

A Study of Anaerobic Organisms in Clinically Suspected Cases of Anaerobic Infections

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Abstracts: Background: Anaerobes are microorganisms grow in tissue with reduced oxidation-reduction potentials, predisposing to infection in absence of oxygen. Most of anaerobic infection occurs when a patient's normal flora gains access to a sterile site as a result of disruption of some anatomical barrier. Moreover, most anaerobic infections involve a mixture of aerobic and anaerobic organisms, so failure of treatment occurs because of mixed infections with both type of bacteria. The aim of study was to isolate and identify anaerobic bacteria in clinically suspected pure anaerobic infection and mixed infection. Also to know antibiotic sensitivity pattern of anaerobes for better treatment of clinical failure cases. Method: Different samples for culture collected either by biopsy or by aspiration using sterile needle and syringe. Anaerobic transport medium was used for sample collected on swab. Liquid anaerobic medium like Thioglycolate medium, Robertson cooked meat medium and solid anaerobic media like Blood agar with antibiotics, hemin, Vit. K, Bacteroides bile esculin agar and Kanamycin- Vancomycin blood agar were used for cultivation. COMPACT TG Anaerobic work station was used for providing oxygen free atmosphere for growth of anaerobic bacteria. Anaerobes isolated in 68(62.96%) out of total 108 samples from different pyogenic lesions of patient admitted in various wards of Gujarat Cancer and Research Institute, Ahmedabad during April 1998 to Dec. 1999. Result: *B. fragilis* and *P. melaninogenicus* were commonest isolate 21(35%) and 23(38.33%) respectively. Metronidazole (93%) and clindamycin(73%) were effective drugs for anaerobic bacteria. 58 samples(53.70%) were positive for both aerobes and anaerobes indicating synergisms for infection between these bacteria. [Jadeja R et al NJIRM 2012; 3(2) : 9-12]

Key words: Bacteroids fragilis, Prevotella melaninogenicus

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Introduction: Anaerobes are microorganisms live and grow in absence of oxygen. Reduction of normal oxidation-reduction potentials of a tissue and a lower oxygen tension resulting from an impaired blood supply, necrosis of tissue, associated with vascular disease, trauma, surgery, presence of foreign body, malignancy, immunosuppressive drugs, steroids and disease such as diabetes mellitus may also predispose to infections with anaerobic organisms.

The common pathogens are moderate obligate anaerobes, they produce virulence factors like superoxide dismutase, catalase, peroxide enzymes that are protective against toxic oxygen reduction products. Several anaerobic bacteria may have capsule that inhibit phagocytosis and promote abscess formation.

As the incidence of anaerobic infections increasing now a days¹² but still the subject is untouched because of no facility for anaerobic culture and no

expertise for isolation and identification of anaerobes.

The aim of study was to know anaerobic bacteria with common infection and antibiotic sensitivity pattern; it can help in treatment of unresponsive infections. Anaerobic bacteria are also associated with other aerobic or anaerobic bacteria as polymicrobial infection leading to mixed infection with more antibiotic resistance. Sometimes inadequate therapy against these anaerobic bacteria may lead to clinical failure.¹¹

Material and Methods : Total 108 samples from different infections were processed at microbiology laboratory of Gujarat Cancer and Research Institute, Ahmedabad during April 1998 to Dec. 1999.

Sample were selected on the bases of following criteria¹⁰

- Foul smelling discharge with blood
- Localization of infection in proximity to mucosal surface

- Presence of necrotic tissue
- Presence of gas in tissue
- Infection associated with malignancy
- Sterile pus
- Clinical history suggestive of anaerobic infection like septic abortion, infection following gastrointestinal surgery
- Lesions not treated with metronidazole oral or local
- Patient not responding to aminoglycosides

Samples were collected from different infections like :

- Post operative wound infection
- Liver abscess
- Pleural effusion
- Peritonitis
- Female genital tract infection
- Diabetic foot ulcer
- Otitis media
- Gas gangrene
- Lung abscess
- Cellulitis

Samples collected by aspiration with sterile needle and syringe and by biopsy. Samples collected on swab placed in thioglycolate media and processed within 30 minutes of collection.

