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Original Research Article

Anaerobic Infection is on Rise: A Hospital Based Study

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Abstract:

Introduction: Anaerobic bacteria cause a wide spectrum of infections varies from local to systemic. The infection is generally polymicrobial. Both sporing and non-sporing anaerobic bacteria are the causative agents. Recovery of anaerobes from clinical specimen is overlooked and neglected. Isolation of anaerobes requires proper sample collection, prompt transport in anaerobic container and anaerobiosis.

Aims and Objectives: The study was conducted to determine the rising trends of anaerobes isolated from various clinical specimens, to review the clinical manifestation and to achieve better clinical outcomes through active surveillance.

Materials and Methods: The study was conducted in the Microbiology Laboratory of a multispeciality corporate hospital of Burdwan town, WB, over a period of 6 months from March,2022 to Sep,2022. Forty samples were collected from suspected patients admitted in different Department. Samples were inoculated onto Anaerobic blood agar and Bacteroides bile esculin agar (Himedia) and placed inside anaerobic Gaspak Jar (BD). Anaerobic indicator was also put inside the jar and the whole system was incubated at 37°C for 24-48hrs. Automated identification of bacteria to spices level was completed by Vitek 2 ANC cards (BioMerieux). Antimicrobial susceptibility testing (AST) - the disc diffusion method was performed on Fastidious anaerobic agar with McF1.0.

Result: A total 40 samples were processed from suspected anaerobic infections over a period of 6 months in the Microbiology Laboratory of a multispeciality corporate hospital of Burdwan. Thirty two (32) anaerobic bacteria were isolated out of 40 samples,8 showed no growth. Sixteen (16) anaerobes were monomicrobial, rest were polymicrobial out of 32 specimens. In our findings, monomicrobial infections were responded by metronidazole and doxycycline followed by clindamycin, whereas polymicrobial infections were treated smoothly by meropenem- colistin combination with clindamycin**.**

Discussion: Our data shows that anaerobic bacteria can be isolated from a variety of infections. The most common sites of isolation were abscesses (40%) followed by soft tissue infections (10%). Anaerobic bloodstream infections (ABSIs) are not very common. Similar results were also observed by Gorbach SL et al. They can be serious and even fatal if left untreated. Here, only one ABSI was there caused by *Bacteroides fragilis*. In our study, 17 (42.5%) were Gram positive bacilli (GPB), 8 (17.5%) were Gram negative bacilli (GNB) and 7 (17%) were Gram positive cocci (GPC). Most commonly isolated organism is *Actinomyces sp.*(10%) followed by *Clostridium sp.*(7.5%) And *Peptococcus sp.*(7.5%). However, other reports have shown that Gram-positive anaerobic cocci (GPAC) are the most frequently isolated pathogens**.** In monomicrobial anaerobic infection, Actinomyces sp. (37.5%) is the most commonly isolated sp. followed by Fusobacterium sp. (25%).

Keywords: Anaerobic Bacteria, Polymicrobial Infections, Anaerobiosis, Antimicrobial Resistance.

Introduction

Anaerobic bacteria comprises a pivotal part of commensal in human body. They cause a wide spectrum of infections varies from local to systemic [1]. Some life-threatening anaerobic infections are very critical and fatal. [2] The source is both endogenous and exogenous. [1] The infection is generally polymicrobial. Common anaerobic infection includes empyema, periodontitis, deep seated abscess like brain abscess, liver abscess, gas gangrene, peritonitis, aspiration pneumonia, antibiotic associated diarrhoea etc. [2] The predisposing factors are trauma, foreign bodies, surgery, ischemia, necrosis, animal bite, root canal, diabetes, malignancy favours the infections. [3]

