ORIGINAL ARTICLE

ASCITIC FLUID CULTIVATED ORGANISMS AND THEIR ANTIMICROBIAL RESILIENCE PATTERN IN PATIENTS WITH LIVER CIRRHOSIS

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ABSTRACT

Background: Spontaneous bacterial peritoinitis is one of the life threatening complications of Cirrhosis of liver. Mortality and morbidity are high because of sepsis, hepatorenal syndrome and liver failure. International societies recommend the use of 3rd generation Cephalosporin as first line and quinolones and Amox-clav as second line of therapy. Development of resistance among microbials against these antibiotics has been reported during last several years. The purpose of this research is to determine the frequency of micro-organism cultivated in ascitic fluid and pattern of their resistance to antimicrobials at a tertiary care hospital.

Methods: Ascitic fluid samples were received from both in-patients and out-patients in sterile leak proof containers. All micro-organisms isolated from ascitic fluid samples were included in the study. Ascitic fluid samples were inoculated on sheep blood agar, chocolate agar, MacConkey agar, according to standard microbiological protocol. Antimicrobial susceptibility testing was performed on MHA medium (Oxoid Ltd, England) using modified Kirby Bauer's disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: Out of 356 ascitic fluid samples, 54(15.1%) of samples were culture positive. Esherichia coli (38.9%) was the most prevalent pathogen isolated, followed by Staphylococcus aureus(11.1%) and Acinetobacter species(7.4%). Frequency of strains resistant with Cefotaxime (100%), Ciprofloxacin (68.4%) and Amox-clav (57.1%) were remarkably high. Esherichia coli was mostly responsive with Amikacin, Meropenum, Cefoperazone/Sulbatum and Piperacillin/Tazobactum.

Conclusion: Gram –ve bacteria has been remained main prevalent infectious organisms causing Spontaneous Bacterial Peritonitis. A high resistance pattern with Cephalosporins and Quinolones is frightening as these drugs have been considered as first line therapy in the management of Spontaneous Bacterial Peritonitis. Resistance profile is better with Amikacin, Meropenem, Cefoperazone/sulbactum and Piperacillin/Tazobactum.

KEYWORDS: Cultivated Organisms, Antimicrobial Resilience Pattern, Ascites, Liver Cirrhosis

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INTRODUCTION

Ascites is abnormal collection of fluid within the peritoneal cavity. It is the most frequent complication of Portal hypertension secondary to liver cirrhosis.[1,2]About 85% of cases with ascites are secondary to cirrhosis of liver and 10% are secondary to malignancies.[3,4]

One of the life threatening complication of Cirrhosis of liver and ascites is Spontaneous Bacterial Peritonitis (SBP), which has an incidence of 7 - 30% per

year.[5] Symptoms are vague and highly non-specific. Mortality is high and may reach up to 40% owing to sepsis, hepatorenal syndrome and liver failure.[6]Also there is a poor prognosis associated with it. Once patient develop SBP, mortality may reach up to 70% at 1 year.[7] Early identification of SBP and treatment may cause remarkable reduction in mortality and morbidity.[8]

SBP is classically diagnosed on the basis of positive ascitic fluid culture and high neutrophilic counts of more than 250/cmm in the ascitic fluid.[8] Based on these counts and culture analysis, there are two variants of SBP i.e. Culture negative neutrocytic ascites (CNAA) and Bacterascites (BA). CNAA is ascites with high neutrophilic count (i.e. more than 250/cmm) but there is no growth on culture medium, while BA is culture positive ascites with neutrophilic count of less than 250/cmm.[9]

Impaired humoral and cellular immune responses allows translocation of bacteria from intestine into ascitic fluid cause SBP.[9] This is the reason most cases of SBP are secondary to infection from gram negative aerobic family of Enterobacteriaceae. Second most common bacterial pathogen which is isolated from asctic fluid is non enterococcal streptococcus species particularly Streptococcus Pneumoniae.[10] In recent studies SBP caused by gram positive organisms have been reported.[11,12]

European Association of Study of Liver disease (EASL) and some other international liver societies recommend the use of 3rd generation Cephalosporin as first line therapy for SBP and quinolones and Amox-clav as second line.[8,13] But the resistance with antibiotics specially with 3rd generation cephalosporins and quinolones have been increasingly reported during the last several years.[14,15] The mortality and morbidity increases significantly when this first line therapy fails. Therefore, for effective treatment one should be familiar with local epidemiological pattern of antibiotic resistance.[16]

In order to identify the best possible antimicrobials in our population we conducted this study with the aim to identify the distribution of cultivated micro-organism in ascitic fluid and pattern of their resilience with antimicrobials.

