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Spontaneous bacterial peritonitis: Management and identification of commonest bacterial species

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ABSTRACT

Background: Spontaneous bacterial peritonitis is a serious problem in cirrhotic patients, and changes in the microbiological profile reported in the last years are impacting the choice of antibiotic used for treatment. **Objective:** The aim of this study was to investigate the causative bacteria and their susceptibility patterns to antimicrobial agents in patients with SBP in our locality in order to clarify the empirical antimicrobial treatment. **Methods:** Seventy-two (72) cirrhotic patients with primary and recurrent spontaneous bacterial peritonitis were included in the study, peritoneal aspirate was cultured on blood culture and isolates were identified by VITEK II. The most frequent infecting organisms and the sensitivity in vitro to antibiotics were registered. **Results:** The patients' age ranged from 50-65 years, 41 were males (56.9%) and 31 were females (43%). *Staphylococcus epidermidis* and *Micrococcus luteus* were the most prominent Gram-positive bacteria, whereas *Escherichia coli* (*E.coli*) was the most prominent Gram-negative bacteria. 100% of Gram-negative bacteria were resistant to all tested antibiotics. Many strains among Gram-positive isolated bacteria were multidrug resistant (MDR): *Staphylococcus nishinomiyaensis* with rate of 11.1% (3/27), *Enterococcus faecalis* 7.4% (2/27), *Enterococcus faecium* 14.8% (4/27), *Micrococcus luteus* 22.2% (6/27), *Staph. epidermidis* 22.2% (6/27) and *Staph. lentus* 11.1% (3/27). *Kocuriarosea* were extremely drug resistant (XDR) with a ratio of 11.1% (3/27). **Conclusion:** Frequent detection of the organisms' causing peritonitis is a must to avoid haphazard use of antibiotics for prophylaxis and treatment to decrease morbidity and mortality.

Introduction

Spontaneous bacterial peritonitis (SBP) is an infection caused by pathogenic microorganisms that invade the abdominal cavity and cause notable damage [1]. In people with end-stage liver diseases, the incidence rate of SBP has been shown to reach

40% to 70% [2]. Recently SBP is defined as an ascites with polymorphonuclear (PMN) count greater than 250 cells/mm³ [1-3]. However, about 60% to 80% of patients with a PMN less than 250 cells/mm³ have signs and symptoms [4]; of those patients, 38% develop SBP [5]. However, empirical antibiotic therapy that is based on the patient's

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clinical symptoms and PMN count can lead to the massive use of antibiotics and the development of multi-drug resistant organisms[6,7].

The most common aetiological bacteria isolated from ascitic fluid of patients with SBP are *E. coli*, *Klebsiellapneumoniae* (*K. pneumoniae*) and other Gram-positive cocci such as *Streptococcus* and *Enterococcus* species[8]. *Escherichia coli* and *K. pneumoniae* are the causative agents in nearly 50% of cases which could be explained by that intestinal flora are the source of the infection. Gram-positive bacteria [*Staphylococcus aureus* (2-4%), *Enterococcus* species. (6-10%)] are responsible for approximately 25% of cases. Anaerobic bacteria are detected at a rate less than 1% due to relatively high oxygen content in ascitic fluid. However, recent studies point to an increase in the proportion of SBP cases caused by Gram-positive bacteria[9]

According to guidelines from the American Association for the Study of Liver Diseases and European Association for the Study of the Liver, a third-generation cephalosporin such as cefotaxime should be initiated immediately after the diagnosis of neutrocytic ascites (ascites with PMN count greater than 250 cells/mm³) without waiting for culture results[10]. Cefotaxime appears to cover 95% of the intestinal facultative anaerobic flora, which includes the most common isolates, *E. coli* and *K. pneumoniae*, and it reaches high concentrations in ascitic fluid. The reduction in clinical and microbiological response to third-generation cephalosporins over the last decade necessitates the classification of infections into community-acquired and nosocomial infections. Many studies indicate that up to 33-75% of patients with nosocomial infection fail to respond to third-generation cephalosporins due to multidrug-resistant bacteria[11]. MDR: Acquired non-susceptibility (resistant or intermediate) to at least one agent in three or more antibiotic categories. XDR: Non-susceptibility to at least one agent in all but bacterial isolates remain susceptible to only one or two antimicrobial categories. PANDR: Pandrug-resistant (PDR): Non-susceptibility to all agents in all antibiotic categories [12].

