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Emergence of Antimicrobial Resistance among Anaerobic Bacteria

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Abstract Polymicrobial infections are predominated by anaerobes accompanied by facultative anaerobes and aerobes. Failure in providing appropriate antibiotic coverage for anaerobes in mixed aerobic, anaerobic infections and increasing resistance to antimicrobial agents among anaerobic bacteria lead to increased morbidity and mortality. Antibiotic resistance among clinically important obligate anaerobic bacteria is going unnoticed because of inadequate isolation, identification, and susceptibility testing. The increasing resistance among several species emphasizes the need to survey the susceptibility patterns of anaerobic organisms. The aims of this study were, firstly, to determine the most common anaerobic bacteria originating from several abscesses and, secondly, to analyze their susceptibility patterns. This prospective study included 50 samples, either pus aspirates or tissue sections from patients with deep visceral abscesses, attending surgical and medical departments over a period of one year. Both aerobic and anaerobic cultures were done, and all isolates were subjected to antibiotic sensitivity using Kirby-Bauer disc diffusion method. A total of 33 samples showed the presence of obligate anaerobes with a rate of isolation of 66%.The obligate anaerobes isolated were *Bacteroides, Prevotella, Fusobacterium, Porphyromonas, Peptococcus, Peptostreptococcus* and *Bifidobacterium* species. *Bacteroides* showed resistance to penicillin G (76.9%), ciprofloxacin (61.5%), erythromycin (61.5%), metronidazole (46.1%), amoxicillin & clavulanic acid (46.1%) and clindamycin (38.4%). *Prevotella* showed resistance to penicillin G (69.2%), erythromycin (30.7%), metronidazole (15.3%) and clindamycin (7.6%). *Porphyromonas, Peptostreptococcus,* and *Bifidobacterium* showed susceptibility to all the drugs tested. *Fusobacterium* showed resistance to penicillin G (63.6%), metronidazole (54.5%), ciprofloxacin (36.3%) and erythromycin (27.2%). *Peptococcus* showed resistance only to ciprofloxacin (33.3%). As the anaerobic bacteria play a significant role in critical infections, all the preliminary laboratory measures are to be taken for their isolation such as proper sample collection, using appropriate media for their growth, and system for anaerobiosis. Their sensitivity pattern has to be studied as there are several reports of the emergence of resistance to various antibiotics. This antibiogram pattern helps the clinician to treat these infections with appropriate & effective therapy resulting in excellent clinical outcomes.

Keywords: *abscess, obligate anaerobes, antibiotic susceptibility testing, antimicrobial resistanc*

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1. Introduction

Purulent infections such as wound infections, cellulitis, and abscesses are known to be caused, not only by aerobes and facultative anaerobes but also by obligate anaerobes [1]. Many clinical studies, done in developed countries have shown the association of anaerobes with abscesses, wound infection, cellulitis, and diabetic foot ulcer. The anaerobic bacteria can be isolated from diabetic foot infection (30.1%), necrotizing fasciitis (18%), empyema, lung abscesses (14.5%) and Deep abscesses (10.7%). According to many studies done, most common obligate anaerobes isolated from different samples are *Bacteroides, Peptococcus, Fusobacterium, Clostridium, Peptostreptococcus* and *Veilllonella* [1]. According to Rosenblatt, there has been a tremendous increase in the role of anaerobic bacteria in clinical infections. However, the exact incidence of anaerobic infection is still unknown. This unknown prevalence is because in routine Clinical Microbiology there is a lack of anaerobic awareness and as a result, aerobes are incriminated as the sole cause of infections, and antibiotic treatment is directed against them. This under treatment results in delayed healing, the persistence of infection and morbidity [2]. Difficulties in isolating these anaerobes from clinical samples have hampered assessment of their frequency and significance in human diseases. Further, there are reports of antimicrobial resistance among anaerobes [3]. Hence there is a need for the study of isolation and antibiogram pattern of obligate anaerobes in human diseases so that an appropriate anaerobic treatment can be given and antibiotic for aerobic bacteria may be withdrawn or modified along with suitable surgical measures. This study was carried out to find out the prevalence of aerobes and anaerobes in deep-seated abscesses, the synergistic association of anaerobes and facultative anaerobes and their antimicrobial susceptibility pattern.