METHODS

This observational study was conducted over a period of two and half years from December 2015 to March 2018 at the Department of Gastroenterology and the Department of Clinical Microbiology of Ziauddin University Hospital Karachi.

Patients who had liver cirrhosis and ascities clinically or on the basis of ultrasound were included after taking written consent from them or any of their relative. Patients with any other etiology of ascites like secondary to tuberculosis or intra-abdominal source of infection, those who were taking antibiotics already, those who had growth of yeast in their ascitic fluid sample and those who did not give consent to get involved in the study were excluded. Diagnostic paracentesis was done either at bed side or under ultrasound guidance using all standard protocols for all participants of the study. 10-20 cc of ascitic fluid was collected from each patient and sent to laboratory in either sterile leak proof containers or in sterile syringes. The fluid analysis included cell count with differentials, cultures and antimicrobial susceptibility pattern. All microorganisms isolated from ascitic fluid samples were included in the study.

Ascitic fluid samples were inoculated on sheep blood agar, chocolate agar, MacConkey agar, according to standard microbiological protocol.17These plates were incubated at 37°C aerobically for 24 to 48 hours. The primary sample was also inoculated in Robertson cooked medium and incubated at ambient air with temperature of 33-37 ◦ C for 24 hours. After 24 hours of incubation the samples from Robertson cooked medium were inoculated on anaerobic sheep blood agar and incubated for 48 hours with a temperature of 33-37◦C in an anaerobic environment. After incubation plates were examined for colonial growth. The initial identification was performed by aid of gram stains and biochemical tests. Antimicrobial susceptibility testing was performed on MHA medium (Oxoid Ltd, England) using modified Kirby Bauer's disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[18]Esherichia coli American Type Culture Collection (ATCC®) 25922 was used as control.

Data analysis was performed by using SPSS version-20. Frequency and percentages were computed for presentation of all categorical variables like micro-organisms, sex, and antimicrobial sensitivities. Mean and standard deviation was calculated for quantitative variables like age of patients.

RESULTS

Three hundred and fifty six (356) ascitic fluid samples of in and out patients were processed for culture and antimicrobials susceptibilities during the study period. From those samples a total of 54(15.1%) clinical isolates of different micro-organisms cultivated. Mean age of patients with positive ascitic fluid culture was 48.6 (+43.6) years. Predominantly isolates were from female patients 29/54(53.7%), while isolates for male patients were 25/54(46.29%). Male to female ratio was 1:1.16. There was marked preponderance towards gram negative organisms that were 35/54 (64.8%), while gram positive organisms cultivated in 12/54 (22.2%) of samples. Seven

samples out of fifty-four (12.9%) showed growth of coagulase negative Staphylococci, which were considered as probable skin contaminants. The most commonly cultivated organism was Esherichia Coli (E.Coli) i.e. 21/54 (38.9%). Table 1 represents different micro-organisms and their frequency isolated from ascitic fluid samples.

TABLE 1: FREQUENCY OF CULTIVATED MICRO-ORGANISMS FROM ASCITIC FLUID

	FREQUENCY	PERCENT
ESCHERICHIA COLI	21	38.9
ACINETOBACTER SPECIES	4	7.4
ENTEROCOCCUS SPECIES	3	5.6
COAGULASE NEGATIVE STAPHYLOCOCCI	7	13.0
AEROMONAS SPECIES	1	1.9
PSEUDOMONAS AUREGINOSA	2	3.7
KLEBSIELLA SPECIES	3	5.6
STRPTOCOCCUS GROUP D	2	3.7
STAPHYLOCOCCUS AUREUS	6	11.1
ENTEROBACTER SPECIES	3	5.6
GRAM POSITIVE	1	1.9
PSEUDOMONA STUTZERI	1	1.9
TOTAL	54	100.0

The pattern of resistance with commonly used antimicrobials for gram negative and gram positive micro-organisms is shown in Table 2 and Table 3, respectively, which shows significantly higher rates of resistance with first line and second line antimicrobials i.e. Cefotaxime, Cefixime, Ciprofloxacin and Ofloxacin. While resistance level was quite low with Amikacin, Meropenem, and Cefoperazone/-sulbactum in case of gram –ve organism and with Linezolid and Vancomycin and Tiecoplannin against gram +ve organisms.