The rate of complications and mortality may increase among these patients due to high resistance rates, prompting some experts to suggest the use of broad-spectrum antibiotics such as carbapenem plus daptomycin or linezolid in the empirical treatment of nosocomial SBP[13].

However, this approach may further increase resistance to these antimicrobial agents and reduce the treatment success of complicated infections in the future. Therefore, it seems useful to know the potential regional causative agents and their antimicrobial resistance patterns in order to recommend empirical antimicrobial treatment. The aim of this study was to investigate the causative bacteria and their susceptibility patterns to antimicrobial agents in patients with SBP in our locality in order to clarify the empirical antimicrobial treatment.

Patients and methods

This cross-sectional study was conducted in Tropical Medicine and Gastroenterology Department in association with Medical Microbiology and Immunology Departments, at Sohag University Hospital from January 2019 to June 2020. In this study, 72 patients were included after taking written consents for participation and after approval of the Ethical Committee under IRB registration number: soh-med-21-06-27.

Inclusion criteria

Cirrhotic patients with SBP that is defined as ascites PMN count ≥ 250 cells/mm³ without evidence of intra-abdominal surgically source of infection with or without culture-positive SBP which is defined as isolation of one microorganism in the ascitic fluid.

Exclusion criteria

Patients who received antibiotic treatment or prophylaxis at admission and/or within the last 3 weeks, ascites due to tuberculosis or malignancy, and those with secondary peritonitis were excluded from the study. Secondary peritonitis was suspected when one of the following features is present: a) Selective and persistent localized abdominal pain and tenderness. b) Isolation of more than one microorganism in the ascitic fluid culture. c) Evidence of intra-abdominal surgically treatable source of infection.

To confirm the diagnosis of ascites, abdominal ultrasonography (U/S) and diagnostic paracentesis were done following the standard precaution guidelines and the use of aseptic techniques in the right or left iliac fossa, 3cm above and 3cm medial to the anterior superior iliac spine. A sterile syringe was used to collect 15mls of ascitic fluid (10mls injected into blood culture bottles at the bedside, and 5mls were put into sterile container bottles of ethylenediaminetetraacetate (EDTA) and

delivered to the laboratory to study cellular and protein contents.

The blood culture bottles (blood culture medium, Egyptian diagnostic medium, EDM) were incubated for a period of 5-7 days at 35-37°C aerobically then by using a sterile syringe, two drops from the bottle were obtained; one for Gram stain and the other one for inoculation onto blood agar plate and MacConkey agar. Then more confirmation for the growth was done by Gram stain. The inoculated plates were examined after 24-48 hrs. The bacteria that were isolated were identified and tested for antimicrobial sensitivity using VITEK II AST GP67 for Gram-positive, disc diffusion for Gram-negative bacteria. If ascitic fluid cultures were positive and the neutrophil count was >250 cells/mm³, patients were diagnosed as having culture-positive neutrocytic ascites (CPNA). If ascitic fluid cultures were negative in the presence of neutrocytic ascites, patients were characterized as having culture-negative neutrocytic ascites (CNNA). Also, we took a sample of 15mls of venous blood for hematological, biochemical and serological investigations.

Statistical analysis

The collected data were coded and verified prior to computerized data entry. The collected data was statistically analysed using Statistical Package for the Social Science (SPSS) version 23 program and expressed in tables and graphs. The data were tested for normality by Kolmogorov-Smirnov. Excel was used to get the graphs. Chi-square and Fisher exact test were used for qualitative data difference between groups. Student's t-test used for parametric data and Mann Whitney for non-parametric data to get the p value between groups. In all analyses, $p < 0.05$ indicated statistical significance.

