Original Article

Analysis of Microbiology and Antibiotic Susceptibility for Spontaneous Bacterial Peritonitis in Hepatitis C Positive and Non Positive Patients

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ABSTRACT

Objective: To analyze microbiology and antibiotic susceptibility of bacterial peritonitis in hospitalized patients with or without hepatitis C.

Study Design: Cross-sectional observational study

Place and Duration of Study: This study was conducted at the Pathology department of Bakhtawar Amin Medical & Dental College, Multan, from January to December 2023.

Methods: This study was included 114 observed patients (51 of them were hepatitis C positive), who underwent ascitic fluid specimen analysis through broth culture, agar culture, and blood culture mediums under aseptic conditions to assess bacterial growth, Gram staining, biochemical properties, and antibiotic susceptibility.

Results: Antibiotics sensitivity profile was tested to isolated bacterial species and results was shown in table. I. it was seen that C. freundii, Enterobacter spp, K. aerogenes, P. aeruginosa, S. aureus, S. epidermidis, S. marcescens, Salmonella spp, and shigella spp were the multidrug resistant. A. israelii and E. coli was the most sensitive against antibiotics. Presence of bacterial isolates from ascitic fluid was shown in figure. I. E. coli, salmonella spp and shigella spp was the most common bacterial isolates identified by ascitic fluid.

Conclusion: Gram-positive and Gram-negative MDR bacteria were common causes of SBP in patients with and without Hepatitis C. Eleven bacterial species were isolated, including C. freundii, Enterobacter spp, K. aerogenes, P. aeruginosa, S. aureus, S. epidermidis, S. marcescens, Salmonella spp, and Shigella spp, all multidrug-resistant. A. israelii and E. coli were most sensitive to antibiotics. Meropenem, a broad-spectrum beta-lactam antibiotic, showed the best results against various MDR bacterial isolates.

Key Words: Bacterial peritonitis, Antibiotic susceptibility, Hepatitis C, Microbiology

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INTRODUCTION

Ascites refers to the accumulation of fluid in the peritoneal cavity, the space between the abdominal organs[1]. Spontaneous bacterial peritonitis (SBP) is a serious condition where there's bacterial infection in this fluid. When these two conditions occur together, it can be quite concerning[2]. In SBP, ascites can play a significant role in the disease process. The fluid in the peritoneal cavity provides an environment conducive to bacterial growth[3]. However, in conditions like cirrhosis, which often leads to ascites, the normal defense mechanisms can be compromised. This creates an opportunity for bacteria to multiply and cause infection[4].

Bacteria can translocate from the gut lumen into the peritoneal cavity. This translocation is facilitated by various factors such as impaired intestinal barrier function, altered gut microbiota composition, and increased intestinal permeability, all of which are common in conditions like cirrhosis[5]. In cirrhosis, there is portal hypertension, which leads to increased pressure in the portal vein and its tributaries. This pressure can cause the formation of collateral vessels and portosystemic shunts, allowing bacteria and toxins from the gut to bypass the liver's filtering function and enter the systemic circulation and peritoneal cavity[6]. Patients with liver cirrhosis often have compromised immune function, including impaired phagocytic activity of macrophages and neutrophils. This immune dysfunction contributes to an increased susceptibility to bacterial infections, including peritonitis[7].

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The bacteria responsible for peritonitis in ascites are often those that are part of the normal gut flora, such as Escherichia coli, Klebsiella pneumoniae, Streptococcus species, and Enterococcus species. However, other pathogens may also be involved, especially in healthcare-associated peritonitis[8].

The prevalence of spontaneous bacterial peritonitis (SBP) is low, at below 3.5% in asymptomatic patients, but it can rise significantly in nosocomial infection 8%-36%[9],[10]. Hospitalized cirrhotic patients with ascites can have SBP rates as high as 30%[11], with significant morbidity and mortality associated with the condition. In ascetic fluid polymorphonuclear count ≥ 250/ml is a diagnostic marker for SBP, and approximately 40% of SBP cases show positive cultures.