Direct smear examination performed to know morphology of bacteria, aerobic culture done to rule out aerobic infection and mixed infection. Anaerobic culture done by using liquid media like thioglycolate and robertson cooked meat media (Robertson, 1915). Solid media used were Blood agar with neomycin (100 µg/ml) as selective media, Brain heart infusion blood agar supplemented with 0.5% yeast extract, vit. K (10 µg/ml), Hemin (5 µg/ml) and 5% sheep blood. Bacteroids bile esculin agar used for selective isolation of *B. fragilis* group, Kanamycin-vancomycin blood agar used for selective isolation of species like Bacteroids, Fusobacteria, Prevotella and Veillonella species.

COMPACT-TG Anaerobic work station used for culture and isolation of anaerobic bacteria. Antibiotic sensitivity testing performed by disc diffusion method with control strains

recommended by CLSI on brain heart infusion agar using broth of thioglycolate media having growth of anaerobic bacteria. After 24 hours incubation at 37 °C in anaerobic atmosphere, zone size measured. Identification of bacteria done by colonial morphology, gram stain, subculture done on selective media, aerotolerance test, fluorescence, catalase, spot indole test, sugar fermentation, bile tolerance test, esculin hydrolysis and antibiotic susceptibility test.

Result: Total 108 samples tested in this study. 67(62%) were male and 41(38%) were female. Pure anaerobic infections were positive in 10(9%) cases, mixed infection with aerobes and anaerobes in 58(54%) cases, pure aerobic infection in 24(22%) of cases and sterile in 16(15%) cases.

Table 1 : Results of isolated aerobic strains.

Sr. no.	Name of organisms	Total no. of strain isolated	%
1	<i>S. aureus</i>	36	40.90
2	Streptococci	06	6.81
3	<i>Pseudomonas</i>	09	10.22
4	<i>E. coli</i>	14	15.90
5	<i>Proteus</i>	05	5.68
6	Coagulase negative staphylococci	07	7.95
7	<i>Klebsiella</i>	10	11.36
8	<i>Candida</i>	01	1.13
Total		88	100

Amongst the aerobic infections in 82 cases, commonest bacteria were *S. aureus*, *E. coli*, *Klebsiella* and *Pseudomonas*.

Table 2 : Anaerobic strain isolated from various samples.

Organisms	No. of strain isolated	%
<i>B. fragilis</i>	21	23.86
<i>P. melaninogenicus</i>	23	26.13
<i>P. intermedia</i>	04	4.54
<i>Bacteroids</i> sp.	05	5.68
<i>B. oralis</i>	01	1.13
<i>B. ureolyticus</i>	01	1.13
<i>C. perfringens</i>	04	4.54

Clostridium sp.	03	3.40
Fusobacterium sp.	02	2.27
Peptostreptococci anaerobius	05	5.68
Peptostreptococci	15	17.04
Peptococcus niger	01	1.13
Fusobacterium nucleatum	03	3.40
Total	88	100

Commonest anaerobes isolated were *P. melaninogenicus*, *B. fragilis*, *Peptostreptococci sp.* And *Peptostreptococci anaerobius* in 88 isolates. Out of 40 strains tested, 36(90%) were sensitive to metronidazole, 29(72.5%) were sensitive to clindamycin, but 35(87.5%) strains were resistant to kanamycin and 33(82.5%) strains were resistant to cefoxitin.

Table 3 : Infection wise bacterial isolates.

