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Bacteroides fragilis-Associated Intra-Abdominal Abscess Following Penetrating Trauma

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ABSTRACT

Background: *Bacteroides fragilis* is a clinically significant anaerobic bacterium known to cause abdominal infections, abscesses, sepsis, and skin and soft tissue infections. We report a case of an intra-abdominal wound infection following a penetrating injury, attributed to *B. fragilis*.

Methods: Foul-smelling pus from the abdominal wound was collected and sent for anaerobic culture using Robertson's cooked meat medium. Gram staining and culture were performed on blood agar supplemented with haemin and vitamin K1. The sample's gram stain revealed gram-negative bacilli.

Results: Colony growth was observed on blood agar after 48 hours and was identified as *B. fragilis* through phenotypic biochemical tests, MALDI-TOF MS, and real-time PCR. The patient was successfully managed with metronidazole.

Conclusion: Prompt diagnosis and early treatment were crucial in reducing the morbidity and mortality associated with this infection.

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Introduction

Bacteroides fragilis is an anaerobe which forms part of normal human colon microbiota. The current *Bacteroides fragilis* group (BFG) has >20 distinct *Bacteroides*, *Parabacteroides* and *Phocaeicola* species which accounts for many anaerobic clinical infections (1). The spread of *B. fragilis* in blood or adjacent tissues has been responsible for the infections like intracavitary abscesses, surgical site infections, diabetic foot infections, peritonitis, sepsis (1, 2).

B.fragilis is obligate gram-negative bacilli responsible for causing intra-abdominal sepsis as the most typical infection. As part of normal gut flora, this pathogen penetrate the sterile peritoneal cavity following inflammation, injury or surgery (intestinal disruption, diverticula rupture, or other perforations brought on by surgical wounds, cancers, or appendicitis) (2). The virulence factors include histolytic enzymes, which destroy tissue, tissue adhesion through fimbriae and agglutinins, polysaccharide capsules, lipopolysaccharides, and a range of enzymes that help to avoid oxygen toxicity and phagocytosis (2).

In the chronic stage of infection, anaerobic *Bacteroides* species start to predominate once enough oxygen has been removed to allow for their replication. If left untreated the mortality rate can be increased to 60%. Therefore, effective management is necessary (3).

Case report

A 20 years male came to emergency with penetrating trauma in left side of lower abdomen on 08/02/2024. On examination, he was conscious and oriented. His vitals were stable and Sp02 was 99%. There was 0.5-1 cm penetrating wound over left iliac region. Chest X-ray was done which had shown air under diaphragm (Figure 1A). Abdominal X-ray showed multiple air-fluid level in abdomen (Figure 1B).

Exploratory laparotomy was done with primary repair of colonic perforation. Patient was put on Inj. Ceftriaxone 1gm BD and Inj. Amikacin 500 mg BD. The patient developed tenderness in site of penetrating trauma with purulent discharge. The foul smelling pus sample was sent to microbiology laboratory for anaerobic culture on 18/02/2023. The sample was transported and cultured within 1 hour. The culture was done on blood agar with 5% haemin and vitamin K1 and incubated in anaerobic jar at 37 °C under anaerobic conditions.

The gram staining from the sample was performed which had shown gram negative bacilli. After 48 hours, the colonies on Blood agar plates were seen to be 2-3 mm in diameter, circular, convex and grey to white in colour. The gram staining from the colony had also shown gram negative bacilli. According to our laboratory protocol metronidazole (5µg) disks are applied to all anaerobic culture plates as a screening method, this strain was found to be sensitive to metronidazole (sensitive >= 25 mm zone diameter according to EUCAST guidelines, Figure 1C).

The standard biochemicals were put and diagnosis of *B. fragilis* was confirmed along with automated test i.e. MALDI-TOF MS (VITEK® MS by bioMérieux, score of 100%) and real-time Polymerase Chain Reaction as shown in Figure 1D-E respectively. Real-time PCR was done by SYBR Green method by using primer and cycle conditions (5 min at 94 °C to denature the DNA, followed by 40 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min and strand extension at 72 °C for 2 min on a real-time thermal cycler) as described by Papaparaskevas J et al.(4).

The patient was put on metronidazole 500 mg TDS and granulation tissue started forming on the injury site. His condition improved due to healing of wound and he got discharged after few days.