TABLE 2: RESISTANCE PATTERN OF COMMON GM –VE ORGANISMS WITH COMMONLY USED ANTIMICROBIALS

		MICRO ORGANISM 1				
		ESCHERICHIA COLI	ACINETOBACTER SPECIES	PSEUDOMONAS AUREGINOSA	KLEBSIELLA SPECIES	ENTEROBACTER SPECIES
		COLUMN N %	COLUMN N %	COLUMN N %	COLUMN N %	COLUMN N %
	RESISTANT	9.5%	75.0%	50.0%	0.0%	0.0%
AMIKACIN	SENSITIVE	90.5%	25.0%	50.0%	100.0%	100.0%
	RESISTANT	57.1%	-	-	66.7%	-
AMOX-CLAV	SENSITIVE	42.9%	-	-	33.3%	-
	RESISTANT	100.0%	-	100.0%	100.0%	100.0%
AZTRONEM	SENSITIVE	0.0%	-	0.0%	0.0%	0.0%
	RESISTANT	27.8%	100.0%	50.0%	0.0%	0.0%
CEF/SUL	SENSITIVE	72.2%	0.0%	50.0%	100.0%	100.0%
	RESISTANT	100.0%	-	-	100.0%	66.7%
CEFIXIME	SENSITIVE	0.0%	-	-	0.0%	33.3%
	RESISTANT	100.0%	-	-	100.0%	66.7%
CEFOTA XIME	SENSITIVE	0.0%	-	-	0.0%	33.3%
	RESISTANT	100.0%	100.0%	-	100.0%	66.7%
CEFTRIOXONE	SENSITIVE	0.0%	0.0%	-	0.0%	33.3%
	RESISTANT	85.7%	100.0%	-	100.0%	33.3%
CO-TRIMOXAZOLE	SENSITIVE	14.3%	0.0%	-	0.0%	66.7%
	RESISTANT	61.9%	100.0%	50.0%	0.0%	0.0%
GENTAMYCIN	SENSITIVE	38.1%	0.0%	50.0%	100.0%	100.0%
	RESISTANT	19.0%	100.0%	50.0%	0.0%	0.0%
MEROPENM	SENSITIVE	81.0%	0.0%	50.0%	100.0%	100.0%
	RESISTANT	68.4%	100%	50.0%	0%	0%
OFLOXACIN	SENSITIVE	36.6%	0%	50.0%	100%	100%
	RESISTANT	19.0%	100.0%	50.0%	0.0%	-
IMIPENEM	SENSITIVE	81.0%	0.0%	50.0%	100.0%	-
	RESISTANT	33.3%	100.0%	50.0%	0.0%	0.0%
TAZO/PIPERA	SENSITIVE	66.7%	0.0%	50.0%	100.0%	100.0%

TABLE 3: RESISTANCE PATTERN OF COMMON GM +VE ORGANISM WITH COMMONLY USED ANTIMICROBIALS

		MICRO ORGANISM 1			
		ENTEROCOCCUS SPECIES	STRPTOCOCCUS	STAPHYLOCOCCUS AUREUS	GRAM POSITIVE ANAEROBIC BACILLI
		COLUMN N %	COLUMN N %	COLUMN N %	COLUMN N %
CLINDAMYCIN	SENSITIVE	-	-	83.3%	100.0%
	RESISTANT	-	-	16.7%	0.0%
ERYTHROMYCIN	SENSITIVE	0.0%	0.0%	33.3%	-
	RESISTANT	100.0%	100.0%	66.7%	-
GENTAMYCIN	SENSITIVE	-	-	83.3%	-
	RESISTANT	-	-	16.7%	-
LEVOFLOXACIN	SENSITIVE	33.3%	50.0%	16.7%	-
	RESISTANT	66.7%	50.0%	83.3%	-
LINEZOLID	SENSITIVE	100.0%	100.0%	100.0%	-
	RESISTANT	0.0%	0.0%	0.0%	-
TEICOPLANIN	SENSITIVE	66.7%	100.0%	100.0%	
	RESISTANT	33.3%	0.0%	0.0%	
VANCOMYCIN	SENSITIVE	66.7%	100.0%	100.0%	100.0%
	RESISTANT	33.3%	0.0%	0.0%	0.0%

Fig 1 and Fig 2 show graphically the combined sensitivity of all Gm +ve organisms and all Gm –ve organisms against applied antimicrobials. Higher sensitivity of gram +ve organisms against Linezolid (100%), Vancomycin (92%) and Teicoplannin (91%) can be observed. While gram-ve organisms has shown a superior sensitivity against Amikacin (82%), Meropenem (73%) and Cefaerazone/Sulbactum (67%).