Both sporing and non-sporing anaerobic bacteria are the causative agents. The commonly encountered anaerobes are *Clostridium sp., Actinomyces sp., Peptococcus sp., Peptostreptococcus sp., Fusobacterium sp., Bacteroides sp., Bifidobacterium sp.* etc. [4] Some are strict obligate anaerobes where as others are moderate obligate anaerobes. Low oxidation reduction potential (Eh) is more important than absence of oxygen to grow the anaerobes. [5] Isolation of anaerobes requires proper sample collection, prompt transport in anaerobic container and anaerobiosis. Recovery of anaerobes from clinical specimen is overlooked and neglected. [6,7] The reasons behind this are transport of sample, exposure to oxygen, cumbersome culture technique, fastidiousness, expensive identification, prolonged turn-around time, communication failure, delayed reporting. [7] Ultimately there is treatment failure. So, high suspicion is necessary. Some significant and valuable clinical clues include anatomical location, foul smell, presence of crepitus, granules, bad breath, pain, discoloration of skin, tissue necrosis, gangrene etc. [8] As there is mixed aerobic-anaerobic infections, antimicrobials should provide the adequate coverage to both. The effective anti-anaerobic antimicrobials are metronidazole, clindamycin, doxycycline, penicillin, vancomycin, doripenem, tigecycline piperacillin tazobactum, chloramphenicol etc. [9] Now-a-days, increasing resistance among antimicrobials is a growing threat. Active surveillance and proper antimicrobial susceptibility testing can overcome this problem. [10] Irrigation, debridement, drainage, improvement of blood supply, tissue oxygenation, alleviating obstruction, surgery are included as adjuvant therapy. [11]

Aims and objectives

The study was conducted to determine the rising trends of anaerobes isolated from various clinical specimens, to review the clinical manifestation and to achieve better clinical outcomes through active surveillance

Materials and Methods

The study was conducted in the Microbiology Laboratory of a multispeciality corporate hospital of Burdwan town, WB, over a period of 6 months from March,2022 to Sep,2022. The facility is 114 bedded hospital. Consent was taken both from hospital authority and participants. Forty samples were collected from suspected patients admitted in different Department. Specimens including tissue, aspirated pus, body fluids, blood culture, high vaginal swab, FNAC material, dental sample were collected aseptically as a bed side procedure inoculated into Robertson's cooked meat medium (RCM) as well as in anaerobic transport container. Gross examination of the sample were noted for any suggestive clinical clues. Samples were inoculated onto Anaerobic blood agar and Bacteroides bile esculin agar (Himedia) and placed inside anaerobic Gaspak Jar (BD). Anaerobic indicator was also put inside the jar and the whole system was incubated at 37℃ for 24-48hrs.The specimens were also cultured aerobically on 5% sheep blood agar and MacConkey's agar for the polymicrobial etiology. Inoculated RCM broth was incubated upto 7 days subculture was done subsequently. Aerotolerence test was performed on chocolate agar. Side by side, Gram's staining was performed direct from sample to see the morphology of bacteria and cells. ZN and fungal stain were also done to exclude the other pathology. Conventional identification was carried out by spot indole test, catalase test, sugar fermentation test, fluorescence under long wave (365 nm) ultraviolet light etc. Automated identification of bacteria to spices level was completed by Vitek 2 ANC cards (BioMerieux). Antimicrobial susceptibility testing (AST) - the disc diffusion method was performed on Fastidious anaerobic agar with McF1.0 (EUCAST-ver1, Jan, 2022)[12] by using the following discs - metronidazole (5µg), clindamycin (2µg), vancomycin (10µg), kanamycin (1000µg), penicillin (10µg), doxycycline (30µg), piperacillin tazobactum (30-6µg), meropenem (10µg), colistin (10µg). Simultaneously, an active surveillance was carried out to see the patient's response towards treatment.

Result

A total 40 samples were processed from suspected anaerobic infections over a period of 6 months in the Microbiology Laboratory of a multispeciality corporate hospital of Burdwan. Thirty two (32) anaerobic bacteria were isolated out of 40 samples,8 showed no growth. Sixteen (16) anaerobes were monomicrobial, rest were polymicrobial out of 32 specimens. Details like nature of specimen, anatomical site, age distribution, clinical features, types of isolates are listed in Table -1.

