ORIGINAL ARTICLE

MICROBIOLOGICAL ANALYSIS AND ANTIBIOTIC SUSCEPTIBILITY FOR SPONTANEOUS BACTERIAL PERITONITIS IN HOSPITALIZED PATIENTS WITH AND WITHOUT HEPATITIS C

Bilal Ahmad¹ ², Muhammad Saqib Ishaq*¹, Qurban Ali², Muhammad Atif Khan¹, Muhammad Ishfaq Khan¹

¹: Department of Health and Biological Sciences, Abasyn University, Peshawar 25000 Pakistan.
²: Department of Microbiology, Quaid-I-Azam University, Islamabad 45320 Pakistan.

Abstract

Spontaneous bacterial peritonitis (SBP) is a common and often fatal bacterial infection with ascites in cirrhotic patients. The pathological accumulation of fluid in the peritoneal cavity is known as ascites or ascitic fluid. Diagnosis of ascites with proper causative agent can be a challenging issue due to bacterial translocation. Initial empirical treatment for SBP may not be effective due to the presence of Multidrug Resistant (MDR) bacteria. The aim of the present study was to investigate the causative agents of ascites in SBP and to check the antibiotic susceptibility of bacterial isolates. For current research, ascitic fluid samples were collected through diagnostic paracentesis from twenty-five hospitalized patients. Ascitic fluid was centrifuged followed by inoculation in bacterial broth, agar and blood culture media. Bacterial isolates were identified through standard biochemical tests and then antibiotic susceptibility testing of bacterial isolates was performed.

Among all tested bacterial species E. coli was prevalent isolate present in 23/25 samples. E. coli was 92% followed by Shigella spp (72%), Enterobacter spp (68%), Enterococcus spp (60%), K. aerogenes (52%), S. aureus (32%), S. epidermidis (30%), A. israelii (28%), P. aeruginosa (24%), C. freundii (20%) and S. marcescens (08%). Six antibiotics (meropenem, moxifloxacin, ciprofloxacin, ceftriaxone, cefixime and vancomycin) were used to check antibiotic susceptibility pattern of bacterial isolates. Our results showed that most of bacterial isolates were MDR except E. coli and A. israelii. Meropenem showed maximum zone of inhibition against S. aureus (34mm), followed by A. israelii (33mm), P. aeruginosa (32mm), C. freundii (31mm), S. marcescens (28mm), S.
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epidermidis (27mm), E. coli (26mm), Salmonella spp (23mm), K. aerogenes (21mm), Enterobacter spp (21mm) and Shigella spp (15mm).
It is concluded that Gram-positive and Gram-negative multi-drug resistant bacteria were the most common cause of SBP in patients with and without hepatitis C and meropenem was the most effective antibiotic against all tested isolates.

1. INTRODUCTION

The term “ascites” is primarily originated from the Greek word Askitos which means bladder or bag (Bodal et al., 2013). Patients with liver cirrhosis and ascites frequently get the infection known as spontaneous bacterial peritonitis (SBP). SBP accounts for 10-30% of all documented bacterial infections in hospitalized patients, making it the most common and frequently fatal bacterial infection in cirrhosis (Wiest et al., 2011). The pathologic accumulation of fluid in the peritoneal cavity that results from liver disorders and cancer is known as ascites. Many patients experience discomfort and suffering due to this challenging clinical issue. In certain cases, there may be an accumulation of fluid that spreads into the chest cavity causing pleural effusion and breathing difficulties (Alzahraa et al., 2011).

SBP develops when there isn't an evident and medically curable intra-abdominal infection source, like perforation or inflammation of an organ. The primary causal factor is bacterial translocation (BT). In cirrhosis, pathological BT is the root cause and origin of SBP, corroborating the theory that the pathological BT-to-SBP pathway is mostly lymphatic. Pathological BT in liver cirrhosis has been linked to three variables, including changes in gut microbiota, increased intestinal permeability, and weakened immunity (Enomoto et al., 2014).