Results

This study conducted on seventy-two patients with SBP, 60 patients had first ever SBP, and 12 patients had recurrent SBP. The age of the patients ranged from 50-65 years. 41 were male (56.9%) and 31 were females (43%).

The culture reveals positive results in SBP patients with older age, history of hematemesis, esophageal band ligation and had normal to mildly enlarged spleen ($p = 0.034$, $p = 0.002$, $p = 0.032$, $p = 0.0001$; respectively). The mean value of AST, prothrombin time, total and direct bilirubin, and platelets count were significantly higher in culture

negative SBP compared to culture positive SBP. While prothrombin concentration and ascitic protein were lower in culture negative SBP compared to culture positive SBP (Table 1).

All samples were diagnosed as SBP according to clinical pictures and neutrophil count in ascitic fluid. Culture-positive neutrophilic ascites was present in 47.3% (34/72) and culture negative neutrophilic ascites was found in 52.7% (38/72). Peritoneal aspirate was cultured and then VITEK II identification revealed the following different Gram-positive and negative bacteria. *Staph epidermidis* and *Micrococcus luteus* were the most prominent as shown in (table 2).

Antibiotic susceptibility of the isolated bacteria was done by VITEK II for Gram-positive bacteria and disc diffusion method for Gram-negative bacteria. MDR bacteria were isolated among Gram positive bacteria: *Dermacoccus nishinomiyaensis* 11.1% (3/27), *Enterococcus faecalis* 7.4% (2/27), *Enterococcus faecium* 14.8% (4/27), *Micrococcus luteus* 22.2% (6/27), *Staph epidermidis* 22.2% (6/27) and *Staph lentus* 11.1% (3/27). *Kocuriarosea* were XDR with a ratio of 11.1% (3/27). as shown in (table 3). All isolated Gram-negative bacteria were resistant to all tested antibiotics as shown in (table 4).

In 34 culture-positive patients, we detected Gram-positive bacteria was more than Gram-negative bacteria in the cultured samples (27 versus 7). Also, we compared the laboratory characteristics differences between Gram-positive bacteria and those with Gram-negative bacteria. There were statistically significant differences both subgroups in the following parameters: the mean values of ascitic lymphocyte, total bilirubin, direct bilirubin, serum creatinine, prolonged prothrombin time, decreased prothrombin concentration, decreased albumin and haemoglobin levels were higher with Gram-positive bacteria than Gram-negative bacterial infection of the ascitic fluid. However, the mean value of ascitic polymorph is higher in Gram-negative bacteria compared to Gram-positive bacteria as shown in (table 5).

Based on univariate binary logistic regression analysis, the significant risk factors associated with ascitic fluid infection with non-pathogenic bacteria as a causative pathogen of SBP were History of hematemesis, serum ALT, albumin, indirect bilirubin, prothrombin time, and ascitic protein ($p = 0.047$, 0.04, 0.017, 0.03, 0.042, 0.002

respectively). However, this relationship disappeared in multivariate analysis

Table 1. Comparison of clinical and laboratory characteristics between culture positive and negative SBP.

