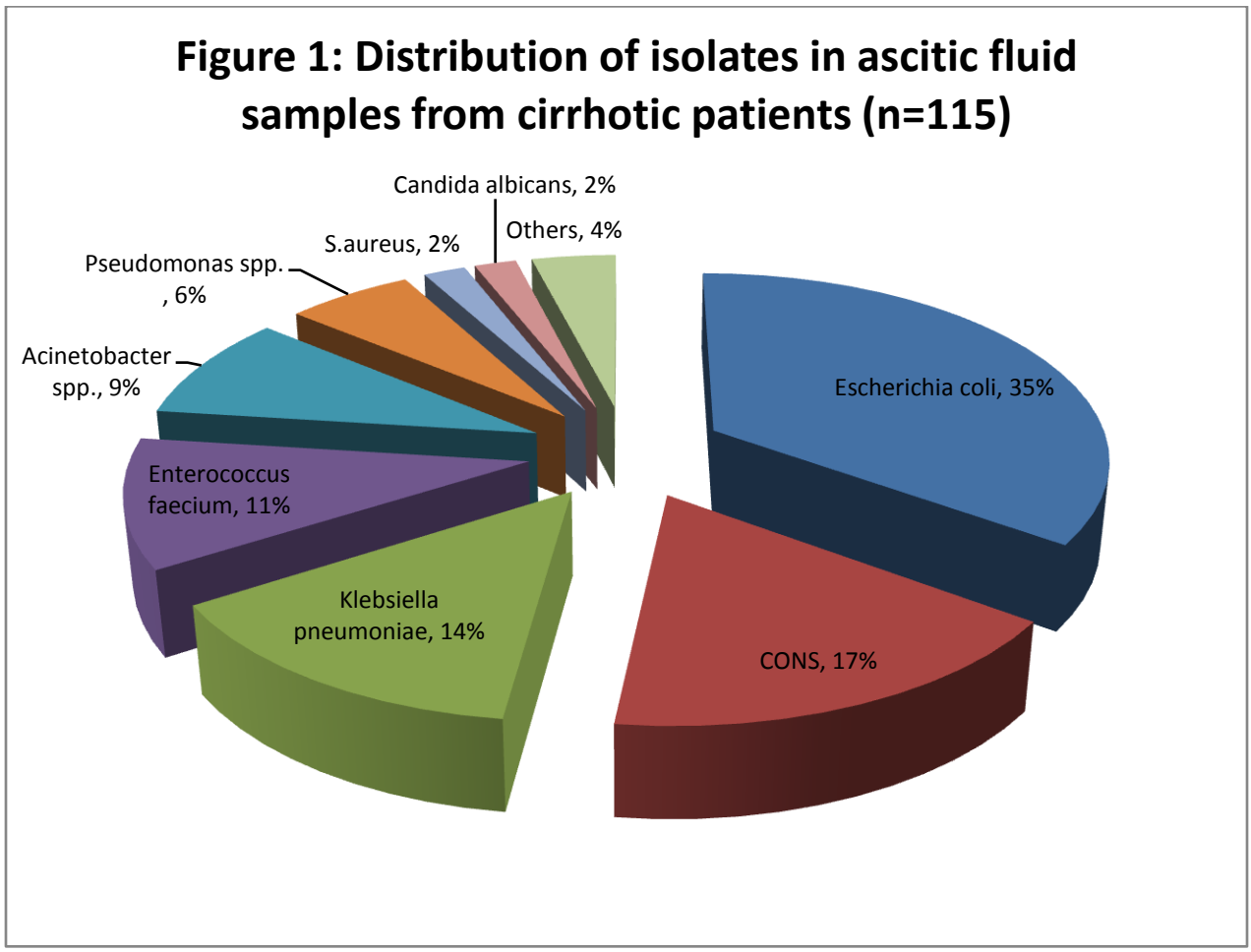


Figure 2: Antimicrobial susceptibility of Gram negative isolates.