Sl. no	Sample	Depart-ment	Patient	Suggestive fea-tures	Anaerobes	Aerobe
1	Bronchoalve-olar lavage (BAL)	Pulmonary Medicine	45yrs/M	Rt. sided mid-lobe cavitary lesion	Fusobacterium nucleatum (GNB)	-
2	Pus	Surgery	65yrs/F	Parietal wall	Peptococcus an-	-

				abscess	aerobius (GPC)	
3	Synovial fluid	Orthopaedics	56yrs/F	Knee abscess	Bacteroides ureolyticus (GNB)	Acinetobacter baumannii
4	Tissue from left Nasal cavity	ENT	23YRS/F	Nasopharyngeal CA	Actinomyces odontolyticus (GPB)	-
5	CSF	Paediatrics	1month/F	Brain Abscess	Atopobium vaginae (GPB)	-
6	Pleural fluid	Pulmonary Medicine	35yrs/M	Pneumonia	Actinomyces meyeri (GPB)	-
7	Pus from abdominal wound	Surgery	28yrs/M	Intestinal obstruction	Bifidobacterium breve (GPB)	-
8	CSF	PICU	1yr/M	Brain abscess	Peptococcus magnus (GPC)	-
9	Tissue from upper limb	orthopaedics	23yrs/M	Gas gangrene by colour doppler	Clostridium septicum (GPB)	Proteus vulgaris
10	Pus	Surgery	11yrs/M	Inguinal abscess	Anaerococcus prevotii (GPC)	-
11	Tissue	Orthopaedics	28yrs/M	condrosarcoma	Actinomyces odontolyticus(GPB)	-
12	Tissue from abdominal wall	Surgery	50yrs/F	Gas gangrene	Hathewaya histolytica(GPB)	-
13	Pus	Surgery	58yrs/ M	Duodenal perforation	Bifidobacterium bifidum(GPB)	-
14	Pus	Paediatrics	10yrs/M	Necrotising lesion on abdomen	Clostridium paraputrificum (GPB)	-
15	Pus	ENT	2 months/F	Periauricular abscess	Actinomyces odontolyticus (GPB)	-
16	Endotracheal secretion	Pulmonary medicine	40yrs/M	Respiratory symptoms	Peptostreptococcus anaerobius (GPC)	-
17.	CSF	NICU	7days/M	convulsion	-	Listeria monocytogenes
18.	Blood culture	ICU	60yrs/M	sepsis	Bacteroides fragilis (GNB)	-
19.	Pus	Dental	43yrs/M	Root canal abscess	Porphyromonas gingivalis (GNB)	MSSA
20	High vaginal swab	Gynaecology	35yrs/F	Vaginosis	Mobiluncus mulieries (GPB)	Escherichia coli, Candida sp.
21	Pus	Dermatology	20yrs/M	Acne vulgaris	Propionibacterium acne (GPB)	MSSA
22	Pus	Dermatology	67yrs/M	Diabetic foot	Actinomyces meyeri (GPB)	Pseudomonas aeruginosa
23	Pus	Medicine	54yrs/M	Perianal abscess	Peptococcus niger (GPC)	Escherichia coli
24.	Bile	Medicine	45yrs/M	Gall bladder stone	Bacteroides fragilis (GNB)	Enterococcus faecalis
25	Pus swab	Dental	53yrs/F	Periodontitis	Prevotella buccae (GNB)	MRSA
26	Pus	Dermatology	23yrs/F	Acne vulgaris	Propionibacterium acne (GPB)	Klebsiella sp.
27	High vaginal swab	Gynaecology	35yrs/F	Vaginosis	Prevotella melaninogenica (GNB)	Proteus mirabilis

28	Pus	ENT	5yrs/F	Periauricular abscess	Peptostreptococcus prevoti (GPC)	MRSA
29	Stool	Medicine	52yrs/M	Diarrhoea	Clostridium difficile (GPB)	Klebsiella sp.
30	Liver abscess	Medicine	55yrs/M	Hepatomegaly	Fusobacterium nucleatum (GNB)	-
31	Pus	Surgery	22yrs/M	Perianal abscess	Peptococcus niger (GPC)	Proteus mirabilis
32	Pus	Surgery	49yrs/ F	Duodenal perforation	Bifidobacterium bifidum (GPB)	Escherichia coli

Effectiveness of antimicrobials are plotted according to AST and surveillance in chart -1.

