British Microbiology Research Journal
17(4): 1-6, 2016; Article no.BMRJ.29869
ISSN: 2231-0886, NLM ID: 101608140

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Identification of Ascitic Fluid Bacterial Pathogens in Spontaneous Bacterial Peritonitis in Nile Delta and Its Impact on Clinical Outcome of these Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author HSK managed literature searches and reviewed the manuscript. Authors HSK and MAA managed the practical work of the study. All authors collaborate in the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/29869
Editor(s):
(1) Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.
Reviewers:
(1) Einar Arnbjornsson, Skåne University Hospital and Lund University, Lund, Sweden.
(2) Wagih Mommtaz Ghannam, Mansoura university, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/16688

Original Research Article

Received 1st October 2016
Accepted 19th October 2016
Published 27th October 2016

ABSTRACT

Aims: This study aimed to identify ascetic fluid bacterial pathogens and their antibiotic resistance profile in Spontaneous Bacterial Peritonitis (SBP) patients in Nile delta and its impact on the clinical outcome of these patients.
Study Design: Retrospective observational study.
Place and Duration of Study: Patients enrolled in this study were admitted to Tropical Medicine and Infectious Diseases Department, Faculty of Medicine, Tanta University, Egypt. Further laboratory work was carried out at Microbiology and Immunology Department, Faculty of Medicine, Tanta University, Egypt, from July 2015 to June 2016.
Methodology: 247 patients with liver cirrhosis and ascites who met the clinical criteria for

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suspicion of SBP including: fever, encephalopathy, refractory ascites and abdominal pain were enrolled in the study. Patients were subjected to thorough history and clinical examination. Ascetic fluid sampling was done for every patient and ascetic fluid analysis was done including cell counts and differential counts. Also, ascitic fluid culture, microbiological testing and antimicrobial sensitivity tests were done.

Results: Out of 247 patients enrolled in this study with liver cirrhosis, ascites and clinical suspicion of SBP, 138 patients were excluded. These excluded patients included: 91 patients had ascetic fluid neutrophils below 250 cells/mm3, 4 patients were cases of secondary peritonitis with polymicrobial culture and 43 patients were found to started empirical antibiotics within 5 days of admission. Out of 109 patients who had SBP, 28 only were culture positive. Among culture positive SBP, 16 (57.1%) were Gram positive and 12 (42.9%) were Gram negative. The most common organism isolated was Gram positive *Enterococci* followed by *E. coli* and *Staph aureus*.

Conclusion: While Gram negative bacteria were the main infectious agents causing SBP a few decades ago, and are still reported to be so in the most recent recommendations and reviews, Gram positive bacteria are now predominant and there is a rising prevalence of bacteria with reduced susceptibility to cephalosporins and fluoroquinolones as regarding this study only and not including previous data or speculations. Current international guidelines recommend the use of a third-generation cephalosporin for empirical treatment of SBP which raise the questions about these guidelines and if they are still valid.

Keywords: Spontaneous bacterial peritonitis; gram positive bacteria; gram negative bacteria; third generation cephalosporins; bacterial resistance.

1. INTRODUCTION

Spontaneous bacterial peritonitis (SBP), defined as an infection of ascites in the absence of a contiguous source of infection [1]. SBP is a common and potentially fatal bacterial infection in patients with decompensated cirrhosis and ascites [2,3]. The short term mortality may reach up to 40% mainly due to sepsis, hepatorenal syndrome, and liver failure [4].

Translocation of bacteria from I ntestine into the ascitic fluid in SBP occurres secondary to impaired humoral and cellular immune responses [5,6]. SBP is also associated with a poor prognosis for patients, with high mortality rates reaching up to 70% at 1 year [3].

Early diagnosis and early optimal treatment of these infections with appropriate antibiotics and the prevention of hepatorenal syndrome with albumin are required [7].

Current European and most other international guidelines recommend the use of a third-generation cephalosporin as the first choice, or amoxicillin-clavulanate acid or fluoroquinolones as an alternative choice [5,8]. However, these recommendations are based mainly on clinical trials that were very often conducted a decade or more ago, and on the assumption that *E. coli* would be involved in nearly half of the cases [9].

