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Bacteriological profile of anaerobes in deep-seated abscess of patients attending a tertiary care hospital

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ABSTRACT

Background: Anaerobes are recognized as important human pathogens causing severe life threatening exogenous and endogenous infection if left untreated. Anaerobes are one of the most neglected pathogens in various clinical samples due to the problem in sample handling, technical difficulties in their cultivation, and identification along with prolonged turnaround time. **Aims and Objectives:** The present study was undertaken to identify the different anaerobic organisms associated with deep seated abscess and their association with various risk. **Materials and Methods:** Pus and fluid sample collected in sterile syringe or swab stick were immediately put in RCM and taken to the laboratory. Gram staining, ZN stain, and culture--both aerobic and anaerobic were done. Obligate anaerobes were checked for aerotolerance. Subcultures were done for identification of species level by Gram stain, colony morphology, biochemical tests, and final identification that were done by the Vitek 2 system. **Results:** Out of the 170 samples, 144 (84.70%) were culture positive and the rest 26 (15.29%) were culture negative; 101 (70.1%) were aerobic, 23 (16%) anaerobes, and 20 (13.9%) mixed aerobic and anaerobic. A total of 51 obligate anaerobes were isolated from various samples. Out of which 32 (62.74%) anaerobic Gram-positive cocci-*Peptostreptococcus anaerobious* being the most common and 13 (25.49%) anaerobic Gram-negative bacilli --*Bacteroids fragilis* being most common and 6 (11.76%) were anaerobic Gram-positive bacilli - *Actinomyces meyeri* being the most common. Diabetes mellitus was a significant associated factor. Maximum number of anaerobes was isolated from abscess over oral cavity followed by gangrenous foot, scrotal abscess, and diabetic foot. **Conclusion:** Anaerobes are an important cause of deep-seated abscess—mostly being polymicrobial in nature. Incision--drainage and proper antibiotic therapy is necessary for their early control and prevention of complications.

Key words: Anaerobic bacteria; Deep-seated abscess; Anaerobic Gram positive bacilli; Anaerobic Gram negative bacilli

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INTRODUCTION

Anaerobes are important members of the normal flora of the body in the skin and mucous membranes. They are responsible for various endogenous bacterial infections in any organ or system of body leading to severe life-threatening infections if left untreated.[1-3] Conditions such as trauma, poor blood supply, vascular stasis leading to tissue necrosis, and lowered oxidation – reduction potential in tissue provide favorable conditions for the anaerobes to multiply.[3] Handling of specimen containing anaerobic organisms is very much challenging due to their susceptibility to environmental oxygen, technical difficulties in cultivation, cost, and most importantly prolonged

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turnaround time leading to delayed report to the clinicians. This leads to failure of detection of the different anaerobes responsible for various infections.[4]

Abscess is a localized collection of purulent inflammatory tissue suppuration in a tissue or organ developing because of introduction of commensal flora into a sterile body site due to some cause and becoming fatal if left untreated.[5,6] Deep-seated abscesses are collection of pus or microorganisms in the deep spaces of body, commonly encountered in surgical wards. They are usually polymicrobial in nature with both aerobic and anaerobic bacteria being the causative agents of infections such as liver abscess, splenic abscess, appendicular abscess, perianal abscess, orofacial infections, empyema, clostridium myonecrosis, peritoneal, and pleural spaces.[7,8] Identification of the causative agents, appropriate antibiotic therapy, and surgical drainage is the treatment of choice.[9] Commonly encountered anaerobic pathogens in the clinical samples include *Peptostreptococcus* species, *Bacteroids fragilis*, *Prevotella* species, *Porphyromonas* species, and others.[10]

Aims and objectives

The aim of the present study was to determine the frequency of anaerobic isolation from various deep-seated abscesses and their association with various risk factors.

MATERIALS AND METHODS

A prospective study was conducted in the microbiology department of Dr. D.Y. Patil Medical College, Hospital, and Research Centre, Western India, for 2 years from July 2011 to September 2013. Deep seated pus and fluid samples were collected from 170 suspected patients admitted to the surgical, medicine, orthopedics, otolaryngology, gynecology, and pediatrics ward along with other intensive care units of hospital. The study was conducted after Institutional Ethical Committee clearance. The demographic information, clinical presentations, risk factors (comorbidities), and other laboratory parameters were collected along with the specimens--tissue, pus aspirate, and wound swab.