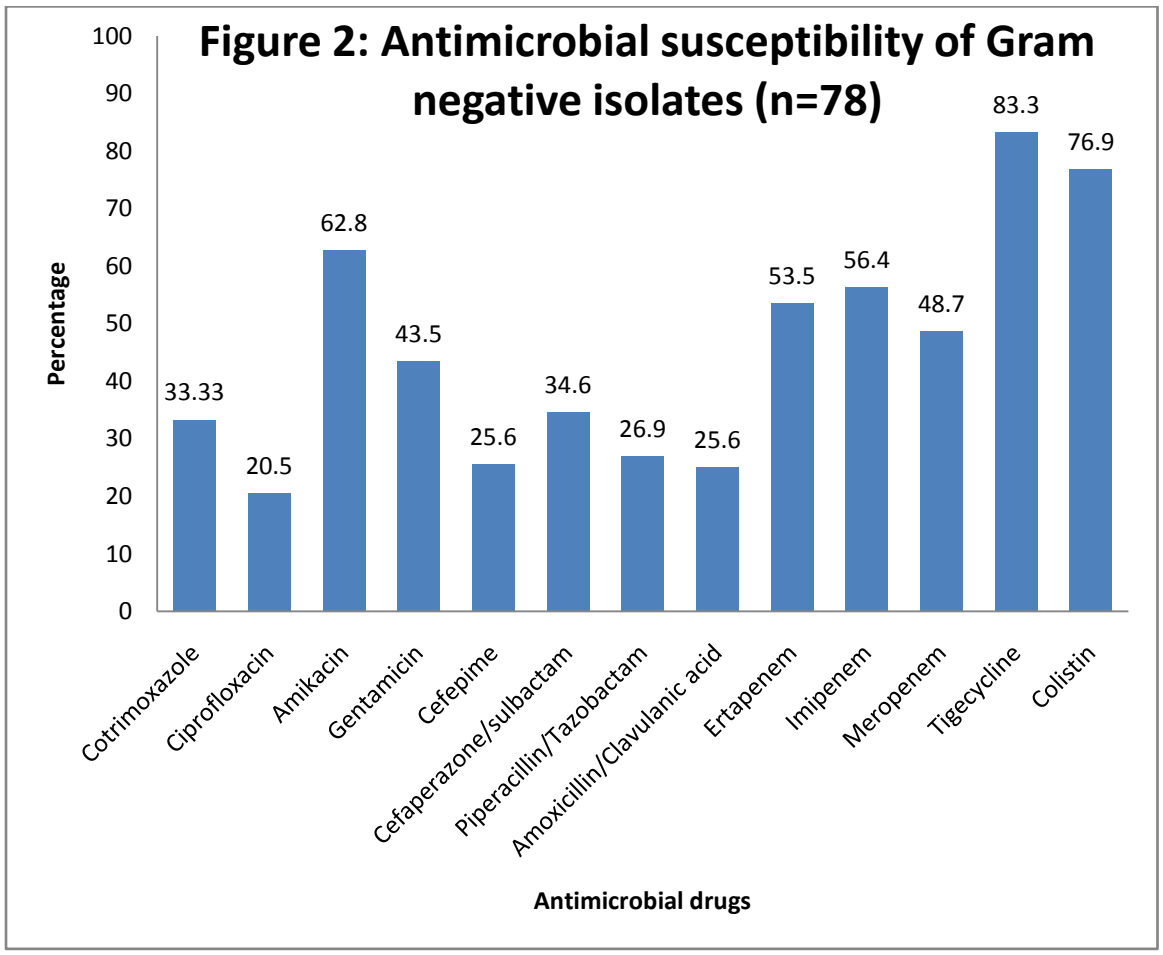
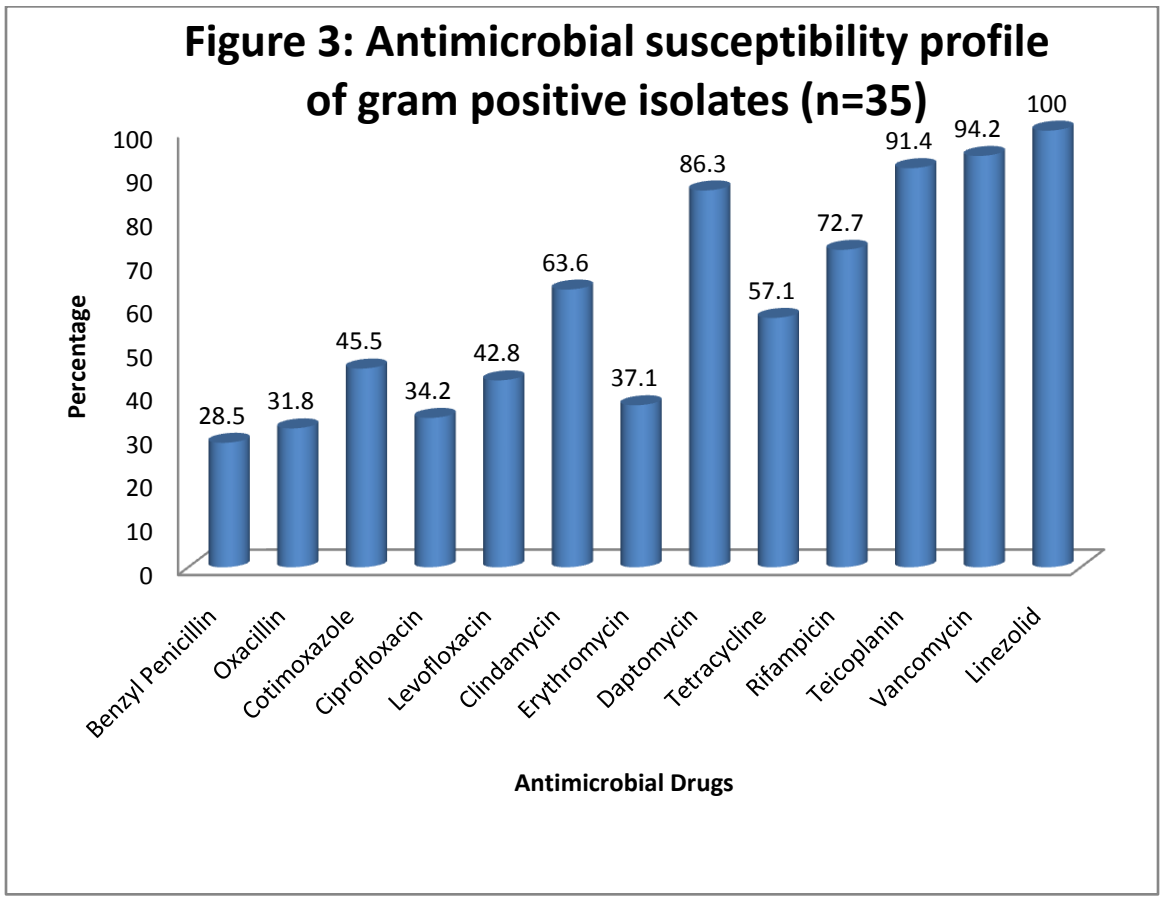


Figure 3: Antimicrobial susceptibility profile of gram positive isolates (n=35)



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