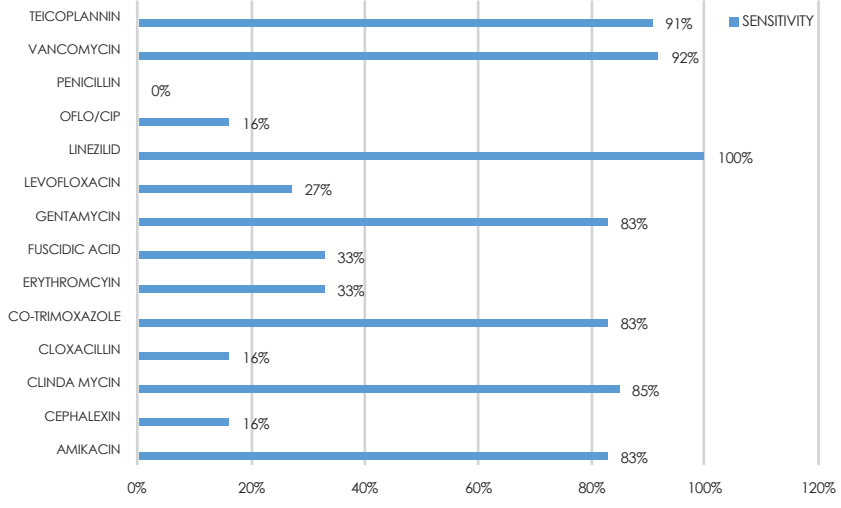

Figure 1: Antimicrobial sensitivity pattern of all Gm +ve organism

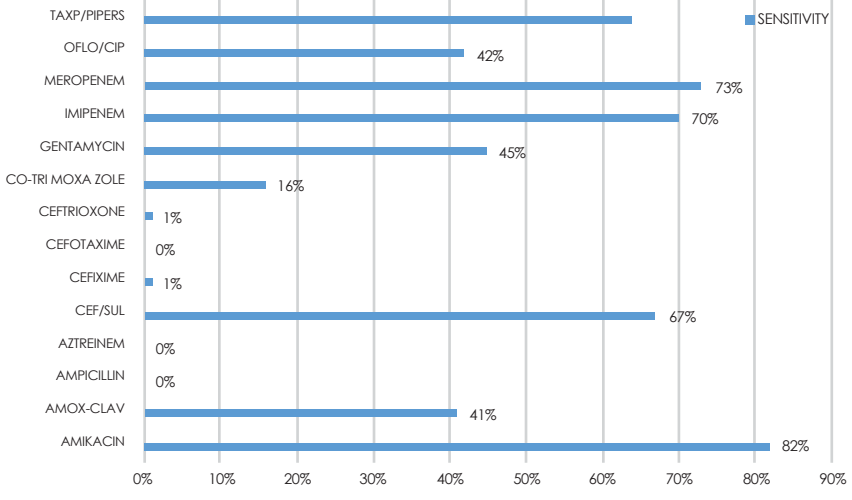

Figure 2: Antimicrobial Sensitivity pattern of all Gm –ve organisms

DISCUSSION

One of the important and grave complications of Liver Cirrhosis and ascities is SBP. As it has high mortality and likelihood of deterioration is higher, early identification of patient is crucial for prognostic improvement.[19]Clinical decisions are also impacted by the recognition of culprit micro-organism cultivated. Timely selection of antimicrobial which ensure sufficient coverage is critical in management of SBP.

There is an obvious need of figures and statistics in our part of our world on on-going microbials spectrum causing SBP and identification of their sensitivity with antimicrobials. In this study, we identified the frequency and distribution of cultivated micro-organism and determined the pattern of their resilience with commonly used antimicrobials using data collected over 3 years.

In this study, out of 356 ascitic fluid samples, a total of 54(15.1%) clinical isolates of different micro-organisms were cultivated, this ratio is similar to other studies in the region.[20,21]Mean age of patients with positive ascitic fluid culture was 48.6 (+43.6) years, this is closer to a similar study done in Gujrat, India.[20] Predominently isolates were from female patients 29/54(53.7%), while isolates for male patients were 25/54(46.29%). Male to female ratio was 1:1.16.