Several studies have pointed out marked changes in the causative bacteria of SBP and extreme changes in antibiotic resistance profiles. In particular, the potential emergence of *Enterococci*, *methicillin-resistant S. aureus* (MRSA), or fluoroquinolone-resistant bacteria, following norfloxacin prophylaxis, is also a cause of concern since they may be associated with a higher risk of therapeutic failure [10].

The antibiotic resistance rate especially for third-generation cephalosporins (including cefotaxime and ceftazidime), ciprofloxacin, and ofloxacin are increasing dramatically. Failure of first-line empirical therapy for SBP is associated with poor survival and increased mortality, therefore, early identification of patients with SBP due to cefotaxime or other third generation cephalosporins resistant bacteria is crucial [10,11].

So, the aim of the study was to identify ascitic fluid bacterial pathogens in Spontaneous Bacterial Peritonitis in Nile delta and its impact on clinical outcome of these patients.

2. PATIENTS, MATERIALS AND METHODS

The patients in the present study were enrolled from Tanta University, Tropical medicine department. A written consent was taken from all participants in this research and the study was approved by the local Ethical Committee.

The study included patients with Liver cirrhosis with ascites and proved to have SBP with ascitic

fluid PMNL≥250 cells/mm^3 while those with ascetic fluid PMNL<250 cells/mm^3 were excluded from the study. Patients with ascitic fluid culture showing polymicrobial infections were also excluded from the study. Moreover, patients who started empirical antibiotics without prior culture were excluded from the study.

Ascitic fluid sampling was done for every patient by the standard diagnostic paracentesis method, and ascitic fluid analysis was done including cell counts and differential counts. Also, ascitic fluid culture and microbiological testing were done. Peritoneal fluid sampling was performed under complete aseptic precautions to ensure the sterility of the samples. Culture-positive SBP was diagnosed in the presence of ascitic fluid PMNL ≥ 250 cells/mm3 and positive ascitic fluid culture for a single organism. Culture-negative neutrocytic ascites was diagnosed when the ascitic fluid culture results are negative, but the PMNL counts are 250 cells/mm3 or higher. A code number for each patient was used, symbols to the name and address were kept in a special file. Patients' names were hidden during the research work.

3. RESULTS

The study enrolled 247 patients with liver cirrhosis, ascites and clinical suspicion of SBP. Ninety one patients had ascetic fluid neutrophils below 250 cells/mm3. Four patients were cases of secondary peritonitis with polymicrobial culture. Forty three patients were found to started empirical antibiotics within 5 days of admission. So, they were excluded from the study (Fig. 1).

The microbiological spectrum found in our patients was 57.1% (16/28) Gram-positive organisms while 42.9% (12/28) were Gram-negative organisms. Amongst the Gram positive cocci, *Enterococcus* bacteria (56.2%) was the commonest followed by *S. aureus* (43.8%). *E. coli* (58.3%) was the commonest bacteria amongst the Gram negative bacilli followed by *Enterobaceria* (25%), *Pseudomonas spp.* (8.3%) and *Klebsiella spp.* (8.3%) (Table 1).

In our study, (54.7%) of the isolated bacteria were third-generation cephalosporin-resistant. Resistance to quinolones was observed in (58.9%) microorganisms. Amoxycilline-clavulanate resistance was demonstrated in (53.6%). The least resistance was observed to gentamicin (17.9) and impenem (7.14%) (Table 2).

Table 1. Bacteria isolated from ascitic fluid in culture positive SBP

Type of bacteria	Number	%
Gram-negative bacteria	12	42.9%
Escherichia coli	7	58.3%
Klebsiella spp.	1	8.3%
Pseudomonas spp.	1	8.3%
Enterobacter spp.	3	25%
Gram-positive bacteria	16	57.1%
Enterococcus spp.	9	56.2%
Staphylococcus aureus	7	43.8%

Table 2. Patterns of antibiotic resistance among all positive cultures

Antibiotic	Resistance rate
Ampicillin	78.6%
Amoxicillin	75%
Ampicillin-sulbactam	53.6%
Cefotaxime	60.7%
Ceftriaxone	57.1%
Ceftazidime	46.4%
Ciprofloxacin	64.3%
Ofloxacin	53.6%
Gentamicin	17.9%
Impenem	7.14%

4. DISCUSSION

Cirrhotic patient with ascites are particularly susceptible to spontaneous bacterial peritonitis due to altered gut permeability, suppression of the reticuloendothelial system and bacterial overgrowth [5]. Gram-negative bacteria (most frequently *E. coli*) through translocation from the intestinal lumen are responsible for the majority of SBP cases [12].

