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Clinical significance of anaerobic microbiology in ascites of chronic liver disease

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ABSTRACT

Background: Hundreds of anaerobes are part of the normal flora of the skin, mouth, and gastrointestinal tract. Disruption can lead to severe infections. Anaerobes are rarely reported in peritoneal fluid due to cultivation challenges. Spontaneous bacterial peritonitis (SBP) is a major cause of morbidity and mortality in chronic liver disease (CLD). **Objective:** To determine the clinical significance of anaerobic bacteria in the ascitic fluid of CLD patients. **Methods:** A prospective study at a tertiary care institute included 200 CLD patients with ascites. Ascitic fluid samples were cultured for anaerobic bacteria using Robertson's cooked meat media and identified with VITEK 2 system. Antimicrobial susceptibility was tested with E-tests in ascitic fluid were included in the study. **Results:** Anaerobic bacteria were found in 7 of 200 samples (3.5%). Isolated bacteria included *Clostridium subterminale*, *Clostridium difficile*, *Clostridium sordellii*, *Clostridium perfringens*, *Staphylococcus saccharolyticus*, *Clostridium* group, and *Actinomyces naeslundii*. High resistance was noted for ciprofloxacin. Imipenem and vancomycin showed 85% sensitivity, while chloramphenicol, tetracycline, and linezolid showed 71% sensitivity. **Conclusion:** Anaerobic bacteria can cause SBP in CLD patients. Routine paracentesis should be emphasized for effective diagnosis and treatment. High antibiotic resistance rates call for careful use and monitoring of antibiotics.

Introduction

Hundreds of species of anaerobes are part of the normal commensal flora of the skin, mouth, and gastrointestinal tract [1]. If this commensal correlation is interrupted, anaerobic bacteria can cause infections with high morbidity and mortality [2]. Anaerobes are not commonly reported from the peritoneal fluid as their cultivation is a challenge and antibiotic testing of anaerobic bacteria is exacting.

Spontaneous bacterial peritonitis (SBP) is defined as an infection of initially sterile ascitic fluid

(AF) in the absence of any intra-abdominal surgically treatable source of infection [3]. SBP is a major cause of mortality and morbidity. The World Health Organization has projected that CLD is responsible for 1.1% of all deaths worldwide [4]. According to a recent meta-analysis, the mortality rate in patients with SBP was as high as 44.4% from 1978-1999 but dropped to 31.5% from 2000 to 2009 [5]. Excessive alcohol consumption, obesity, and hepatitis infection were all preventable causes of CLD, according to the Chief Medical Officer's 2012

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annual report [6]. In the Western world, liver damage due to excessive alcohol consumption is the most common cause of CLD, whereas CLD caused by hepatitis B infection is more common in Asia [7]. Sydney M. Finegold describes anaerobes as microorganisms unable to grow on solid media in an atmosphere containing 18% O₂ and 10% CO₂ [8]. It has been postulated that aerobic bacterial infection is more common in patients with liver disease followed by anaerobes [9]. This study aimed to find the clinical significance of anaerobic bacteria in the ascites of chronic liver disease patients by isolating them in the laboratory.

Material and methods

The present prospective study was conducted in Vardhman Mahavir Medical College & Safdarjung Hospital New Delhi. Ethical approval was obtained from the institutional ethics committee (IEC/VMMC/SJH/THESIS/2019-10/205). A total of 200 patients with a confirmed diagnosis of chronic liver disease admitted in an emergency as well as indoor patients in the Medicine Department were included in the study. Chronic liver disease was diagnosed based on clinical history, physical examination, laboratory tests including liver function tests (LFTs), ultrasound imaging, and, where necessary, liver biopsy [1]. Criteria included elevated liver enzymes (AST, ALT), evidence of liver dysfunction (e.g., coagulopathy, hypoalbuminemia), imaging findings consistent with liver cirrhosis or fibrosis, and confirmation of etiology such as viral hepatitis markers or history of alcohol abuse. The sample size was determined based on the prevalence of anaerobic bacteria in SBP from previous studies, targeting a confidence level of 95% and a margin of error of 5%. Using the formula for sample size calculation for proportions, $n = Z^2 \times P \times (1-P) \div d^2$ where $Z = 1.96$ for 95% confidence, P is the estimated prevalence (3.5%) [10], and d is the margin of error (0.05). This resulted in a required sample size of approximately 200 patients.