In different geographical areas the etiological order of peritonitis differ.[22] Most of the culture positive fluid samples, historically, have shown prevalence towards the growth of gram negative organisms.[23] In our study, the main etiological factor isolated from ascitic fluid samples were also gram negative bacteria (64.8%), followed by gram positive bacteria 22.2%. This pattern is similar to the pattern of a similar study in Egypt, where gram –ve bacteria isolated was 57.1%.[21]In the preset study, the most frequent organism isolated was E. coli (38.9%), followed by Staphylococcus aureus (11.1%), Acinetobacter species (7.4%), Enterococcus species (5.6%), Klebsiella (5.6%), Enterobacter Species(5.6%), and Pseudomonas Aureginosa (3.7%). In our study, E. coli has remained the most cultivated organism in culture positive ascitic fluid, independent of wards. These results are correspondent to similar studies done in Karachi, Rawalpindi, Bannu and Peshawar.[24,25,26,27] The isolation of Psuedomona Aureginosa in 2 (3.7%) cases, which is not a common isolate of SBP, was a distinct feature in our study. It was in contrast with the most of the similar studies done in Pakistan.[24,25,27] But study done in Bannu and another study done in Iran, showed isolation of Pseudomona Aureginosain ascitic fluid with a frequency of 22.2% and 4.8%, respectively.[14,26] Recently a rise in isolation of Enterococcus associated SBP was noticed in Euorpe.[27,28] A study in Germany showed a rise in Enterococcal SBP from 11% to 33% and was associated with higher resistance to 3rd generation Cephalosporins.[29]In contrast, a current study didn't show such a significant rise in isolation of Enterococcal species which was 5.6%, and it is correlated with most of the Asian studies.[24,25,27]

Antimicroibial susceptibilities and pattern of their resilience was also evaluated in our study. As a total, this study underlines emergence of bacterial resistance with the first line and second line antimicrobials, recommended for treatment of SBP. Most of the strains of bacteria, isolated showed their resilience with third generation cephalosporins, Quinolones and Co-Amoxiclav. The pattern of resistance specially with third generation Cephalosporin in our study is much higher than the literature published in other countries of the region.[20,21,30,31]

In our study, 84% of the gram +ve organisms and 99% of gram –ve organisms were resistant with Cephalosporins. Resistance with quinolones was observed in 84% and 58% for gram +ve and gram –ve organisms respectively. Frequency of resistance with Cephalosporins are much higher in our study compared to other recent similar studies of the area.[24,32,33] Assorted use of antimicrobials specially cephalosporins in last few decades explains the emergence of higher level of resistance. In contrast better resistance profile noticed with Amikacin, Meropenem, ImipenemCefperazone/sulbactum and Piperacillin/Tazobactum in case of gram –ve organisms, while gram positive organisms revealed better sensitivity with Linezolid, Teicoplannin, Vancomycin, clindamycin, Amikacin and Co-trimoxazole. Low resistance with these drugs may be because of auxiliary use of these drugs. Similar sensitivity profile is also notice in literature published from Lahore and JPMC, Karachi.[24,34] Facts in current study advocate the use of Amikacin as compelling possibility in treating patients with SBP. Even higher estimates of sensitivity against Meropenem have been noticed, but its possible contribution in development of hepatorenal syndrome limits it recommendation as a first line drug in SBP. The emergence of resistance with antimicrobials among pathogens which are isolated is fearsome. Proper planning is required to intercept the escalation of drug resilient strains and injudicious practice of antibiotics must be avoided to arrest antimicrobials resistance

CONCLUSION

The present analysis suggests the development of resistance with regularly used antimicrobials to manage SBP, which also includes antibiotics recommended by EASL and some other international guidelines. The situation is worrying, especially in a region where Cirrhosis of liver and SBP is a common medical condition. Higher proportion of resistance with Cephalosporins, Co-Amoxiclav and Quinolones is concerning, as these drugs have been consid-

ered as first line. Nevertheless, Amikacin, Meropenem, Piperacillin/Tazobactum and Cefaperazone/-Sulbactum are yet eminently efficacious for treatment of SBP. In order to arrest further spread of resistance, antimicrobial use should be wise and judicious. Further studies are also required to search for effective alternate antimicrobials which can assist in managing SBP successfully.

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