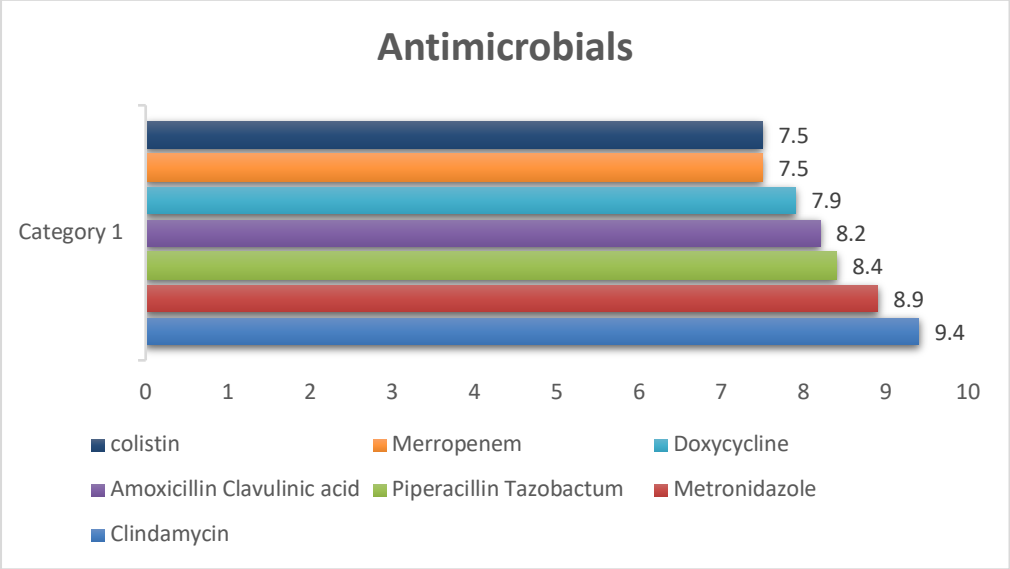

Chart 1: Ast Pattern

Discussion

Anaerobic infections can be very dangerous and can lead to death if not treated promptly. It can take several days or more to diagnose this type of infection. When anaerobic and aerobic bacteria are mixed together, they can work together to cause more severe infections. This is why it is important to use antibiotics that can kill both types of bacteria when treating anaerobic infections. [13] Anaerobic bacteria can form biofilms, which are protective layers that make them more difficult to kill with antibiotics. [14]

Our data shows that anaerobic bacteria can be isolated from a variety of infections. The most common sites of isolation were abscesses (40%) followed by soft tissue infections (10%). Anaerobic bloodstream infections (ABSIs) are not very common. Similar results were also observed by Gorbach SL et al. [15] They can be serious and even fatal if left untreated. Here, only one ABSI was there caused by *Bacteroides fragilis*. In our study, 17 (42.5%) were Gram positive bacilli (GPB), 8 (17.5%) were Gram negative bacilli (GNB) and 7 (17%) were Gram positive cocci (GPC). Most commonly isolated organism is *Actinomyces sp.*(10%) followed by *Clostridium sp.*(7.5%) And *Peptococcus sp.*(7.5%). However, other reports have shown that Gram-positive anaerobic cocci (GPAC) are the most frequently isolated pathogens**.** [16] In monomicrobial anaerobic infection, Actinomyces sp. (37.5%) is the most commonly isolated sp. followed by Fusobacterium sp. (25%). In our findings, monomicrobial infections were responded by metronidazole and doxycycline followed by clindamycin, whereas polymicrobial infections were treated smoothly by meropenem- colistin combination with clindamycin. It varies with hospital and region. [17]

It is important for clinicians to be aware of the signs and symptoms of anaerobic infections. Anaerobic bacteria are often difficult to grow in the lab, so they can be easily overlooked. By staying up-to-date on changes in bacteria, microbiologists and clinicians can provide the best possible care for their patients. [18] The rise of antimicrobial resistance among anaerobes has made it important. Different anaerobes have different patterns of antimicrobial resistance. [19] Many studies have shown the correlation between clinical failure and antibacterial resistance. [20] Clinicians can make better decisions about how to treat patients The

choice of antibiotics for the initial treatment of anaerobic infections is often based on local or national surveillance data.

Conclusion

Anaerobic pathogens can be isolated from a variety of infection sites. However, the true extent of antimicrobial resistance among these pathogens is unknown unless they are routinely cultured and susceptibility tests are performed.

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