2. Materials and Methods

The Study was conducted from August 2015 to December 2016 with a total sample size of fifty. Either pus aspirate or tissue samples were collected from critical cases like brain abscess, empyema thoracis, gluteal abscess, dental abscess, liver abscess, breast abscess, pelvic abscess, Fournier's gangrene and Ludwig's angina. Samples like wound swabs, drain fluid, sputum, orotracheal secretion, vaginal & cervical swabs, voided urine, throat swab were not included.

Pus aspirate was collected in Operation Theater during surgery or bedside under sterile aseptic conditions and was inoculated immediately on pre-reduced anaerobically sterilized *Bacteroides* Bile Esculin agar, Laked Kanamycin Vancomycin *Brucella* blood agar biplate (Hardy Diagnostics, USA) and 5% sheep blood agar. Anaerobiosis was created for both the media plates using GEN bag anaer with clip seal (Biomerieux) and after transporting to the laboratory incubated at 37°C for 48 hours. *Pseudomonas aeruginosa* ATCC 27853 on citrate agar medium was used as a control for anaerobiosis. The pus was simultaneously inoculated in thioglycollate broth, blood agar and Mac Conkey agar and incubated aerobically at 37°C for 24 to 48 hours. Thioglycollate broth was used as a transport media as well as used as a backup broth for subculturing.

A part of the pus aspirate was examined for foul odor, purulence, blood tinge, black necrotic discharge or sulfur granules. Direct smear examination which plays a crucial role in identifying anaerobic bacteria and empirical therapy, was performed by making a thin smear of the pus sample. The smear was air dried & methanol fixed. For tissue sample, the tissue was crushed in motor & pestle, and a thin smear was prepared, air dried & methanol fixed. Gram staining of fixed smears was done with safranin as counter stain and examined. Wherever necessary depending on the differential diagnosis, Ziehl-Neelsen staining for acid fast bacilli, potassium hydroxide mount examination for fungal elements and wet mount examination for trophozoites were done.

The primary plates were examined for all different colony types. The colony description was given as size, shape, edge, profile, color, opacity, pigment and hemolysis. The anaerobic growth on selective media was quantified as - heavy growth, moderate growth and few colonies [4].

Confirmation of obligate anaerobes was done by subculturing of the suspected colonies onto blood agar along with Gram stain examination. Aerotolerance test of the suspected colonies was done to differentiate between facultative & obligate anaerobes.

Further identification of suspected colonies grown on blood agar was made by recording characteristics like colony morphology, pigment production, hemolysis and presumptive identification using special potency discs (Hardy Diagnostics, USA). The special potency discs used were Vancomycin 5µg, Kanamycin 1000µg, and Colistin10µg. For presumptive identification, a lawn culture was made on two quadrants of blood agar using 3 to 4 isolated colonies. The other two quadrants were streaked for isolated colonies. The special potency discs were placed within 20 minutes on the first and second quadrant and anaerobically incubated at 35°C for 48 hours. A zone of inhibition of more than 10 mm was interpreted as sensitive and less than 10 mm as resistant.

Antimicrobial susceptibility testing of anaerobes was performed on 5% sheep blood agar plate by Kirby-Bauer Disc diffusion method [5]. Turbidity was adjusted to 0.5 Mc Farland for rapid growing anaerobes & 1 Mc Farland for slow growing anaerobes. Anaerobic incubation was done for 24 to 48 hours depending on the growth rate of the organism [4]. Quality control of anaerobic media and susceptibility testing was performed by using ATCC 23745 *Bacteroides fragilis*.

For aerobic culture, the sample was inoculated on 5% sheep blood agar & Mac Conkey agar media and Gram staining is done. The media plates were incubated at 37°C for 24 to 48 hours. After the growth, the predominant colonies were processed further & identified using a set of biochemical reactions. Antimicrobial susceptibility testing for the aerobic bacteria isolated was done on Mueller Hinton agar medium by Kirby-Bauer disk diffusion method in accordance with CLSI guidelines. After overnight incubation, the zone diameters around the antimicrobial discs were measured. Sensitivity and resistance pattern was reported according to CLSI guidelines. ATCC 25922 *Escherichia coli*, ATCC 25923 *Staphylococcus aureus*, ATCC 27853 *Pseudomonas aeruginosa*, ATCC 29212 *Enterococcus faecalis* were used for Quality control of aerobic media and antimicrobial susceptibility testing.

Table 1. Interpretation of special potency disc patterns of obligate anaerobic bacteria

Bacteria	Vancomycin (5µg)	Kanamycin (1000µg)	Colistin (10 µg)
Bacteroides fragilis group	R	R	R
Fusobacterium species	R	S	S
Prevotella species	R	R^S	V
Porphyromonas species	S	R	R
Gram positive anaerobic cocci	S	V	R
Gram negative	R	V	V

S-sensitive, R-Resistant, R^S–mostly resistant but can be sensitive, V-Variable sensitivity.