The findings from this study can directly impact clinical practice by informing guidelines for empiric antibiotic therapy, highlighting the need for tailored approaches in HCV-positive individuals, and potentially identifying emerging trends in antibiotic resistance patterns that require proactive management strategies.

METHODS

This study conducted at the Pathology Department of Bakhtawar Amin Medical and Dental College, Multan from January to December 2023 included 114 observed patients (51 of them were hepatitis C positive), who underwent ascitic fluid specimen analysis through blood culture, agar culture and broth culture mediums in sterile environment to evaluate growth of bacteria, antibiotic susceptibility, biochemical properties and gram staining. Additionally, samples of fresh ascites fluid were taken through diagnostic paracentesis from patients with SBP, with ascitic fluid PMN counts exceeding 250/mm³, using sterile syringes of 20 ml.

This study included cirrhotic patients who had cloudy-colored ascitic fluid in their peritoneal cavity with a polymorphonuclear leukocyte (PMN) count greater than 250/mm³. A 10 ml sample of peritoneal/ascitic fluid was centrifuged at 2000 revolutions per minute (RPM) for at least 10 minutes. The inoculated cultures were then placed in a controlled environment, typically an incubator set at 37°C (body temperature), to mimic the conditions conducive to microbial growth. The incubation period lasted for 72 hours, allowing sufficient time for the organisms to multiply and form visible colonies or growth in the broth.

Two 10 ml portions of peritoneal fluid were centrifuged at for 10 minutes at > 2000 RPM to concentrate them and then placed in thioglycolate medium. Subsequently, cultures from Brain Heart Infusion (BHI) were utilized for subcultures, which were then inoculated onto blood agar media. These media plates were finally incubated at 37°C for 24 hours to allow bacterial growth and observation.

Different subcultures were performed using specific agar media and incubation conditions to support the growth and differentiation of various bacterial species. MacConkey agar was utilized to promote the growth of Gram-negative bacteria and differentiate between lactose fermenters and non-fermenters after incubation at 37°C for 24-48 hours. Eosin Methylene Blue (EMB) agar was used to identify Escherichia coli growth following incubation at 37°C for 18-24 hours. The Salmonella Shigella (SS) agar method was employed for the identification and differentiation of Salmonella and Shigella species, with incubation at 37°C for 12 to 18 hours. Mannitol Salt Agar (MSA) was used to obtain positive cultures of gram-positive bacteria like S. aureus and S. epidermidis, incubated at 37°C for 18 to 24 hours. Finally, Pseudomonas Cetrimide Agar (PCA) selective media were used for isolating gram-negative bacteria such as Pseudomonas aeruginosa, with incubation at 37°C for 48 hours. At the end bacterial growth was examined with Gram's staining method and antibiotic susceptibility was examined with Kirby-Bauer disc diffusion technique.

RESULTS

Antibiotics sensitivity profile was tested to isolated bacterial species and results was shown in table. I. it was seen that C. freundii, Enterobacter spp, K. aerogenes, P. aeruginosa, S. aureus, S. epidermidis, S. marcescens, salmonella spp, and shigella spp were the multidrug resistant. Whereas A. israelii and E. coli was the most sensitive against antibiotics. (Table. I).

Presence of bacterial isolates from ascitic fluid were shown in figure. I. E. coli, salmonella spp and shigella spp was the most common bacterial isolates identified by ascitic fluid. (Figure. I).

