

Clinico-microbiological spectrum of anaerobic pyogenic infections in an Indian tertiary care teaching hospital: A two-year study

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ABSTRACT

Introduction: Anaerobes are important however the most neglected pathogens. Timely isolation of anaerobes can guide the clinician about the correct course of clinical treatment and thus reduce the mortality and also the problem of antimicrobial resistance. **Materials and Methods:** Tissue and/or pus aspirates were collected aseptically from infectious sites in the Robertson's cooked meat medium (RCM) and sent to anaerobic bacteriology laboratory for culture. Subcultures from RCM for each sample were done on neomycin blood agar and 5% sheep blood agar along with metronidazole disc (5µg). The plates were incubated in an anaerobic jar using GasPak for 72 hrs. The preliminary identification was performed by standard biochemical tests for both obligate and facultative anaerobic isolates. Speciations of obligate anaerobes were performed by Vitek 2 automated system. **Results:** Obligate anaerobes either single or polymicrobial were obtained in 38/216 (14.5 %) samples processed during the study period. Polymicrobial infections were reported in 21/216 (55.26%) samples and most commonly with obligate anaerobic gramnegative bacilli i.e. *Prevotella-Porphyrmonas* and *Bacteroides fragilis* group. Most common monomicrobial anaerobic infections were observed with *Veillonella spp.* (n=4) and *Porphyrmonas spp.* (n=4) followed by *Bacteroides fragilis* (n=3). Obligate anaerobes were predominantly isolated from skin and soft tissue infections (n=14) followed by surgical site infections (n=8). **Conclusion:** Although most of the infections are polymicrobial, a rise in the incidence of monomicrobial anaerobic infections has been noticed. Therefore, the performance of anaerobic cultures along with aerobic cultures is much needed for complete bacterial work-up of specimens from infectious sites and better patient management.

Keywords: Anaerobes, pyogenic infections

Introduction

Anaerobic bacteria are causative agents for numerous infections involving different body sites such as oro-dental, intra-abdominal, pulmonary, gynaecological/obstetric, post-operative wound infections and various skin and soft tissue infections.^[1] These

infections are mainly endogenous. The most commonly reported anaerobes in clinical specimens include *Bacteroides fragilis* group, *Clostridium spp.*, pigmented *Prevotella* and *Porphyrmonas* group, *Peptostreptococcus spp.*, *Fusobacterim spp.*, and *Actinomyces spp.*^[1] However, variations in the distribution and isolation rate of anaerobic isolates have been reported across the globe depending on the infected anatomic sites.^[2-4] The infections can be serious and life-threatening but often overlooked. Though mostly polymicrobial, a rise in the incidence of monomicrobial infections due to anaerobes has been observed in recent times.^[2-5] Their accurate identification is important considering the innate resistance of anaerobes to selective antimicrobial

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agents.^[6] Emerging antimicrobial resistance to commonly used antimicrobial agents such as metronidazole, clindamycin, penicillin etc., has also been reported.^[6] Timely isolation and identification of anaerobes can guide the clinician about the correct course of clinical treatment and thus reduce the morbidity and length of hospital stay. However, anaerobes remain the most neglected pathogens due to laborious culture techniques with longer turn-around times. The delay in isolation of anaerobic bacteria from the specimen often leads to early initiation of broad-spectrum antibiotics which ultimately worsen the problem of antimicrobial resistance.

The knowledge about the spectrum of anaerobic bacteria with various pyogenic infections helps to decide the empirical antibiotic therapy for better management of patients. Hence, the present study was conducted to study the role of anaerobic bacteria and their profile in various pyogenic infections.

Materials and Methods

Ethical considerations

The study was initiated after obtaining approval from the Institutional Human Ethics Committee.

A cross-sectional observational study was conducted in the anaerobic bacteriology laboratory, department of microbiology attached to a tertiary care teaching institute for two years from April 2018 to March 2020. The demographic details and clinical diagnosis were recorded from medical records. Specimens such as tissue and pus aspirates collected aseptically from superficial or deep-seated pyogenic infections in the Robertson's cooked meat medium (RCM) and sent to anaerobic bacteriology laboratory for bacterial culture. Wound swabs were rejected. If the specimens were transported to the laboratory in a sterile syringe, the aspirates were immediately transferred into the RCM medium in the lab.