	Culture positive SBP N=34	Culture negative SBP N=38	P value
Age	60.15±12.24	56.11±7.49	0.034
SBP:			0.124
First ever SBP	34 (89.5%)	26 (76.5%)	
Recurrent SBP	4 (10.5%)	8 (23.5%)	
Diabetes Mellitus	21(61.78%)	16(42.11%)	0.096
History of hematemesis	18(52.94%)	7(18.42%)	0.002
Esophageal variceal ligation	15(44.12%)	7(18.42%)	0.032
Spleen size:			0.0001
Normal to mild enlarged	28(82.35%)	18(47.37%)	
Moderately to markedly enlarged	6(17.65%)	20(52.63%)	
Liver size:			0.936
Normal	14(41.18%)	16(42.11%)	
Reduced	20(58.82%)	22(57.89%)	
ALT	33.14±17.96	43.15±28.79	0.236
AST	46.73±25.16	97.31±80.05	0.008
Albumin	2.21±0.55	1.98±0.36	0.042
Prothrombine time	16.56±2.67	18.96±4.31	0.024
Prothrombine concentration	56.20±8.87	47.43±17.07	0.000
Total bilirubin	2.62±1.22	4.89±3.4	0.006
Direct bilirubin	1.67±0.79	3.40±2.46	0.005
Hemoglobin	10.37±1.76	10.05±1.90	0.451
Platelets count	98.53±15.71	125.42±73.62	0.034
WBCs	8.12±3.43	8.54±2.45	0.550
Urea	55.73±14.04	50.52±24.62	0.09
Creatinine	1.69±0.68	1.71±1.21	0.152
Ascitic protein	1.66±0.51	1.3±0.49	0.008
Ascitic cell count	1321.18±832.39	2047.24±253.66	0.681

N= Number, ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Table 2. Distribution of the major pathogens in the ascites samples in patients with spontaneous bacterial peritonitis.

Organisms	First SBP	RBP	Total 72
<u>Culture negative</u>	<u>34 (56.7%)</u>	<u>4 (33.3%)</u>	<u>38(52.7%)</u>
<u>Culture positive</u>	<u>26(43.3%)</u>	<u>8(66.7%)</u>	<u>34(47.3%)</u>
• <i>Dermacoccusnishinomiyaensis</i>	3 (5.0%)	0 (0.0%)	
• <i>E.coli</i>	0 (0.0%)	4 (33.3%)	
• <i>Enterococcus Faecalis</i>	2 (3.3%)	0 (0.0%)	
• <i>Enterococcus faecium</i>	0 (0.0%)	4 (33.3%)	
• <i>Kocuriarosea</i>	3 (5.0%)	0 (0.0%)	
• <i>Micrococcus luteus</i>	6 (10.0%)	0 (0.0%)	
• <i>Pandoraesa spp.</i>	3 (5.0%)	0(0.0%)	
• <i>Staph epidermidis</i>	6 (10.0%)	0(0.0%)	
• <i>Staph lentus</i>	3 (5.0%)	0(0.0%)	

SBP= Spontaneous bacterial peritonitis. RBP= recurrent bacterial peritonitis

Table 3. Drug resistance rate of Gram-positive bacterial isolates to commonly used antibiotics.

Antibiotic Resistance	Type of organism						
	<i>Dermacoccus nishinomiyaensis</i> N=3	<i>Enterococcus Faecalis</i> N=2	<i>Enterococcus faecium</i> N=4	<i>Kocuriarosea</i> N=3	<i>Micrococcus luteus</i> N=6	<i>Staph epidermidis</i> N=6	<i>Staph lentus</i> N=3
	MDR: 100% TOTAL MDR: 11.1%(3/27)	MDR:100% TOTAL MDR: 7.4%(2/27)	MDR:100% TOTAL MDR: 14.8%(4/27)	XDR:100% TOTAL XDR: 11.1%(3/27)	MDR: 100% TOTAL MDR: 22.2%(6/27)	MDR: 100% TOTAL MDR: 22.2%(6/27)	MDR: 100% TOTAL MDR: 11.1%(3/27)
R	R	R	R	R	R	R	
Benzyl-penicillin	3 100%	2 100%	4 100%	3 100%	6 100%	6 100%	0 0%
Ampicillin Sulbactam	3 100%	2 100%	4 100%	3 100%	6 100%	0 0%	3 100%
Oxacillin	3 100%	2 100%	4 100%	3 100%	6 100%	6 100%	3 100%
Gentamicin	3 100%	2 100%	4 100%	0 0%	6 100%	0 0%	0 0%
Ciprofloxacin	3 100%	0 0%	4 100%	3 100%	0 0%	0 0%	0 0%
Levofloxacin	3 100%	0 0%	4 100%	3 100%	0 0%	0 0%	0 0%
Moxifloxacin	3 100%	0 0%	4 100%	3 100%	0 0%	0 0%	0 0%
Erythromycin	3 100%	2 100%	4 100%	3 100%	6 100%	4 66.7%	3 100%
Quinupristin Dalfopristin	3 100%	2 100%	0 0%	3 100%	6 100%	4 66.7%	3 100%
Clindamycin	3 100%	2 100%	4 100%	3 100%	6 100%	4 66.7%	3 100%
Linezolid	0 0%	2 100%	0 0%	0 0%	6 100%	4 66.7%	3 100%
Vancomycin	3 100%	2 100%	4 100%	3 100%	0 0%	4 66.7%	3 100%
Tetracycline	0 0%	0 0%	4 100%	3 100%	0 0%	4 66.7%	3 100%
Tigecycline	0 0%	0 0%	0 0%	3 100%	0 0%	4 66.7%	0 0%
Nitrofurantoin	0 0%	0 0%	4 100%	3 100%	6 100%	4 66.7%	0 0%
Rifampicin	3 100%	0 0%	0 0%	3 100%	6 100%	4 66.7%	0 0%
Trimethoprim Sulfametho-Xazole	3 100%	0 0%	0 0%	3 100%	6 100%	0 0%	0 0%