Table No. 1: Antibiotic susceptibility profile with respect to bacterial isolates

Bacterial isolates	MXF (mm)	CIP (mm)	CRO (mm)	CFM (mm)	VA (mm)	MEM (mm)
A. israelii	111	99	58	R	52	66
C. freundii	R	R	R	R	54	68
E. coli	112	101	45	R	43	57
Enterobacter spp	R	R	43	R	R	41
K. aerogenes	R	R	53	R	R	74
P. aeruginosa	R	62	R	R	48	86
S. aureus	76	R	R	R	105	104

S. epidermidis	R	R	R	R	74	59
S. marcescens	R	R	R	74	R	86
Salmonella spp	98	106	R	R	R	104
Shigella spp	113	56	R	R	R	76

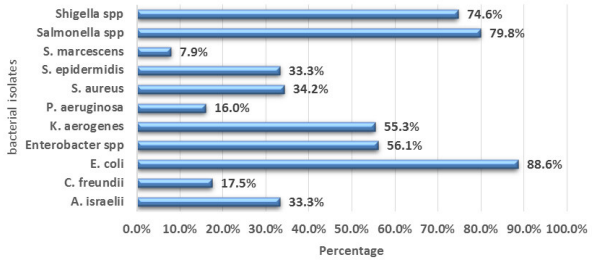

Figure No. 1: Presence of bacterial isolates from ascitic fluid

DISCUSSION

Spontaneous bacterial peritonitis (SBP) is a common infection in liver cirrhosis patients with ascites, affecting the gastrointestinal and peritoneal areas. The turbidity of peritoneal fluid depends on microorganism presence due to bacterial translocation (BT), where enteric bacteria migrate through intestinal and abdominal walls to infect the peritoneal cavity, leading to ascites.

In this study, the culture sensitivity of ascitic fluid was assessed using various bacterial growth media and blood culture bottles, resulting in the isolation of a total of 11 bacterial species. This investigation aligns with previous studies by Ahmad et al[12] who isolated same number and species of bacteria from ascites fluid and found E. coli most prevalent among them.

This study aligns with previous research conducted by Oey et al[13] and Shizuma et al[14] which evaluated the frequent and common causes of spontaneous bacterial peritonitis (SBP). They found that Gram-negative enteric bacteria, mostly Klebsiella spp, Pseudomonas aeruginosa, Enterobacter aerogenes and E. coli were the primary culprits, while Gram-positive bacteria like Staphylococcus epidermidis and coagulase-negative Staphylococcus aureus were less commonly associated with SBP.

Most of the bacterial isolates from the antibiotic susceptibility testing were found to be resistant, particularly displaying multidrug resistance (MDR) and resistance specifically to ceftriaxone. However, Fernández et al[15] reported that intravenous ceftriaxone shows greater efficacy compared to oral norfloxacin and others. Treatment recommendations for spontaneous bacterial peritonitis (SBP) vary based on whether the infection is nosocomial or community-acquired, as evidenced by studies such as Lutz et al[16] which found that about one third of healthcare-related and nosocomial SBP infections showed resistance to third-generation cephalosporins.

Ariza et al[17] findings revealed varying rates of resistance to third-generation cephalosporins in different categories: 7.1% in community-acquired SBP, 21.1% in health care-related SBP, and 40.9% in nosocomial SBP, based on 246 episodes observed in 200 patients with LC and SBP between 2001 and 2009. Due to these findings, Tandon et al[18] suggest refraining from using third-generation cephalosporins as empirical treatment for health care-related SBP.

In a recent randomized, controlled study, it was found that empirical treatment of nosocomial spontaneous bacterial peritonitis (SBP) with meropenem plus daptomycin showed higher effectiveness in resolving SBP and demonstrated better 3-month survival rates compared to treatment with third-generation ceftazidime, particularly in cases involving Gram-positive Enterococci resistant to vancomycin and MRSA[19,20].

CONCLUSION

Gram-positive and Gram-negative MDR bacteria were common causes of SBP in patients with and without Hepatitis C. Eleven bacterial species were isolated, including C. freundii, Enterobacter spp, K. aerogenes, P. aeruginosa, S. aureus, S. epidermidis, S. marcescens, Salmonella spp, and Shigella spp, all multidrug-resistant. A. israelii and E. coli were most sensitive to antibiotics. Meropenem, a broad-spectrum beta-lactam antibiotic, showed the best results against various MDR bacterial isolates.

Author's Contribution:

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Drafting or Revising Critically:	Kanwar Sajid Ali, Muhammad Umar Ali Khan, Amjad Ali
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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