The samples were subjected to gram staining and anaerobic culture. The staining was performed by Kopeloff's - Beerman Modification of gram staining for anaerobic bacteria.^[7] Subcultures from RCM for each sample was done on both selective media and non-selective media i.e., neomycin blood agar (NBA) and 5% sheep blood agar respectively. A metronidazole disc (5 µg) was kept in each subculture plate for preliminary identification [Figure 1]. The plates were incubated anaerobically in an anaerobic jar using GasPak (HiMedia, Mumbai) for 72 hrs.

The preliminary identification was performed by the conventional method for both obligate^[7] and facultative anaerobic isolates.^[8] Obligate anaerobes were preliminarily identified based on gram staining morphology using Kopeloff's-Beerman modification, aerotolerance test, special disc potency testing (Kanamycin 1000 µg, Vancomycin 5 µg and Colistin 10 µg), biochemical identification parameters such as catalase test using 15% H₂O₂,

Table 1: Demographic profile of study subjects (n=38)

Characteristics	No. of patients	%
Age (yrs)		
01-20 Y	10	26.31
21-40 Y	11	28.94
41-60 Y	11	28.94
60-80 Y	6	15.78
Gender		
Male	24	63.15
Female	14	36.84
Type of Specimen		
Aspirates	32	84.21
Tissue sample	6	15.78
Type of microbial growth		
Monomicrobial anaerobic growth	17	44.73
Polymicrobial (Obligate anaerobe + Facultative anaerobe) growth	21	55.26

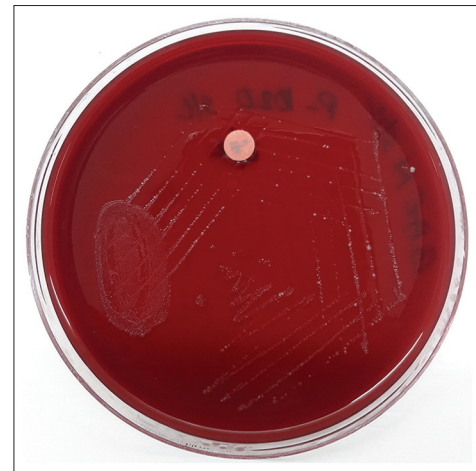


Figure 1: Brucella Blood Agar plate showing Polymicrobial bacterial growth of obligate and facultative anaerobes (Obligate anaerobes showing zone of inhibition around metronidazole disc)

indole test, urease test, nitrate reduction test, sugar fermentation tests in Viande-Levure broth, growth in the presence of 20% bile, esculin hydrolysis, lipase and lecithinase production and susceptibility to sodium polyanethol sulfonate (SPS). Vitek 2 automated system (bioMerieux Inc., USA) was used for species identification. The RCM broth and inoculated culture plates were discarded if no growth was observed after seven days of incubation.

Statistical analysis

Data was entered in MS Excel and descriptive statistics such as frequencies were calculated.

Results

A total of 262 samples were processed for anaerobic culture during the study period. Obligate anaerobic isolates either single or polymicrobial were obtained in 38 (14.5%) samples. Although polymicrobial anaerobic infections by obligate and facultative