Pus samples from deep-seated abscesses were aspirated aseptically with a sterile syringe and needle. In case of insufficient samples, swabs were collected from the floor of ulcer or depth of abscess, immediately put into Robertson's cooked meat (RCM) medium and immediately taken to the microbiology laboratory.

Macroscopic examination of the samples was done. A foul odor, presence of necrotic tissue, and sulfur granules were valuable clues for possible presence of anaerobes. Microscopic examination was done for every sample for cellular characteristics and Gram stain were done which provided idea about bacteria along with their shape and size. Other definitive morphological features of bacteria such as presence of spore, branching filaments, and pointed ends were noted. ZN stain was performed for each sample to exclude presence of acid fast bacilli.

The specimens were inoculated for anaerobic culture into RCM broth, non-selective media Brucella blood agar enriched with Vitamin K and hemin and selective and differential media Bacteroides bile esculin agar for preliminary identification. Inoculated RCM broth was incubated for 7 days and subculture was done on 5% sheep blood agar. All the plates were incubated in anaerobic gaspak jar (BD diagnostics) at 37°C and opened 48–72 h later for inspection of the plates. The specimens were also inoculated on Maconkey agar and 5% sheep blood agar for identification of aerobic organisms, if any.

Preliminary identification of anaerobic isolates was done by colony morphology, Gram stain, aerotolerance test on chocolate agar, fluorescence under long wave (365 nm) ultraviolet light, biochemical reactions such as catalase test, indole test, nitrate reduction test, and sugar fermentation tests. Automated microbial identification systems--VITEK 2 ANC (Anaerobic and Corynebacterium) ID card (BioMérieux) was used for species level identification.

RESULTS

A total 170 samples were tested for anaerobic culture from various anatomical sites during the study period in microbiology laboratory in our tertiary care hospital. Out of this, 144 (84.70%) samples showed positive culture of growth of aerobic, anaerobic, or mixed growth. The remaining 26 (15.30%) samples did not show any growth and thus were culture negative; hence, were considered as sterile cultures (Table 1).

Out of 170 cases, 40 patients (23.5%) presented with diabetics mellitus which was significantly higher than other history of illness. About 6 patients (3.5%) presented with vascular abnormalities and 3 patients (1.8%)

Table 1: Distribution of type of culture positive cases (n=144)

Type of culture positivity	Number	%
Aerobic isolates	101	70.1
Anaerobic isolates	23	16.0
Mixed aerobic and anaerobic infections	20	13.9
Total	144	100.0

presented with pre-existing malignancy for which they had received chemotherapy for prolonged period. Rest of the 121 (71.2%) cases did not give history of any illness (Table 2).

Out of 23 anaerobic infections, 17 (73.91%) infections were only anaerobic monomicrobial infections which was significantly higher (P<0.01) and 6 (26.08%) samples were anaerobic polymicrobial infections (Table 3).

Table 2: Distribution of history of illness (risk factors) in the patients of deep seated abscesses

History of illness	Number	%
Diabetes mellitus	40	23.5
Vascularity compromised	06	3.5
Malignancy	03	1.8
Nil	121	71.2
Total	170	100.0

Table 3: Distribution of pure anaerobic polymicrobial infections

Sr. No	Type of abscess	Anaerobes isolated
1.	Gangrenous foot	*Peptostreptococcusniger*, *Peptostreptococcusanaerobious*
2.	Pyometra	*Peptostreptococcusanaerobious*, *Bifidobacterium* species
3.	Perforative peritonitis	*Peptostreptococcus magnus*, *Bcteriodes fragilis*
4.	Gangrenous foot	*Peptostreptococcus anaerobious*, *Bacteroids fragilis*
5.	Brain abscess	*Peptostrepococcus magnus*
6.	Palatal abscess	*Peptostreptococcus anaerobious*, *Prevotella melaninogenica*

Out of 144 positive samples, 20 (13.88 %) samples showed mixed aerobic and anaerobic bacteria--30 aerobic 22 anaerobic bacteria (Table 4).