Table 2. Antibiotics used for anaerobic antimicrobial susceptibility testing

DRUG	DISC POTENCY
Penicillin G	10 IU
Erythromycin	15 µg
Metronidazole	5 µg
Chloramphenicol	30 µg
Clindamycin	2 µg
Amoxicillin & Clavulanic acid	30 µg
Imipenem	10 µg
Ciprofloxacin	5 µg

3. RESULTS

A total of 50 samples of deep-seated abscesses obtained during the study period were processed. Out of this, 40 (80%) samples have shown growth of either aerobic or anaerobic or mixed growth. The remaining 10 (20%) samples have not demonstrated any observable growth and were considered sterile. Among 40 culture positive specimens, 33 (66%) samples showed anaerobic growth out of which 17 (34%) samples showed pure anaerobic growth and 16 (32%) samples showed mixed anaerobic and aerobic bacterial growths. 7 (14%) samples out of 40 positive samples showed only aerobic bacterial growth.

Anaerobes are known for their polymicrobial type of infection wherein a synergy exists between the obligate anaerobes & facultative anaerobes. Similar synergism was found in our study wherein 51.2% samples had grown both obligate & facultative anaerobes or among 2 or more obligate anaerobes. Total synergism is seen in 21 culture positive specimens & single organism was found in 10 culture positive samples. Synergy was taken into consideration based on culture results than a direct smear. If the results of direct smear were taken into account, then the percentage of synergism would still go up, since in some of the samples multiple morphotypes were seen & all were not cultivable.

3.1. Association of Predisposing Conditions

Most of the mixed infections involving aerobic and anaerobic organisms were found to be commonly associated with some predisposing conditions like chronic suppurative otitis media, congenital heart diseases, diabetes mellitus, tooth decay, trauma, tobacco chewing, pneumonia, etc. Percentage of association of predisposing conditions among positive samples in the present study was 87.5%.

Table 3. Association of predisposing conditions with infections

Total positive cultures	40
Cultures associated with predisposing conditions	35
Cultures with no predisposing conditions	5

3.2. Microscopy versus Culture

The sensitivity of microscopy depends on the number of organisms in the specimen (10^3 CFU/ml). In our study, the Gram's stain was positive in eight culture-negative cases, while it was negative in eleven culture-positive cases. There is a need to adopt methods to improve the detection rates especially by microscopy, which includes fluorescent staining using acridine orange (sensitivity (10^2 CFU/ml) [13]. In our study increased isolation of anaerobes was seen in dental abscesses. Out of 18 samples processed, all samples showed the growth of obligate anaerobes in which 11 samples showed mixed infection, only one obligate anaerobe was present in seven samples, and six facultative anaerobes were grown. The total rate of isolation was 100%. The most common organisms were *Prevotella* species (13), *Fusobacterium* species (11) followed by *Porphyromonas* species, *Peptococcus* species and *Bifidobacterium* species. A study done by Don Walter Kannangara showed that out of sixty-one cases of pyogenic dental infection considered Forty-five (74 percent) patients had anaerobic infections.

3.3. Isolation of Obligate Anaerobes

Out of 50 clinical samples, 33 samples showed positivity for obligate anaerobic bacterial growth. *Bacteroides* species (13), *Prevotella* species (13), *Fusobacterium* species (11) were commonly found in the abscesses followed by *Peptococcus* species (3), *Peptostreptococcus* species (1), *Porphyromonas* species (1) and *Bifidobacterium* species (1).

3.4. Isolation of Aerobes and Facultative Anaerobes

Of total 50 clinical samples, five aerobic organisms were isolated out of which four are facultative anaerobes, and one (*Pseudomonas aeruginosa*) is an obligate aerobe. *Escherichia coli* (7), *Klebsiella pneumoniae* (3) and *Staphylococcus aureus* (7), were more commonly found followed by *Pseudomonas aeruginosa* (7), and *Enterococcus faecalis* (1) in synergism with obligate anaerobes.