N= Number

Table 4. Drug resistance rate of major gram-negative bacteria to commonly used antibacterial agents.

Antibiotic resistance	Type of organism	
	<i>E. coli</i> N=4	<i>Pandoraea spp.</i> N=3
	R	R
Ampicillin	4 100%	3 100%
Meropenem	4 100%	3 100%
Levofloxacin	4 100%	3 100%
Doxycycline	4 100%	3 100%
Cefepime	4 100%	3 100%
Cefazolin	4 100%	3 100%
Streptomycin	4 100%	3 100%
ampicillin/sulbactam	4 100%	3 100%
Tobramycin	4 100%	3 100%
Aztreonam	4 100%	3 100%
Cefotaxime	4 100%	3 100%
Ceftazidime	4 100%	3 100%
Gentamicin	4 100%	3 100%
Tetracycline	4 100%	3 100%
Nitrofurantion	4 100%	3 100%

N= number

Table 5. Laboratory characteristic differences between patients with Gram-positive bacteria and those with Gram-negative bacteria.

Characteristics	Gram positive N=27	Gram negative N=7	P-value
Ascitic protein: Mean ± SD Median (IQR)	1.5±0.4 1.8 (1.0:2.0)	1.9±0.58 1.5 (1.5:2.6)	0.251
Ascitic cell count: Mean ± SD Median (IQR)	1236.7±658.8 950 (750:1800)	1542.8±1363 450.0 (450.0:3000)	0.94
Ascitic polymorph: Mean ± SD Median (IQR)	77.5±10.6 80 (70:85)	89.2±5.3 85 (85:95.0)	0.004
Ascitic lymphocyte: Mean ± SD Median (IQR)	22.4±10.6 20 (15:30)	10.7±5.3 15 (5:15)	0.004
Total bilirubin: Mean ± SD Median (IQR)	2.8±1.2 2.9 (2.3: 4)	2.6±0.3 1.4 (1.4:2)	0.024
Direct bilirubin: Mean ± SD Median (IQR)	1.8±0.8 1.8(1.3:2.3)	1.0±0.16 0.9 (0.9:1.2)	0.01
Indirect bilirubin: Mean ± SD Median (IQR)	1.0±0.56 1 (0.4:1.7)	0.6±0.16 0.5 (0.5:0.8)	0.07
Prothrombin time: Mean ± SD Median (IQR)	16.9±2.8 17 (16:17)	15.1±1.06 16 (14:16)	0.024
Prothrombin Concentration: Mean ± SD Median (IQR)	54.8±9.3 54 (50:59)	61.57±3.2 59.0 (59:65)	0.017
Urea: Mean ± SD Median (IQR)	57.7±15 60 (45:70)	47.8±2.6 50 (45:50)	0.224
Creatinine: Mean ± SD Median (IQR)	1.8±0.7 1.7 (1.3:2.6)	1.2±0.0 1.2 (1.2:1.2)	0.004
ALT: Mean ± SD Median (IQR)	35.3±19.6 35 (18:50)	24.5±0.5 25 (24:25)	0.176
AST: Mean ± SD Median (IQR)	45.5±27 39 (22:67)	51.2±17.1 65 (33:65)	0.53
Albumin: Mean ± SD Median (IQR)	2.1±0.5 2 (1.6:2.5)	2.5±0.4 2.2 (2.2:3)	0.034
Hemoglobin: Mean ± SD Median (IQR)	9.9±1.6 10 (8.7:11.4)	12.1±0.2 12 (12:12.4)	0.002
Platelets: Mean ± SD Median (IQR)	96.7±13.63 91 (90:100)	105.4±21.9 123 (82:123)	0.56
WBCs: Mean ± SD Median (IQR)	8.000±3.200 9.000(5.500:10.400)	8.400±4.400 12.000 (3.600:12.000)	0.33