Fifty-one anaerobes were isolated from various deep seated abscesses. Of these 51, 32 (62.74%) were Gram-positive anaerobic cocci, 13 (25.49%) were Gram-negative anaerobic bacilli, and 6 (11.76%) were Gram-positive anaerobic bacilli. In our study, Gram-positive anaerobic cocci were predominantly isolated (62.74%) and *Peptostreptococcus anaerobius* 13 (25.49%) was the most common isolate followed by *Peptostreptococcus micros* 06 (11.76%). Among the Gram-negative anaerobic bacilli, *B. fragilis* 07 (13.72%) was the most common isolate. Among Gram-positive anaerobic bacilli, *Actinomyces meyeri* 03(5.88%) was predominant (Table 5).

Figure 1 demonstrates the frequency of the samples isolated from the different sites of the body.

DISCUSSION

Anaerobes are significant component of normal flora mostly present over the mucosal membrane generally arising from the host's own endogenous flora. They get entrance into the body of the host through penetrating wound as a result of trauma, accident, or surgical procedures for abscesses of the liver, brain, lung or appendicitis, peritonitis, chronic otitis media and sinusitis, endophthalmitis, endocarditis, myonecrosis, and gas gangrene. They may also enter the body causing different dental and oral infections sometimes leading to serious life-threatening septicemia.

Table 4: Distribution of organisms in mixed aerobic and anaerobic infections

Sl. No	Type of abscess	Aerobic bacteria	Anaerobic bacteria
1.	Scrotal abscess	*MRSA, E. coli* *MSSA*	*Peptoniphilus asaccharolyticus* *Propionibacterium acne*
2.	Chronic osteomyelitis	Group D streptococcus	*Peptococcus niger, Bacteroids fragilis*
3	Gangrenous foot	*Proteus mirabilis, Group D Streptococcus* *Proteus mirabilis*	*Bacteroids fragilis* *Peptostreptococcus magnus*
4.	Diabetic foot	*E. coli, Pseudomonas aeruginosa* *MSSA, Klebsiellapneumonia*	*Peptoniphilus asaccharolyticus* *Actinomyces meyeri*
5.	Infected socket	*MSSA, Streptococcus viridians* *Streptococcus viridians* *MSSA, Streptococcus viridians* *MSSA, Streptococcus viridians* *Streptococcus viridians*	*Fusobacteriumn ucleatum* *Fusobacterium nucleatum* *Fusobacteriumn ucleatum* *Peptostreptococcus micros* *Actinomyces meyeri*
6.	Appendicular abscess	*E. coli* *E. coli*	*Peptostreptococcus anaerobius, Bacteroids fragilis* *Bacteroids fragilis*
7.	Perianal abscess	*E. coli*	*Prevotella melaninogenicus*
8.	Space infection	*Klebsiellaoxytoca* *MSSA, Acinetobacter* sp. *MSSA, Acinetobacter* sp. *d) Citrobacterfreundii*	*Peptostreptococcus anaerobius* *Fusobacterium nucleatum* *Actinomyces meyeri* *Peptostreptococcus micros*
9.	Palatal abscess	*MSSA, Pseudomonas aeruginosa*	*Peptoniphilus asaccharolyticus*

Table 5: Spectrum of anaerobic isolates from various deep seated abscesses

Total no of anaerobes isolated	51
A. Gram-positive anaerobic cocci	32 (62.74%)
Peptostreptococcus anaerobius	13
Peptostreptococcus micros	6
Peptoniphilus asaccharolyticus	5
Peptostreptococcus magnus	5
Peptococcus niger	2
Finegoldia magna	1
Gram-negative anaerobic bacilli	13 (25.49%)
Bacteroids fragilis	7
Fusobacterium nucleatum	4
Prevotella melaninogenica	2
Gram-positive anaerobic bacilli	6 (11.76%)
Actinomyces meyeri	3
Propionibacterium acnes	2
Bifidobacterium spp	1
Total	51