The prevalence is low (3.5% or less) in asymptomatic outpatients, but it rises to 8% to 36% in nosocomial settings. Bacterascites, which are characterized by a positive culture but no rise in the Polymorphonuclear (PMN) count in the ascitic fluid, affect 2-3% of outpatients and up to 11% of patients overall (Wiest et al, 2011). It has been reported that up to 30% of hospitalized cirrhotic patients with ascites can be identified with this illness. Significant morbidity and mortality are linked to SBP. A polymorph-nuclear neutrophil (PMN) count of higher or equal to 250/ml in ascitic fluid is a diagnostic marker for SBP. About 40% of SBP instances revealed positive culture. Many researchers, especially the older ones, revealed that the majority of SBP episodes were caused by Gram-negative intestinal bacteria (Oey et al., 2017). However, proper detection, diagnosis, and effective treatment are highly recommendable (Saud et al., 2010).

There are specific alterations in the fecal microbial composition linked to liver cirrhosis (Chen Y et al., 2011) including a rise in the prevalence of potentially harmful bacteria like Enterobacteriaceae. Advanced stages of liver cirrhosis are often associated with small intestinal bacterial overgrowth (SIBO), which is defined as more than 105 colony-forming units/ml of jejunal aspirate and/or species of the colonic type. SIBO has been associated with pathological BT and SBP (Bauer et al., 2001). In this study, ascitic fluid from twenty-five SBP patients was analyzed for microbiological investigation and antibiotic susceptibility testing.

2. METHODOLOGY

A total of 25 patients (13 of them were diagnosed with Hepatitis C) were observed for this study. Ascitic fluid specimens were subjected to broth culture, agar culture, and blood culture medium under aseptic conditions to check bacterial growth, Gram staining, biochemical testing, and antibiotic susceptibility tests.

Sample Collection

Fresh Peritoneal fluid samples were drained from patients having SBP with the help of diagnostic paracentesis. Ascitic fluid PMN >250/mm³ was collected in 20 and 50 ml sterile syringes from gastroenterology and hepatology wards Hayatabad Medical Complex (HMC), Peshawar. The labeled samples were transferred to Microbiology Research laboratory Abasyn University Peshawar for further investigation and Microbiological Diagnosis.
Inclusion criteria

Cirrhotic patients having cloudy-colored ascitic fluid in the peritoneal cavity reported with PMN count >250/mm³ were included in this study.

Culturing Methods

The protocols for the culturing method were followed as mentioned by Alzahraa et al (2011) and Vas & Law (1985) with some modifications.

Brain Heart Infusion (BHI) Method

Peritoneal/Ascitic fluid (10 ml) was centrifuged at 2000 revolutions per minute (RPM) at least for 10 minutes. After 10 minutes the centrifuge was allowed to stop, then supernatant was discarded and the pellet was inoculated into brain heart infusion (BHI) broth medium to support growth of fastidious organisms. Cultures were incubated at 37°C for 72 hours and organisms were identified by standard microbiological methods.

Thioglycolate Method

Different volumes of peritoneal fluid (two 10 ml portions) were taken and concentrated by centrifugation at > 2000 RPM for 10 minutes. After the centrifugation, the pellets were inoculated into a thioglycolate medium and incubated for 72 to 96 hours to support the growth of aerobic and anaerobic bacteria.

Blood Agar Base Method

Cultures from BHI were used for subcultures and inoculated into blood agar media for the growth of fastidious organisms and to differentiate bacteria based on their hemolytic properties, after inoculation the media plates and incubated at 37°C for 24 hours.

MacConkey Agar Method

Subcultures of bacterial growth were performed on MacConkey agar and incubated at 37°C for 24-48 hours to support the growth of Gram-negative bacteria and to differentiate lactose fermenting and non-fermenting bacterial species.

Eosin Methylene Blue (EMB) Dye Agar Method

Subcultures were performed on EMB agar media and incubated at 37°C for 18-24 hours to check and identify the growth of Escherichia coli.

Salmonella Shigella (SS) Agar Method

Subcultures were performed on SS agar enrichment media and incubated the media plates at 37°C for 12 to 18 hours identification and differentiation of Salmonella and Shigella species.

Mannitol Salt Agar (MSA) Method

Positive ascitic fluid cultures for Gram-positive bacteria such as S. aureus and S. epidermidis can be obtained on MSA media while incubated media plates at 37°C for 18 to 24 hrs.

Pseudomonas Cetrimide Agar (PCA)

To achieve isolates of Gram-negative bacteria, Pseudomonas aeruginosa, inoculation on PCA selective media were performed and incubated at 37°C for 48 hrs.
Identification Methods

After culturing, sub-culturing, and positive growth examination, two identification methods (Gram’s staining for morphology and biochemical tests for biochemical characteristics) were followed. Kirby-Bauer disc diffusion (Ishaq et al., 2015) method for the antibiotic susceptibility of bacterial isolates was performed.