Table 6. Univariate and multivariate analysis of variables in SBP caused by non-pathogenic bacteria.

Baseline variables	Univariate analysis		Multivariate analysis		
	Odds Ratio (95% CI)	P value	Significant variables	Odds Ratio (95% CI)	P value
Diabetes Mellitus	0.74(0.441-1.24)	0.255	History of hematemesis	0.4(0.08-1.83)	0.239
History of hematemesis	2.91(1.01-8.34)	0.047	Albumin	1.09(0.23-5.06)	0.914
Esophageal variceal ligation	1.26(0.66-2.41)	0.48	AST	0.96(0.95-1)	0.101
ALT	0.96(0.95-1)	0.07	Prothrombine time	1.07(0.73-1.57)	0.72
AST	0.97(0.93-1)	0.04	Indirect bilirubin	0.49(0.15-1.59)	0.24
Albumin	3.94(1.28-12.18)	0.017	Ascitic protein	1.9(0.52-7.08)	0.32
Prothrombine time	0.82(0.68-0.97))	0.03			
Prothrombine concentration	1.04(1-1.78)	0.45			
Total bilirubin	0.78(0.59-1.01)	0.63			
Direct bilirubin	0.73(0.51-1.05)	0.09			
Indirect bilirubin	0.43(0.19-0.97)	0.042			
Urea	1.09(0.6-1.82)	0.73			
Creatinine	1.01(0.99-1.04)	0.22			
Ascitic protein	6..84(1.99-23.5)	0.002			
Ascitic cell count	1(0.99-1)	0.72			
Ascitic polymorph	1.05(0.96-1.09)	0.07			
Ascitic lymphocyte	0.96(0.91-1.01)	0.08			
WBCs	0.86(0.72-1.02)	0.101			

Discussion

Liver cirrhosis is a global health and economic burden, causing significant morbidity and mortality[13]. One of the severe complications in patients with liver cirrhosis is bacterial infection that is a major cause of acute decompensation which is a key prognostic determinant and is significantly associated with mortality[14]. One of these infections is spontaneous ascitic infection that is caused by various microorganisms.

In the present study, ages of the patients ranged from 50-65 years with a mean age of 57.8± 11;this is in agreement with the study done by **Nguyen et al.**[15] in which the mean age of patients with SBP was 55.36 ± 12.32 years. In our study, we found SBP was more prevalent in females than other studies where SBP reported in 41(56.9%) males and 31(43%) females. Similarly, **Nouman et al.**[16] found that patients with SBP were 45% male and 54% female. This was different from the study done by **Nguyen et al.**[15] in which there were 53 males (91.3%) and 5 females (8.6%), and the study done

by **Kim et al.**[17] in which there were 61 males (79.2%) and 16 females (20.7%).