All patients with CLD and ascites, above 18 years of age and of either gender was enrolled. The ascitic fluid samples were received in a microbiology laboratory in a Robertson's cooked

meat media for anaerobes from suspected cases of spontaneous bacterial peritonitis in chronic liver disease. After 48 hours of incubation of Robertson's cooked meat media (RCM), subculture was done on 5% sheep blood agar, hemin blood agar, and kanamycin-vancomycin laked blood agar. All cultured plates were placed in an anaerobic jar. Anaerobiosis was generated by Anaero Gas Pack sachet in a sealed anaerobic jar. The anaerobiosis was observed using a biological indicator i.e., *Pseudomonas aeruginosa*, which is a strict aerobe. Following incubation, after 24-48 hours, if growth was present, colony characteristics were observed. Identification was done using the automated VITEK 2 microbial identification system. Antimicrobial susceptibility testing was performed by Gradient Tests (E-Test). The bacterial inoculum was homogeneously spread over a blood agar plate supplemented with 5% sheep blood. After a 15-minute E-strip placement, the minimum inhibitory concentration (MIC) was noted after 24-48 hours of anaerobic incubation and interpreted as recommended by CLSI.

Results

During the 2 years of the study period, 200 ascitic fluid samples were analysed. Anaerobic bacterial growth was observed in 7/200 (3.5%) samples. The anaerobic bacteria responsible for spontaneous bacterial peritonitis were *Clostridium subterminale* (1), *Clostridium difficile* (1), *Clostridium sordellii* (1), *Clostridium perfringens* (1), *Staphylococcus saccharolyticus* (1), *Clostridium* group (1), and *Actinomyces naeslundii* (1). The MIC of penicillin, ampicillin, doripenem, imipenem, meropenem, metronidazole, cefotaxime, chloramphenicol, tetracycline, moxifloxacin, cefixime, ciprofloxacin, trimethoprim-sulfamethoxazole, linezolid, ceftioxin, and vancomycin was determined using E-strip.

The antibiotic resistance pattern showed that all isolates were resistant to ciprofloxacin. The anaerobic bacteria were 85% sensitive to imipenem and vancomycin, followed by 71% sensitivity to chloramphenicol, tetracycline, and linezolid. Detailed antibiogram findings are shown in **table (1)**.

Table 1. Detailed antibiogram findings.

E-strip	Sensitive %	Resistant %
Penicillin	28.57	71.43
Ampicillin	14.28	85.72
Doripenem	28.57	71.43
Imipenem	85.72	14.28
Meropenem	42.85	57.15
Metronidazole	14.28	85.72
Cefotaxime	42.85	57.15
Chloramphenicol	71.43	28.57
Tetracycline	71.43	28.57
Moxifloxacin	28.57	71.43
Ciprofloxacin	0	100
Trimethoprim-sulfamethoxazole	14.28	85.72
Linezolid	71.43	28.57
cefixime	28.57	71.43
cefoxitin	28.57	71.43
vancomycin	85.72	14.28

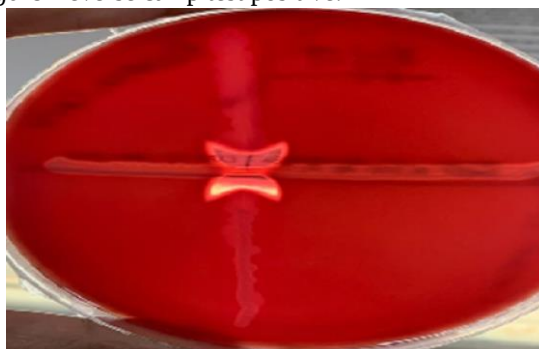
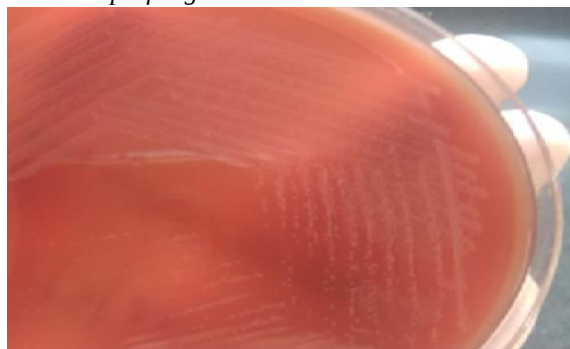
Figure 1. *Clostridium perfringens*- reverse camp test positive.**Figure 2.** Culture plate of *Clostridium perfringens***Figure 3.** Culture plate of *Clostridium difficile*.