3. RESULTS

The ascitic samples were treated and streaked over different bacterial growth media like nutrient agar for initial culturing and then streaked over enriched, enrichment, selective and differential media for sub-culturing (Figure 2). After positive bacterial growth on culture media, biochemical tests were performed for bacterial identification and antibiotic susceptibility tests were checked.

Identification and confirmation of biochemically tested bacterial species

After biochemical tests bacterial species (Figures 1 a-h, Table 1) isolated from the culturing of ascitic fluid were E. coli, K. aerogenes, C. freundii, Enterobacter spp, Salmonella spp, S. marcescens, A. israelii, S. aureus, S. epidermidis, Shigella spp, P. aeruginosa. Among all tested bacterial species, E. coli was the most isolated bacteria present in 23 samples out of 25. The percentage presence of E. coli was 92% followed by Shigella spp (72%), Salmonella spp (68%), Enterobacter spp (60%), K. aerogenes (52%), S. aureus (32%), S. epidermidis (30%), A. israelii (28%), P. aeruginosa (24%), C. freundii (20%) and S. marcescens (08%). Table 2 shows the measurement of zone of inhibition.
Table 1: Biochemical tests for bacterial identification.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gram staining</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Coagulase</th>
<th>TSI</th>
<th>Gas</th>
<th>H2S</th>
<th>Urease</th>
<th>Citrate</th>
<th>Indole</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>K. aerogenes</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Unknown</td>
<td>A/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. freundii</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Unknown</td>
<td>K/A</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Unknown</td>
<td>A/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>A/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>A/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. israelii</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
<td>A/A</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>A/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>A/A</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shigella spp</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
<td>K/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>K/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: - = Negative; + = Positive; K = Red / Pink (alkaline); A = Yellow (acidic)
in millimeters (mm).

Drug Resistance Profile of Bacterial Isolates

The isolated bacterial species were tested for antibiotics sensitivity profile against Meropenem (MEM), Moxifloxacin (MXF), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefixime (CFM) and Vancomycin (VA). Our results showed that most of the isolated bacterial species were MDR except E. coli and A. israelii which were sensitive against used antibiotics (Figure 3, Table 2).

Table 2: Antibiotic susceptibility profiling of bacterial isolates from ascitic fluid.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>MXF mm</th>
<th>CIP mm</th>
<th>CRO mm</th>
<th>CFM mm</th>
<th>VA mm</th>
<th>MEM mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>25</td>
<td>22</td>
<td>R</td>
<td>R</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>K. aerogenes</td>
<td>R</td>
<td>R</td>
<td>15</td>
<td>R</td>
<td>R</td>
<td>21</td>
</tr>
<tr>
<td>C. freundii</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>14</td>
<td>31</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>R</td>
<td>R</td>
<td>17</td>
<td>R</td>
<td>R</td>
<td>21</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>19</td>
<td>21</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>23</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>17</td>
<td>R</td>
<td>28</td>
</tr>
<tr>
<td>A. israelii</td>
<td>23</td>
<td>21</td>
<td>16</td>
<td>R</td>
<td>14</td>
<td>33</td>
</tr>
<tr>
<td>S. aureus</td>
<td>32</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>25</td>
<td>34</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>15</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>Shigella spp</td>
<td>20</td>
<td>15</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>15</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>R</td>
<td>15</td>
<td>R</td>
<td>R</td>
<td>14</td>
<td>32</td>
</tr>
</tbody>
</table>

Key: R = Resistant; mm = millimeter; MXF = Moxifloxacin; CIP = Ciprofloxacin; CRO = Ceftriaxone; CFM = Cefixime; VA = Vancomycin; MEM = Meropenem
Antibiotic Susceptibility Testing

Antibiotic susceptibility tests were performed with Mueller Hinton Agar (MHA) described by Ishaq et al. (2015) and six antibiotics were tested against isolated bacterial species. The antibiotics that were used against tested bacterial isolates showed varying results. Among six antibiotics, Meropenem showed maximum effect against all tested bacterial species. The maximum zone of inhibition against *S. aureus* (34 mm), followed by *A. israelii* (33 mm), *P. aeruginosa* (32 mm), *C. freundii* (31 mm), *S. marcescens* (28 mm), *S. epidermidis* (27 mm), *E. coli* (26 mm), *Salmonella* spp (23 mm), *K. aerogenes* (21 mm), *Enterobacter* spp (21 mm) and *Shigella* spp (15 mm). The most effective antibiotics were Meropenem followed by moxifloxacin (Figure 3).