Type of infection	No. of sample	No. of isolate	<i>B. fragilis</i>	<i>p. melaninogenicus</i>	<i>p. intermedia</i>	<i>Bacteroids sp.</i>	<i>B. oralis</i>	<i>B. ureolyticus</i>	<i>c. perfringens</i>	<i>C. tetani</i>	<i>Clostridium sp.</i>	<i>P. niger</i>	<i>P. anaerobius</i>	<i>Peptostreptococcus sp.</i>	<i>F. nucleatum</i>	<i>Fusobacterium sp.</i>
Cellulitis	10	7	-	-	-	2	-	-	-	-	-	-	1	4	-	-
Perianal abscess	4	4	3	-	-	1	-	-	-	-	-	-	-	-	-	-
Lung abscess	7	7	1	1	1	-	-	-	-	-	-	-	1	1	2	-
Liver abscess	7	7	2	4	-	-	-	-	-	-	-	-	-	1	-	-
Gas gangrene	6	6	1	0	-	-	-	-	3	1	1	-	-	-	-	-
Post operative wound infection	28	16	5	5	1	-	-	-	-	-	-	-	1	3	-	1
Periodontitis	8	8	-	6	1	-	1	-	-	-	-	-	-	-	-	-
Pyometra	5	7	1	1	1	-	-	-	-	-	1	1	-	2	-	-
Pericardial effusion	1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Peritonitis	4	4	2	-	-	1	-	-	-	-	-	-	-	1	-	-
Diabetic foot ulcer	5	10	3	1	-	-	-	1	1	-	-	-	1	2	1	-
Otitis media	5	3	1	1	-	-	-	-	-	-	-	-	1	-	-	-
Pleural effusion	6	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Others	12	8	2	4	-	1	-	-	-	-	-	-	-	-	-	1
Total	108	88	21	23	4	5	1	1	4	1	2	1	5	15	3	2

Discussion: Anaerobes are fastidious, so isolation of bacteria requires appropriate method of collection, transportation and cultivation of specimen. Treatment is further complicated by 3 factors : slow growth, increasing resistance to antibiotics and polymicrobial synergistic nature of the infection.¹¹

The main aim of study was to isolate anaerobes from different infections and to perform antibiotic susceptibility testing. Amongst 108 clinically suspected anaerobic cases studied, the isolation of anaerobes was in 68(62.96%) of the cases, mere compared to Gupta's study(1972)³ as out of 258(10.8%) and Reena et al in (1997)⁷ shown 50 out

of 267(18.7%). Mixed infection also shown high as 53.7% compared to Gupta's study 66(12.5%)³ and Reena et al study 0(0%)⁷ and also compared with Serpil Ercis et al(20.5%).¹³ This could be due to fact that the study performed at Cancer institute having patients with immunocompromised status either due to disease or therapy. This study shows mere prevalence of anaerobic bacteria in immunocompromised patient.

Most common infection with anaerobes was post operative wound infections. Out of 28 cases studied 8(28.57%) were positive for anaerobes with

common isolate were *P. melaninogenicus*(31.25%), *B. fragilis*(31.25%), and *Peptostreptococci*(25%), while only 14% were positive for anaerobic infection with *B. fragilis* commonest as per Kazue ueno et al study.¹⁴ The result are quite similar with Menon et al in 1989⁸ and Reena in 1997⁷ as 22(10.3%) out of 213 case and 20(14.7%) positive out of 136 case with similar isolates.

In the present study, total 40 strains were included for antibiogram. Here *B. fragilis* was found to be resistant to penicillin, an observation which is quite similar with the finding of Mitchell in 1973⁹. Metronidazole is still a drug of choice for anaerobic infection as *B. fragilis* (87.5%) and *Peptostreptococci*(100%) were found sensitive as compared with Beena et al (1992)⁵ and Jindal (1993)⁶ indicated *Bacteroides*(100%), *Clostridia*(100%) and *Peptostreptococci*(85%) were sensitive. Clindamycin is very active bactericidal antibiotic against gram positive organisms and gram negative organisms. In our study 100% of gram negative bacilli and 61.53 % of gram positive cocci were sensitive , similar results were recorded by Mitchell(1973)⁹ and Beena et al(1992)⁵. Cephalosporin group of drugs now becoming resistant in our study. 25% of *B. fragilis* were sensitive. For *Clostridia* group of organisms, vancomycin(10%) and Metronidazole(67%) were effective against nearly all strains.

Conclusion: In present study of anaerobic infection of 108 cases, Anaerobes were isolated in 68(62.96%) of cases with most common anaerobes like *B. fragilis*(35%),*P.melaninogenicus* (38.33%), *Clostridia sp.*(42.85%), *Cl. Perfringens*(37.14%) and *P. anaerobius* in (23.80%) of cases. Anaerobic susceptibility testing indicated metronidazole(93%) and clindamycin(73%) effective antibiotics for anaerobic bacteria. Pure isolation of anaerobic bacteria from culture is definitely an indication that they are directly associated with the pathogenesis. However 58(53.70%) cases were in mixed growth aerobes and anaerobes, indicating that there is synergism between aerobes and anaerobes for pathogenicity of infections.

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