In our study, CPNA was present in 47.2% (34/72) and this percent is more than that obtained by **Nguyen et al.**[15] who found culture-positive SBP in 29.3% (17/58) patients but less than that reported by **Oladimeji et al.**[18] who found it 66.7%. Also, we found CNNA was present in 52.7% of the patients which is near to the result obtained by **Duah et al.**[19] who found CNNA in 63.33% but our result was higher than that obtained by **Oladimeji et al.**[18] who found CNNA in 33.3% of the patients. The previous differences may be explained by differences in culture methods and techniques used, also recent use of antibiotics may also contribute to the relatively low prevalence of culture positive SBP.

In the current study, we found that liver function tests were more impaired and ascitic protein was lower in patient with CNNA compared to patients with CPNA. Also, we found patients with CPNA were older in age with history of hematemesis, esophageal band ligation and had

normal to mildly enlarged spleen compared to patient with CNNA. On the other hand **Yassen et al.** [20], reported that no significant differences as regards, age, sex, abdominal pain, bleeding esophageal varies, serum albumin, bilirubin, ALT, AST, PT were found between both culture positive and negative groups. However, several studies stated that bacterial infection in cirrhotic patients is an important cause of liver function deterioration and development of complications [21,22].

In our study, the most prevalent pathogens were Gram-positive bacteria especially *Staph epidermidis* and *Micrococcus luteus*. Our findings were in agreement with several studies that showed a high frequency of Gram-positive bacterial infections associated with SBP as the study of **Cholongitaset al.** [23], and that was done by **Alexopoulou et al.** [24]. Also, **Fernandez et al.** [12] reported that Gram-positive bacterial infections were more frequently in the hospitals than Gram-negative infections (55.6% vs 36.0%, respectively) and this finding was in agreement with our study as our patients were all from the admitted patients in our department. On the other hand, many studies as those done by **Nguyen et al.** [15], and **Bibi et al.** [25], Gram negative bacteria were more prevalent especially *E.coli*.

Kim et al. [17] found 64.9% of the patients were infected with Gram-negative infections and 35.1% with Gram-positive infections. *Escherichia coli* was the most common isolate (32.5%), followed by *Klebsiella pneumonia* (19.5%) and for patients with Gram-positive bacterial infections, *Enterococcus* species and *Staphylococcus aureus* were the most common isolates (13.0%), these findings were opposite to our findings where 76.4% of our culture positive samples were caused by Gram-positive bacterial infections while only 23.5% of these positive samples were caused by Gram-negative infections. Our results were similar to previous studies in which the commonest strain of Gram-negative bacteria among patients infected with Gram-negative organisms was *E.coli*.

The predominance of Gram-positive bacteria in our study and in previous studies may be explained by the fact that patients with cirrhosis frequently require hospital care, recurrent hospitalizations or hospitalizations in intensive care units [26].

As regards to the antibiotic resistance profile of Gram positive bacteria isolated in our

study, we found that the rate of MDR in *Micrococcus luteus* among all isolated Gram-positive bacteria was 22.2% but it was 100% sensitive to each quinolone, vancomycin, tetracyclin and tigecyclin. While among isolated *Dermaococcus* 11.1% were MDR, but still 100% sensitive for each linezolid, tetracycline, tigecyclin and nitrofurantoin. *Kocuriarosea* were XDR with a ratio of 11.1% but still 100% sensitive to both gentamycin and linezolid.

The rate of MDR among *Enterococcus faecalis* and *faecium* were 7.4% and 14.8% respectively, but they had 100% sensitivity to tigecyclin, rifampicin and trimethoprim sulfamethoxazole. These results are different from **Zhang et al.** [27] who found MDR rates was 0.0%, 71.4% in *Enterococcus faecalis* and *faecium* respectively with 100% susceptibility to linezolid but it was similar to us in 100% sensitivity to tigecyclin.