Table 2: Microbiological Profile of Anaerobic Isolates

Organisms Isolated	No. of Isolates
Monomicrobial Growth (Only Obligate Anaerobes)	
<i>Porphyromonas</i> spp.	04
<i>Veillonella</i> spp.	04
<i>B. fragilis</i> group	03
<i>Peptostreptococci</i> spp.	02
<i>Peptoniphilus asaccharolyticus</i>	01
<i>Anaerococcus prevotii</i>	01
<i>Prevotella disenes</i>	01
<i>Clostridium difficile</i>	01
Total	17
Polymicrobial growth (Obligate Anaerobe + Facultative Anaerobe)	
<i>Porphyromonas</i> spp. + <i>Escherichia. Coli</i>	01
<i>Porphyromonas</i> spp. + <i>S. aureus</i>	01
<i>Porphyromonas</i> spp. + <i>Citrobacter</i> spp.	01
<i>Porphyromonas</i> spp. + <i>Klebsiella pneumonia</i>	01
<i>Prevotella disenes</i> + <i>Escherichia coli</i>	01
<i>Prevotella bivia</i> + <i>Escherichia coli</i>	01
<i>Prevotella oris</i> + <i>Escherichia coli</i>	01
<i>Prevotella oralis</i> + <i>Escherichia coli</i>	01
<i>Prevotella intermedia</i> + <i>Escherichia coli</i>	01
<i>Bacteroides fragilis</i> + <i>Enterobacter</i> spp.	01
<i>Bacteroides stercoris</i> + <i>Escherichia coli</i>	01
<i>Bacteroides vulgatus</i> + <i>Escherichia coli</i>	01
<i>Bacteroides fragilis</i> group + <i>Enterococcus</i> spp.	01
<i>Bacteroides fragilis</i> + <i>Escherichia coli</i>	01
<i>Veillonella</i> spp.+ <i>Proteus mirabilis</i>	01
<i>Veillonella</i> spp. + <i>S. aureus</i>	01
<i>Veillonella</i> spp. + <i>Klebsiella pneumonia</i>	01
<i>Veillonella</i> spp. + <i>Klebsiella pneumonia</i>	01
<i>Parabacteroides distasonis</i> + <i>Escherichia coli</i> + <i>Proteus mirabilis</i>	01
<i>Clostridium clostridioformie</i> + <i>Escherichia coli</i>	01
<i>Finegoldia magna</i> + <i>Klebsiella pneumonia</i>	01
Total	21

anaerobes ($n = 21$) were more, infections exclusively by only obligate anaerobes were reported in a significant number of patients ($n = 18$) [Table 1].

Most common anaerobic bacteria which were isolated as monomicrobial infection included *Veillonella* spp. ($n = 4$) and *Porphyromonas* spp. ($n = 4$) followed by *Bacteroides fragilis* ($n = 3$). Mixed infections of obligate anaerobes with facultative anaerobes were obtained in 21 (55.26%) samples. There was no consistent pattern of combinations, although polymicrobial infections were most commonly found with obligate anaerobic gram-negative bacilli i.e., *Prevotella-Porphyromonas* and *Bacteroides fragilis* group. In one of the patients, infection by *Parabacteroides distasonis* (obligate anaerobes) was found with two facultative anaerobic bacteria viz. *Escherichia coli* and *Proteus mirabilis*. Among facultative anaerobes, *E. coli* ($n = 11$) was the commonest isolate obtained followed by *Klebsiella pneumoniae* ($n = 4$) [Table 2].

Anaerobes either as a single or mixed infection were predominantly isolated from the skin and soft tissue infections ($n = 14$) followed

by surgical site infections ($n = 8$). Anaerobic infections by monomicrobial and/or polymicrobial bacteria were also found from various deep-seated pyogenic abscesses such as in the brain, kidney, liver, intra-abdominal and retroperitoneum regions [Table 3].

Discussion

Pyogenic infections caused by anaerobic bacteria are mostly polymicrobial and associated with aerobic and/or other anaerobic bacteria. In the present study, polymicrobial infections were reported in 21 (55.26%) cases of pyogenic infections [Tables 1 and 2]. Saini S, et al.^[9] found mostly secondary peritonitis (80%), necrotizing fasciitis (75%) and wounds with devitalized tissues (66.7%) cases as polymicrobial infection. De A, et al.^[10] also reported 78.2% infections as a mixed infection by anaerobes and aerobes. In a polymicrobial infection, anaerobes and aerobes work in synergy in many ways like by lowering oxidation-reduction potentials in host tissues by aerobic bacteria and thus favouring the growth of anaerobes, protection from phagocytosis, production of capsule and production of β lactamases and various other virulence factors. Microbial synergy leads to enhanced pathogenicity and severity of the infection.^[11] In this study, exclusively obligate anaerobic bacterial pathogens were isolated in 17 (44.73%) of cases [Tables 1 and 2]. Our findings are in concordance with the findings reported by Shenoy P, et al.^[2] and Antony B, et al.^[12] in which monomicrobial anaerobic pathogens were isolated insignificant number of specimens obtained from pyogenic infections.