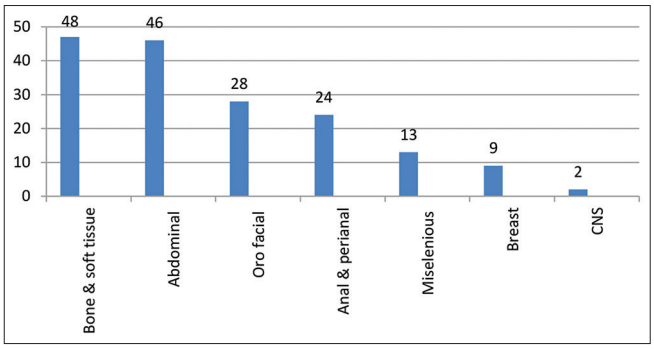

Figure 1: Distribution of samples according to the different anatomical sites

The present study was undertaken to isolate anaerobes from various deep seated abscesses. One hundred and seventy patients presenting with different deep seated abscesses especially close to mucosal surface were included in our study. Out of the 170 samples, 144 (84.70 %) samples were culture positive and 26 (15.29 %) samples were culture negative. In 2009, Zimmerman et al., reported 80% culture positivity of the samples from various operatively drained abscess including aerobe and anaerobes which is similar to our study.[11]

Diabetes was identified as a very important triggering factor for the growth of anaerobes in deep-seated abscesses. The reason may be because of the development of microvascular thrombosis reducing blood supply to the extremity in patients with prolonged diabetes. The reduced oxygen supply creates environment for anaerobic bacteria to grow. Three patients with malignancy developed deep-seated abscess probably because prolonged chemotherapy impaired the cellular defense mechanism of the body making it prone to develop abscess. This is similar to the study conducted by Brook and Frazier.[12]

Peripheral arterial occlusive disease is also associated with deep-seated abscess probably because this leads to reduced blood supply to the lower extremities making them prone to infection by various anaerobes.

We found from our study that aerobic isolates were significantly higher (70%) than the anaerobes. Similar results have been reported by Gupta et al.,[13] and Set et al.,[14] who obtained 14% and 18.7% anaerobes from pyogenic lesions and wound infections concordant to our study. In another study, mixed infections were obtained 35% from intra-abdominal infections which differ from our study probably due to difference in site of infections.[15]

Anaerobic Gram-positive cocci (GAPC) were predominant pathogens in our study isolated by various investigators from 18% to 82.4% from various pyogenic lesions.[16,17] *Peptostreptococcus anaerobius* followed by other species of *Peptostreptococcus* was the commonly reported GAPC in our study. However, *Finegoldia magna* was reported as monomicrobial flora from a case of necrotizing fasciitis. High frequency of isolation of *Finegoldia magna* from chronic wound and ulcer was also reported by Murphy and Frick.[18]

In our study, we have isolated 13 anaerobic Gram-negative bacilli (25.49%) from various clinical specimens — *B. fragilis* (n=7) was most commonly isolated anaerobes from various intra-abdominal and soft-tissue infections. Similar results were reported by others where *B. fragilis* was frequently isolated as microorganism from various surgical infections.[19] If the infection left untreated, the mortality rate may very high up to 60%.[8]

In our study, a wide range of anaerobes was isolated from devitalized tissue or from patients with impaired immune status. Foul smelling discharge, presence of gas or crepitus, and infections confined to mucosal surface are the clinical clues for suspect an anaerobic infection.

Now a days, there is an increased incidence of antimicrobial resistance among anaerobes and knowledge of distribution of organisms that may assist in selection of appropriate empirical therapy for anaerobes.

Limitations of the study

This study was done on small number of sample; larger study are required to confirm the result.

CONCLUSION

Anaerobes are the most neglected and overlooked microorganisms in recent culture-based diagnostics. Role of anaerobes should be recognized by both clinician

and microbiologist to provide adequate patient care and decrease the incidence of therapeutic failure.

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RP- Conception and designing of the work, data collection, statistical analysis and interpretation, and prepared first draft of manuscript; **NC-** Data analysis and interpretation, reviewed the literature, and manuscript preparation; **SC-** preparation of manuscript and revision of the manuscriptcript; **RNM-** preparation of manuscript and review; and **SSC-** preparation of manuscript and review.

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