In our study, *Staphylococci* were 100% resistant to oxacillin, 100% vancomycin resistance in *staph lentus* but 33.3% of *Staph epidermidis* still sensitive to vancomycin. These findings are different from **Zhang et al.** [27] who found 0% resistance to vancomycin. *Staphylococci* in our study were 100% sensitive for each gentamycin, quinolone and Trimethoprim sulfamethoxazole which represent suitable lines for prophylaxis and treatment. Our results were in agreement with others who found the prevalence of infections caused by multiresistant bacteria (e.g., methicillin-resistant *S. aureus* and *Enterococcus faecium*) is increasing in cirrhotic patients [11].

As regards antibiotic profile of Gram-negative bacteria *E.coli* and *pandorecaea* spp. were resistant to all tested antibiotics, In contrast to **Oliveira et al.** [28] who found that 19% of *E. coli* are (MDR), in our study quinolone resistance was 100% in Gram-negative dissimilar to **Zhang et al.** [27] who found 41.5% only quinolone resistance.

When we studied the clinical characteristics of the patients and their relation to the type of bacteria (Gram-positive and Gram-negative), patients with Gram-negative bacteria had statistically higher polymorphonuclear leucocyte count and prothrombin concentration. Patients with Gram-positive bacteria had statistically significant higher level of ascitic lymphocytes, total bilirubin, direct bilirubin, prothrombin time, and serum creatinine, and had statistically significant lower level of albumin and hemoglobin, [27] mentioned that, patients infected with GNB had worse liver

function, higher MELD score, higher inflammatory index, and a higher risk of progressing to ACLF (acute or chronic liver failure). These results suggest that more attention should be paid to patients infected with GNB.

In our study, the association of high total bilirubin, high serum creatinine, and SBP with Gram-positive bacteria is a strong predictor of mortality during hospitalization and this is in agreement with **Coral et al.**[29] who found the mortality rate in SBP infected patients with or without renal impairment was 36% and 6% respectively. Also, **Sort et al.**[30], **Salerno et al.**[31-32]. Found plasma volume expansion with intravenous albumin decreases renal impairment and mortality in patients with cirrhosis and SBP more than use of antibiotic therapy alone.

In this study, there is a significant difference in ascitic protein, ascitic cell count, hemoglobin, liver coarseness and spleen size between first and recurrent peritonitis. Hemoglobin is decreased in patients with first SBP while ascitic cell count and proteins are decreased in recurrent peritonitis. Also, liver coarseness and change in spleen size were more prominent in first SBP.

Limitations of this study

First, the patients included in this study were from a single hospital in Sohag University. Therefore, the results might not be applicable to different hospitals. Second, we detected organisms out of the usual microbiological profile of species: *g Dermacoccus nishinomiyaensis*, *Kocuriarosea* and *Pandoraea* spp. These organisms are found on the normal skin as commensals and are unusual causes of peritonitis. To be accurate that they are the cause, another 10 ml of ascitic fluid should be aspirated and cultured, if revealed the same organism it is surely the cause, but that could not be possible as we cultured the organism on the media and pure colonies were preserved in -80 °C for further identification by VITEK II. Third, this study did not include patients with culture positive ascitic fluid with (PMN) count less than 250/mm³ and in clinical practice; patients with this condition are occasionally treated with antibiotics.

Conclusion

Spontaneous bacterial peritonitis is a serious problem in cirrhotic patients with increased morbidity and mortality. Screening the causative organism periodically is very important to both identify the cause and to select the proper antibiotic

for prophylaxis and treatment. Also, infections caused by MDR and XDR bacteria should be a current concern, and new antibiotic strategies are needed for this special population. Individualized antibiotic treatment based on local epidemiology is the key for success, not neglecting the urge to preserve renal function of these complex patients.

Recommendations

Antibiotic prophylaxis and treatment therapy should be adjusted according to the results of culture and sensitivity of the isolated organisms. Future studies including a direct comparison with another well-validated molecular method of bacterial DNA detection and identification, as well as standard microbiological culture diagnostics are recommended. Further studies conducted in larger patient populations involving multiple hospitals will be necessary.

Conflict of interest

The authors declared no conflict of interest.

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