In this study, anaerobic gram-negative bacteria were most commonly isolated. Infection by *B. fragilis* group and *Porphyromonas* spp., were predominantly reported among the study participants [Table 2]. *Prevotella* spp., was the second most common group isolated. In previous other similar studies, *B. fragilis* was the most frequent anaerobic isolates reported.^[2,9] *Prevotella-Porphyromonas* group predominantly consisted of pigmented bacteria and play a key role in pyogenic infections. Most of these anaerobic gram-negative bacteria are β lactamases producers and are found to be resistant to the commonly used β lactam antimicrobial agents contributing to therapeutic failure.^[6]

In the present study, anaerobic bacteria were isolated from diverse anatomic sites with variable recovery rates [Table 3]. The pathogenic anaerobes were most commonly reported from the skin and soft tissue infections such as diabetic foot, cellulitis, necrotising fasciitis, Fournier's gangrene, folliculitis, hidradenitis suppurativa and various abscesses. The infections were mostly polymicrobial with anaerobic gram-negative bacteria and facultative anaerobes. However, monomicrobial anaerobic infections by *B. fragilis* group, *Veillonella* spp., *Peptostreptococci* spp., and *Clostridium difficile* were also detected from a significant number of cases [Table 3].

Zhao-Fleming HH, et al.^[13] performed 16S rRNA sequencing on necrotizing soft tissue infections (NSTIs) samples and identified

Table 3: Distribution of Anaerobic Bacterial Isolates from Different Infection Sites

Clinical Diagnosis	Anaerobic Isolates	n	Total
Skin and soft tissue infections	<i>B. fragilis</i> group	02	14
	<i>Veillonella</i> spp.	02	
	<i>Peptostreptococci</i> spp	01	
	<i>Clostridium difficile</i>	01	
	<i>Veillonella</i> spp. + <i>S. aureus</i>	01	
	<i>Veillonella</i> spp. + <i>Klebsiella pneumoniae</i>	01	
	<i>Veillonella</i> spp.+ <i>Proteus mirabilis</i>	01	
	<i>Prevotella bivia</i> + <i>Escherichia coli</i>	01	
	<i>Prevotella intermedia</i> + <i>Escherichia coli</i>	01	
	<i>Prevotella oris</i> + <i>Escherichia coli</i>	01	
	<i>Porphyromonas</i> spp. + <i>S. aureus</i>	01	
	<i>Parabacteroides distasonis</i> + <i>Escherichia coli</i> + <i>Proteus mirabilis</i>	01	
	Surgical Site Infections	<i>B. fragilis</i> group	
<i>Porphyromonas</i> spp		01	
<i>Anaerococcus prevotii</i>		01	
<i>Porphyromonas</i> spp. + <i>Klebsiella pneumoniae</i>		01	
<i>Prevotella oris</i> + <i>Escherichia coli</i>		01	
<i>Veillonella</i> spp. + <i>Klebsiella pneumoniae</i>		01	
<i>Fingoldia magna</i> + <i>Klebsiella pneumoniae</i>		01	
<i>Veillonella</i> spp		02	
Intra-abdominal Abscess	<i>Bacteroides fragilis</i> + <i>Enterobacter</i> spp.	01	03
	<i>Bacteroides vulgatus</i> + <i>Escherichia coli</i>	01	
Retroperitoneal abscess	<i>Prevotella disenes</i> + <i>Escherichia coli</i>	01	02
	<i>Porphyromonas</i> spp.	01	
Brain abscess	<i>Bacteroides fragilis</i> group+ <i>Enterococcus</i> spp.	01	02
	<i>Prevotella disenes</i>	01	
Breast abscess	<i>Peptoniphilus asaccharolyticus</i>	01	02
	<i>Prevotella disenes</i>	01	
Uterus with adnexa infection	<i>Porphyromonas</i> spp. + <i>Citrobacter</i> spp.	01	01
Gluteal abscess	<i>Veillonella</i> spp.	01	01
Bone infections	<i>Bacteroides stercoris</i> + <i>Escherichia coli</i>	01	01
Perianal abscess	<i>B. fragilis</i> group	01	01
Liver abscess	<i>Porphyromonas</i> spp.	01	01
Scrotal abscess	<i>Clostridium clostridioforme</i> + <i>Escherichia coli</i>	01	01
Lymph node abscess			
Total			38