Figure 4. Gram stain of *Clostridium difficile*

Discussion

In this study, anaerobic bacteria were isolated in 3.5% of the ascitic fluid samples from patients with chronic liver disease (CLD), indicating their potential role in spontaneous bacterial peritonitis (SBP). Previous studies have also suggested that anaerobes, although less frequently isolated, can contribute to SBP cases [10,11]. Finegold SM reported that anaerobic bacteria are part of the normal human flora and can cause severe infections if the commensal balance is disturbed [1]. This aligns with findings by **Kawale et al.** who noted that SBP occurs in up to 30% of hospitalized patients with CLD and carries significant mortality despite antibiotic treatment [12]. Various case reports, including one by **Ted Butler et al**, have also isolated anaerobic bacteria such as *Clostridium tertium* in clinical infections [13].

Anaerobes and mortality in peritonitis

Anaerobic infections in the context of peritonitis are often associated with high morbidity and mortality rates. Finegold and others have emphasized that anaerobes, although less frequently isolated from clinical samples, can lead to severe and sometimes fatal infections, particularly in immunocompromised patients such as those with CLD [2, 3]. The ability of anaerobic bacteria to thrive in low-oxygen environments, coupled with their inherent resistance to many common antibiotics, makes these infections particularly challenging to treat.

The mortality rate associated with SBP remains significant. Studies have shown that the overall mortality rate for SBP ranges from 20% to 40% despite advancements in antibiotic therapy and supportive care [3-5]. The presence of anaerobic bacteria in ascitic fluid complicates the clinical

picture, as these organisms are not only difficult to culture but also exhibit high levels of antibiotic resistance. The identification of anaerobes in this study suggests that these pathogens contribute to the persistence and severity of SBP, necessitating a comprehensive approach to diagnosis and treatment.

The clinical implications of anaerobic infections in SBP are profound. The high resistance rates observed in this study, particularly to ciprofloxacin, highlight the necessity for judicious use of antibiotics and the need for routine surveillance of antimicrobial resistance patterns. The findings advocate for the inclusion of anaerobic coverage in empirical antibiotic regimens for SBP, especially in regions with high prevalence of antibiotic-resistant strains.

Routine diagnostic paracentesis in CLD patients with ascites is crucial for early detection of SBP and prompt initiation of appropriate antimicrobial therapy. The inclusion of anaerobic cultures in routine diagnostic protocols can improve the detection of these elusive pathogens and guide targeted therapy. This approach can potentially reduce the morbidity and mortality associated with SBP.

Spontaneous bacterial peritonitis (SBP) involves the translocation of bacteria from the gut into the ascitic fluid. Anaerobes, being part of the normal intestinal flora, can translocate under certain conditions such as increased intestinal permeability, bacterial overgrowth, and impaired immune response [4-6]. Factors such as prolonged use of proton pump inhibitors (PPIs), which alter the gut microbiome and decrease gastrointestinal motility, can predispose CLD patients to both aerobic and anaerobic bacterial infections [5]. Despite the predominantly anaerobic intestinal flora, anaerobes

rarely cause SBP due to their inability to translocate the intestinal mucosa and the high oxygen content in the intestinal wall [14]. However, the use of PPIs in CLD patients can lead to bacterial overgrowth in the small intestine, changes in the gut microbiome, and decreased gastrointestinal motility, increasing the risk of both aerobic and anaerobic infections [15].

Anaerobic culture can take several days before antimicrobial susceptibility testing can be carried out. Many laboratories do not routinely perform these expensive tests. The isolation of anaerobic bacteria in this study thus emphasizes the need for improved diagnostic methods and tailored treatment strategies in managing SBP in CLD patients.

Conclusion

In conclusion, anaerobic bacteria play a significant role in SBP among CLD patients, emphasizing the need for heightened clinical suspicion, routine microbiological evaluation, and targeted antibiotic therapy. The study underscores the challenges posed by antibiotic resistance in anaerobic infections and advocates for a prudent approach to antibiotic use in clinical practice. Routine diagnostic paracentesis, including anaerobic cultures, should be a standard practice to ensure early detection and appropriate management of SBP in CLD patients.

Competing interests

Not declared.

Funding

No funding was received for this study.

Data availability

All data generated or analyzed during this study are included in this published article.

Authors' contribution

All authors made significant contributions to the work presented, including study design, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

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