Results

The gram staining from the sample was performed which had shown gram negative bacilli. After 48 hours, the colonies on Blood agar were seen to be 2-3 mm in diameter, circular, convex and grey to white in colour. The gram staining from the colony had also shown gram negative bacilli. According to our laboratory protocol metronidazole (5µg) disks are applied to all anaerobic culture plates as a screening method, this strain was found to be sensitive to metronidazole (sensitive >= 25 mm zone diameter according to EUCAST guidelines, Figure 1C).

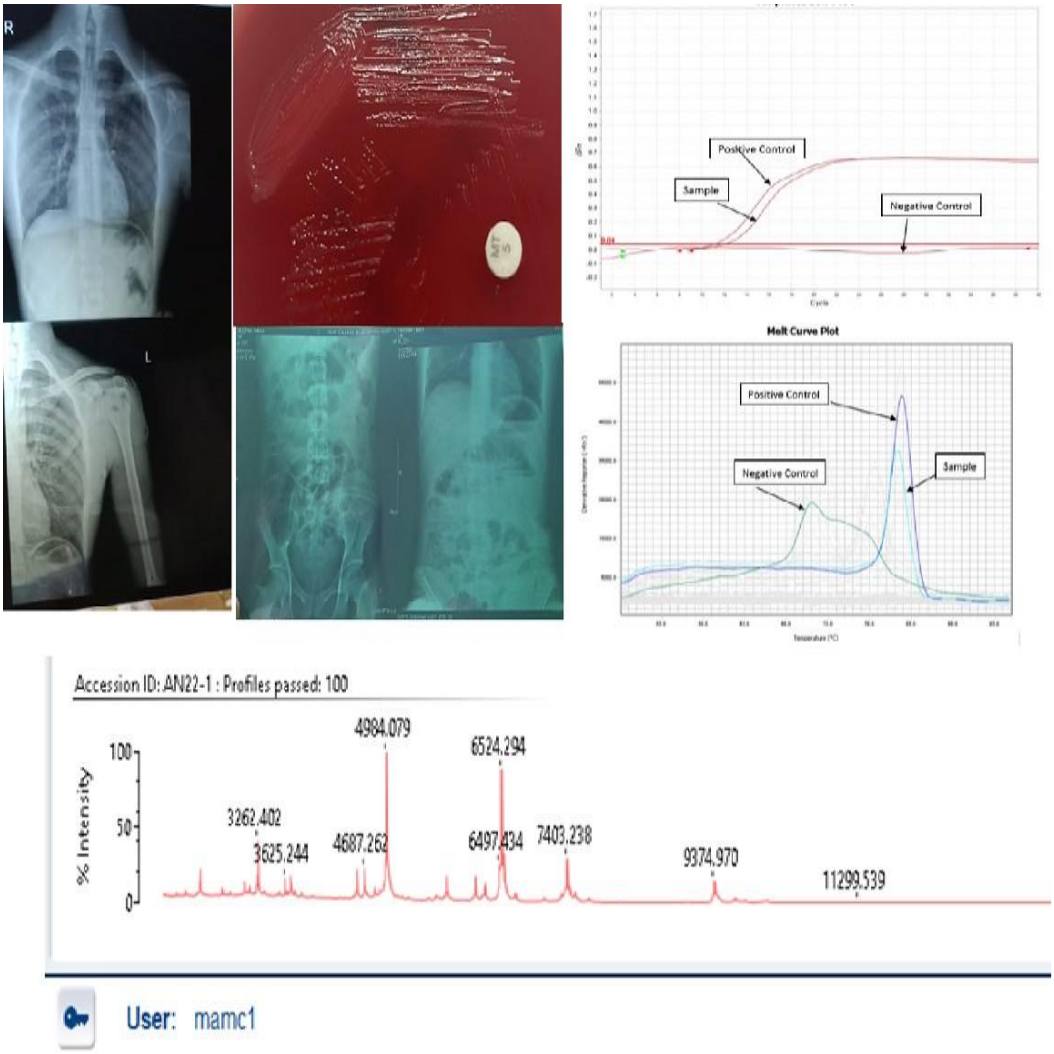

Fig 1. A. X-ray of Chest PA view showing air under diaphragm on left side, B. X-ray of Abdomen with multiple air fluid levels, C. Growth of *B. fragilis* on Blood agar, D. Graph showing protein peaks of identification of *B. fragilis* by MALDI-TOF MS, E. Amplification curve plot of real-time PCR with sample and controls, F. Melt Curve plot of real-time PCR with sample and controls.