![Figure 2: Percentage of bacterial isolates identified from ascitic fluid.](image)

![Figure 3: Zone of inhibition. (A): *S. aureus* against antibiotics; (B): *E. coli* against antibiotics; (C): *K. aerogenes* against antibiotics.](image)

4. DISCUSSION

SBP is a common bacterial infection in patients with liver cirrhosis and ascites with high morbidity and mortality rate. This infection frequently causes in the gastrointestinal tract mostly in peritoneal cavity of cancerous and non-cancerous patients due to the turbid peritoneal fluid. Turbidity of peritoneal fluid depends upon the presence of microorganisms that cause ascites. The pathogenesis of SBP is not entirely understood. A number of possible routes of infection can be proposed. The pathogenic theory of SBP states that this infection cause by the BT in which enteric...
bacteria cross the intestinal and abdominal wall to reach the peritoneal cavity and cause ascites in the peritoneal fluid. SBP is diagnosed by a PMN count ≥ 250/ml in ascitic fluid while conventional bacterial culture is sometimes negative in ascitic fluid due to prolonged antibiotics treatment. In the current study, culture sensitivity of ascitic fluid was checked on different bacterial growth media and blood culture bottles. A total of 11 bacterial species were isolated i.e., E. coli, K. aerogenes, C. freundii, Enterobacter spp, Salmonella spp, S. marcescens, A. israelii, S. aureus, S. epidermidis, Shigella spp and P. aeruginosa. This study is correlated with Alzahraa et al. (2011) and Shizoma (2018). The current study is in line with Oey et al. (2017) who evaluated that frequent and common cause of SBP; Gram-negative enteric bacteria mostly Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Klebsiella spp while Gram-positive bacteria i.e Staphylococcus aureus and coagulase-negative Staphylococcus epidermidis were less common cause. The antibiotic susceptibility testing showed that most of the bacterial isolates were resistant to the antibiotics mentioned above and mostly they were MDR. This study is confirmative with Oey et al. (2017) which stated that frequent isolates were resistant towards CRO (ceftriaxone). The current study is in confirmation with Alzahraa et al. (2011) evaluated that P. aeruginosa was MDR but sensitive towards CIP (ciproflaxcin) and S. aureus was resistant to following antibiotics i.e CFM (Cefixime) and CRO (Ceftriaxone) but sensitive towards VA (Vancomycin). Bacterial species that were isolated in the current study was E. coli (92%) followed by Shigella spp (72%), Salmonella spp (68%), Enterobacter spp (60%), K. aerogenes (52%), S. aureus (32%), S. epidermidis (30%), A. israelii (28%), P. aeruginosa (24%), C. freundii (20%) and S. marcescens (08%).

5. CONCLUSION

As there was minimum effect of pre antibiotic treatment on the patients with SBP, the present study concluded that Gram positive and Gram negative MDR bacteria were the most common cause of SBP in patients with and without Hepatitis C. The present data of culture sensitivity and resistivity showed that 11 bacterial species were isolated from 25 samples; among them 2/11 (18%) were sensitive towards used antibiotics and 9/11 (82%) were MDR. A total of six antibiotics Meropenem, Moxifloxacin, Ciprofloxacin, Ceftriaxone, Cefixime and Vancomycin were used. Among these antibiotics Meropenem which is beta-lactam broad spectrum antibiotic showed best result against different MDR bacterial isolates i.e, K. aerogenes, C. freundii, Enterobacter spp, Salmonella spp, S. marcescens, S. aureus, S. epidermidis, Shigella spp and P. aeruginosa.

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Author contributions

All authors have made a substantial direct and intellectual contribution to the work and approved it for publication. Bilal Ahmad conducted the laboratory work, M. Saqib Ishaq supervised and designed the research project. Qurban Ali, M. Atif Khan and M. Ishfaq Khan helped in writing an initial draft and the revision of the research article. Qurban Ali assisted in manuscript writing.

Conflict of interest

The authors declared no conflict of interest.

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