However, the preponderance of infections caused by Gram-negative bacteria due to epidemiological changes has shifted to a higher prevalence of infections being caused by Gram-positive cocci. This may be explained by increasing antibiotic prophylaxis, exposure to hospital environment, and frequent invasive procedures [13].

In our study which was done on 247 patients presented with symptoms such as low grade fever, rapidly accumulating ascites, unexplained deterioration of general condition and some of them had abdominal pain. They were admitted to the hospital suspected of having SBP and diagnostic paracentesis was done for all patients. PMN was found <250 cells/mm^3 in 138 patients and 250 cells/mm^3 in 109 patients. Ascitic fluid cultures among those 109 patients were found negative in

81 (74.3%) patient and positive only in 28 (25.6%) patients.

The rate of culture-positive cases was obviously low in our study despite the fact that ascitic fluid cultures were performed by the standard (culture-bottle) method. On the other hand, previous studies reported the culture-positive rate of SBP ascites to be ranging about 40% of cases of SBP [14], and Bibi et al. [15] reported the culture-positive rate of SBP ascites to be about 50% [15]. However, similar results to our study were reported with lower rate of culture-positive rate of SBP in about 39% of cases and they suggested that the low rate probably depended on an earlier diagnosis of the infection [16]. Sajjad M et al. [17] has reported much lower rates of culture positivity to even below 25%. This difference could be attributed due to the different culture techniques [17].

The microbiological spectrum found in our patients was 57.1% (16/28) Gram-positive organisms while 42.9% (12/28) were Gram-negative organisms. Amongst the Gram positive cocci, *Enterococcus spp.* bacteria (56.2%) was the commonest followed by S. aureus (43.8%). *E. coli* (58.3%) was the commonest bacteria amongst the Gram negative bacilli followed by *Enterobacer* spp. (25%), *Pseudomonas spp* (8.3%) and *Klebsiella spp.* (8.3%).

Our findings are in agreement with several reports that showed a higher frequency of Gram-positive bacterial infections associated with SBP [18,19]. Gou et al. [20] report also supported the view that Gram-positive pathogens were predominant among ascites fluid samples from SBP patients [20]. Our results were partial agreement with Hardick et al. [21] who found even much higher frequency of Gram positive bacteria; as 83.3% (10/12) were diagnosed as gram –positive organisms by Broad-Range PCR [21]. Our results were also in accordance with Alexopoulou et al. [13], who isolated the majority of pathogens from patients with SBP were gram-positive cocci (55%) [22].