Table 1. Cases of *B.fragilis* identified in last 20 years

TYPE OF INFECTION	References
Endophthalmitis	8
Septic abortion presenting as a right lower trapezius abscess	7
Intra-abdominal abscess	9
Enterotoxigenic diarrhoea	10
Endocarditis	11
Septic thrombophlebitis of superior mesenteric vein	12
Endocarditis	13
Septic arthritis	14
Vertebral Osteomyelitis	15
Portal vein and superior mesentery vein thrombosis secondary to antithrombin III and protein C deficiency	16

The standard biochemicals were put and diagnosis of *B. fragilis* was confirmed along with automated test i.e. MALDI-TOF MS (VITEK® MS by bioMérieux, score of 100%) and real-time Polymerase Chain Reaction as shown in Figure 1D-E respectively. Real-time PCR was done by SYBR Green method by using primer and cycle conditions (5 min at 94 °C to denature the DNA, followed by 40 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min and strand extension at 72 °C for 2 min on a real-time thermal cycler) as described by Papaparaskevas J et al.[4].

The patient was put on metronidazole 500 mg TDS and granulation tissue started forming on the injury site. His condition improved due to healing of wound and he got discharged after few days.

Discussion

The human colon has the highest concentration of anaerobes in the body which has maximum bacteria of *Bacteroides* genus [5]. Bacteroides fragilis is presently thought to be the most virulent pathogen which is present in about 60% of clinical samples of gastrointestinal tract infection followed by *B. thetaiomicron* (17%), *B. ovatus* (<8%) and other species (*B. vulgatus* and *Parabacteroides distasonis*) (5).

Clinically, intra-abdominal abscesses frequently develop after situations that result in bowel perforation and subsequent colonic contents leakage into the abdomen. *Bacteroides fragilis* is the cause of the majority of clinical cases of anaerobic sepsis and intra-abdominal abscesses (6). In this case there has been colonic perforation caused due to the trauma to the abdomen which has thus further led the patient prone to intra-abdominal abscess.

Surgical manipulation of the gastrointestinal tract is an important risk factor due to the abundance of *Bacteroides* spp. in the gut flora besides other aerobes and anaerobes causing abdominal infections. If the intestinal barrier is compromised by surgery, trauma, malignancy and ischemia, *B. fragilis* may escape its gastrointestinal niche and become a dangerous pathogen causing infections or illness (6). Since in this case, the infection is caused to the colon during penetrating wound injury so there is high probability of intra-abdominal abscess which has occurred after trauma and repair of colon by surgery which could have further led to the pus discharge from the site of the wound.

The clinical manifestations of *Bacteroides fragilis* include diarrhoea, synergistic anaerobic infections and necrotizing fasciitis. Unchecked local infection or presence of central line can

spread the bacteria in the blood causing sepsis (7). The various cases since 15 years are shown in Table 1.

The anaerobic pathogen should be considered in the diagnostic protocols and therefore the suspected sample should be processed for anaerobic culture on routine basis so that anaerobic organism like *Bacteroides fragilis* can be diagnosed accurately. Due to quick turnaround time and excellent sensitivity, molecular test (PCR) can help in improved diagnosis (4). It typically only takes a few minutes for MALDI-TOF MS to quickly identify species of various microorganisms, greatly reducing the detection time and increasing the diagnostic effectiveness of infectious diseases (17).

Metronidazole is considered as the preferred antimicrobial medication against the B.fragilis (18). The other antibiotics which can be given for treating B.fragilis infections are clindamycin, fluoroquinolones and tigecycline (19).

Conclusion

In conclusion, the present case highlights the need of anaerobic culture in abdominal infections particularly involving penetration wound so that anaerobes like Bacteroides fragilis and others can be identified and targeted antibiotic therapy can be started. This will significantly reduce morbidity and mortality in such patients.

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Ethics approval and consent to participate

Written consent to publish manuscript was obtained from the patient.

Conflict of interest

Oves Siddiqui (First Author), Ashish William (corresponding author), Nilakshi Gupta, Swati Nirmal, Sonal Saxena, Prabhav Aggarwal declare that they have no conflict of interest.

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