five most common bacterial genera of NSTIs (*Prevotella*, *Bacteroides*, *Peptoniphilus*, *Porphyromonas*, and *Enterococcus*). They reported the association of high relative abundance of obligate anaerobes with a worse outcome. Kamble S, et al.^[14] found the predominance of anaerobic gram-positive bacteria such as *Clostridium* spp., followed by *Peptostreptococcus* spp., and *Propionibacterium* spp., from cutaneous and subcutaneous wound infections. The second most common anaerobic infections reported in this study are surgical site infections (SSIs). Out of 8 cases of SSIs reported in this study, 4 were found to be infected exclusively by obligate anaerobic bacteria viz. *B. fragilis* group, *Porphyromonas* spp., and *Anaerococcus prevotii* [Table 3]. Akhi MT, et al.^[15] reported polymicrobial infection among SSI patients with *B. fragilis* as the predominant anaerobic isolate.

Anaerobic bacteria are important etiological agents causing deep-seated pyogenic infections. In this study, intra-abdominal infections by the endogenous anaerobes viz. *Veillonella* spp and *B. fragilis* were reported [Table 3]. Pricop GR, et al.^[16] investigated the

antibiotic resistance profiles as well as the virulence determinants of anaerobic bacteria isolated from intra-abdominal infections. The species of *Bacteroides* genus dominated the anaerobic etiology, followed by *Clostridium* spp., strains. The *Bacteroides* spp., isolated from the abdominal abscesses and ascites fluid found out to be the most virulent. In the present study, *Bacteroides vulgatus* and *Prevotella disenes* each were isolated as a mixed infection with *E. coli* from 2 cases of retroperitoneal abscesses [Table 3]. Brook I, et al.^[17] observed the polymicrobial aerobic-anaerobic nature of retroperitoneal abscesses and the predominant anaerobes recovered were *Peptostreptococcus* species, *Bacteroides fragilis* group, *Prevotella* species and *Clostridium* species. Two cases of brain abscesses caused by *Porphyromonas* spp., and *Bacteroides fragilis* group were diagnosed in the present study [Table 3]. *Porphyromonas* spp., are mainly associated with oro-dental infections and have reported causing brain abscesses of odontogenic origin.^[18] Shruthi U, et al.^[19] studied the anaerobic profile of brain abscess in southern India and found *Bacteroides* spp., as the most common anaerobic isolate. Rare anaerobic isolates such as *Fusobacterium*

*nucleatum*²⁰¹ and *Actinomyces meyeri*²¹¹ have also been reported previously from brain abscess. The majority of previously reported studies on breast abscess revealed the polymicrobial nature of infection predominantly with gram-positive anaerobic cocci and *Bacteroides fragilis* group.^[22,23] In the present study, two cases of only monomicrobial infections by *Peptoniphilus asaccharolyticus* and *Prevotella disenes* were reported from breast abscesses [Table 3]. Anaerobes were also recovered from the less common sites such as bone, lymph node, uterus and scrotum in this study [Table 3].

Since the samples were not processed for aerobic culture, the polymicrobial infection of obligate anaerobes with obligate aerobic bacteria could not be commented in the present study.

Conclusions

Anaerobes are important causative agents for pyogenic infections and are isolated from diverse infection sites. Therefore, the performance of anaerobic cultures along with aerobic cultures is much needed for complete bacterial work-up of specimens from infectious sites. With increasing rates of antimicrobial resistance amongst anaerobic bacteria, knowledge about their distribution may assist in the selection of appropriate empirical therapy for better management of pyogenic infections.

Key points of the research

Although most of the infections are polymicrobial, a rise in the incidence of monomicrobial anaerobic infections has been noticed. There was no consistent pattern of combination of obligate and facultative anaerobes obtained. Polymicrobial infections were most commonly found with obligate anaerobic gram-negative bacilli i.e. *Prevotella-Porphyrromonas* and *Bacteroides fragilis* group predominantly in combination with *E. coli*. Most common monomicrobial anaerobes isolated were *Veillonella spp.*, and *Porphyrromonas spp.* Anaerobes either as a single or mixed infection were predominantly isolated from skin and soft tissue infections and also from various deep-seated pyogenic abscesses such as abdominal and other visceral organs.

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Conflicts of interest

There are no conflicts of interest.

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