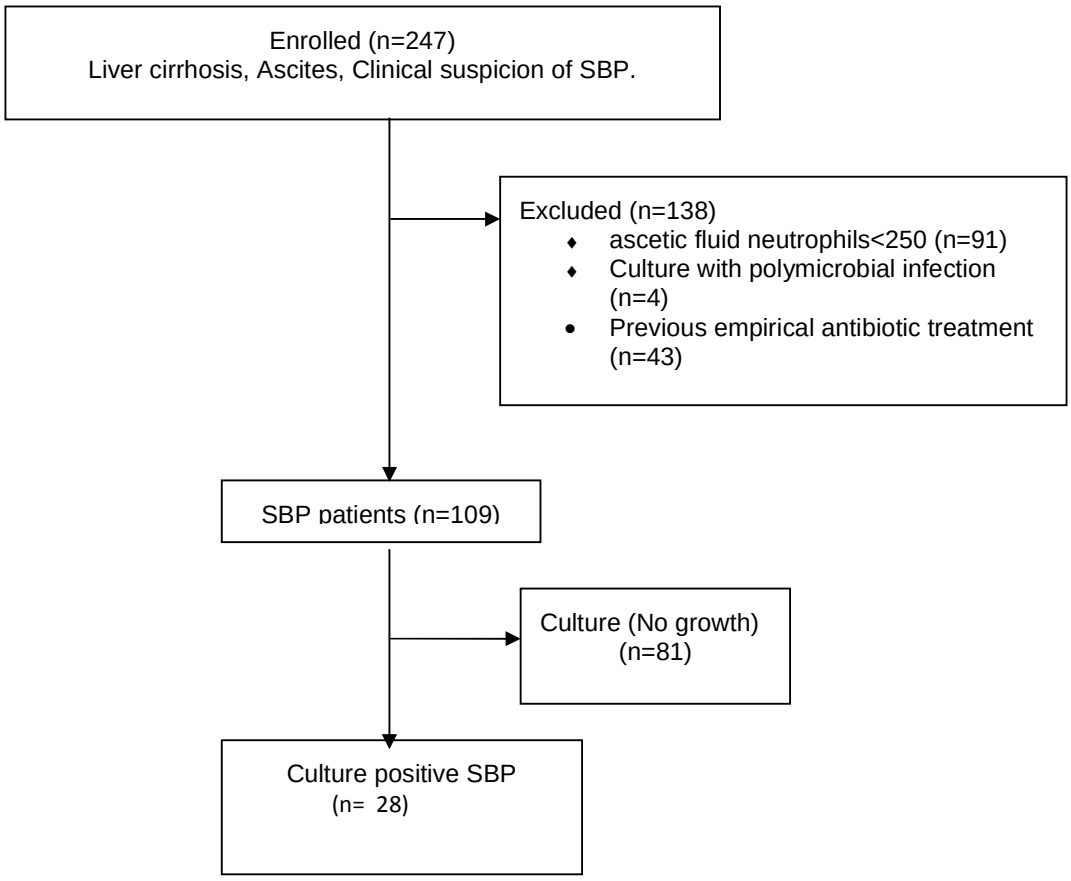

Fig. 1. Flow diagram of Identification of ascitic fluid bacterial pathogens in spontaneous bacterial peritonitis in Nile Delta

In contaray, Iqbal et al. [23] reported from Khyber Teaching Hospital, Peshawar that Gram negative bacteria was predominant with *E. coli* were 58.13% in SBP ascites followed by *Streptococcus pneumoniae* in 18.60%, *S. aureus* in 9.13% and *Acinetobacter* in 4.63% cases [23]. Also, Haider et al. [24] found 60% of the cultured bacteria as Gram negative bacilli and 24% as Gram positive cocci, amongst these *E. coli* were 30%, *Klebsiella spp.* 14% and *Enterobacter spp.* and *Pseudomonas spp* 4% each [24].

The development of new antibiotics and the possibility of an earlier diagnosis of SBP have dramatically changed the natural history of resolution from 25% before 1980 to 70% - 90% in the last few years [25]. The administration of an inappropriate therapy is associated to an increased mortality. The choice of the empirical therapy should be based not only on the severity and the origin of the infection, but also on the local microbiological profile [5].

In our study, (54.7%) of the isolated bacteria were third-generation cephalosporin-resistant. Resistance to quinolones was observed in (58.9%) microorganisms. Amoxycilline-clavulanate resistance was demonstrated in (53.6%). The least resistance was observed to gentamicin (17.9) and impenem (7.14%).

As third-generation cephalosporins were the first choice empirical antibiotics against SBP and other infections. The bacterial resistance to these antibiotics was evaluated and 36.2% to 50% of isolates from cirrhotic patients were resistant [26].

A position statement based on the EASL Special Conference 2013 recommended as empirical treatment for community acquired infections either cefotaxime (or ceftriaxone) or amoxicillin/ clavulanate and for nosocomial either piperacillin/ tazobactam or meropenem ± glycopeptides [27].

5. CONCLUSION

While Gram negative bacteria were the main infectious agents causing SBP a few decades ago, and are still reported to be so in the most recent recommendations and reviews. Gram positive cocci are now predominant and there is rising prevalence of bacteria with reduced susceptibility to cephalosporins and fluoroquinolones as regarding this study only and not including previous data or speculations. Current international guidelines recommend the use of a third-generation cephalosporin for empirical treatment of SBP which raise the questions about these guidelines and if they are still valid.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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