3.5. Antimicrobial Susceptibility Pattern of Anaerobes

Bacteroides species were the most encountered clinically significant isolates among the gram negative anaerobes. The *Bacteroides* species showed maximum resistance to penicillin G (76.9%) followed by ciprofloxacin (61.5%) and erythromycin (61.5%). Resistance was also found to metronidazole (46.1%), amoxicillin & clavulanic acid (46.1%) though seven isolates showed susceptibility to metronidazole (53.9%) and amoxicillin & clavulanic acid (53.9%). Five isolates showed resistance (38.4%) and eight isolates (61.6%) were sensitive to clindamycin. All the *Bacteroides* species were uniformly susceptible to imipenem and chloramphenicol.

Prevotella species showed maximum resistance to penicillin G (69.2%) and erythromycin (30.7%). Few isolates of *Prevotella* showed resistance to metronidazole (15.3%). 11 isolates were susceptible to metronidazole (84.7%). Out of 13 *Prevotella* isolates only one isolate was resistant to clindamycin (7.6%) and remaining 12 isolates (92.4%) were susceptible. All the *Prevotella* isolates were uniformly susceptible to chloramphenicol, amoxicillin & clavulanic acid and imipenem.

Table 4. Antimicrobial susceptibility pattern of anaerobes

	Metronidazole		Clindamycin		Chloramphenlcol		Amoxicillin Clavulanic acid		Erythromcin		Ciprofloxacin		Lmipenem		Penicillin G	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Bacteroides (13)	7	6(46.1%)	8	5(38.4%)	12	1	7	6(46.1%)	5	8(61.5%)	5	8(61.5%)	13	0	3	10(76.9%)
Prevotella (13)	11	2(15.3%)	12	1(7.6%)	13	0	9	4	5	8(30.7%)	7	6(46.1%)	13	0	4	9(69.2%)
Porphyromonas (1)	1	0	1	0	1	0	1	0	0	1	0	1	1	0	1	0
Fusobacterium (11)	5	6(54.5%)	10	1	11	0	10	1	8	3	7	4	11	0	4	7(63.6%)
Peptococcus (3)	3	0	3	0	3	0	3	0	3	0	2	1	3	0	3	0
Peptostreptococcus(1)	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
Bifidobacterium (1)	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0

Porphyromonas species, *Peptostreptococcus* species and *Bifidobacterium* species showed susceptibility to all the drugs tested.

Fusobacterium species showed maximum resistance to penicillin G (63.6%) and metronidazole (54.5%) followed by ciprofloxacin (36.3%) and erythromycin (27.2%). All *Fusobacterium* isolates were susceptible to imipenem and chloramphenicol. Out of 11 *Fusobacterium* isolates, only one strain (9%) is resistant to clindamycin and remaining ten isolates showed susceptibility. Similarly only one strain (9%) out of 11 showed resistance to amoxicillin & clavulanic acid.

Peptococcus species showed susceptibility to all the drugs tested except one isolate which showed resistance only to ciprofloxacin.

3.6. Antimicrobial Susceptibility Pattern of Aerobes and Facultative Anaerobes

A total of 23 aerobic organisms were isolated, out of which 15 were Gram negative bacilli and 8 were Gram positive cocci. All the Gram negative bacilli were uniformly sensitive to imipenem (100%). Sensitivity pattern of *Escherichia coli* showed maximum resistance to gentamicin (71.4%) followed by cephalexin (42.8%). The resistance profile of *Escherichia coli* with ceftriaxone and ciprofloxacin were 28.5% and 42.8% respectively. All the *E. coli* isolates showed sensitivity to piperacillin & tazobactam. *Klebsiella pneumoniae* showed high resistance to cephalexin (66.6%) and gentamicin (66.6%) followed by ceftriaxone (33.3%) and ciprofloxacin (33.3%). All the isolates of *Klebsiella pneumonia* were sensitive to piperacillin & tazobactam. *Pseudomonas aeruginosa* showed high resistance to ceftriaxone (60%) and gentamicin (60%) followed by ciprofloxacin (40%) and piperacillin & tazobactam (20%). Regarding the antimicrobial susceptibilities of Gram positive cocci, *Staphylococcus aureus* was highly resistant to ampicillin and with 100% isolates showing resistance to this drug. All the isolates showed sensitivity to piperacillin & tazobactam (100%). Resistance to ciprofloxacin was 28.5% and to ceftriaxone was 14.2%. In the present study, no MRSA isolates were encountered. Only one isolate of *Enterococcus faecalis* was isolated from a case of an abscess forming post cholecystectomy which was sensitive to high-level gentamicin. Resistance was seen with amoxicillin & clavulanic acid, ciprofloxacin, imipenem and levofloxacin.

4. Discussion

Anaerobic bacteria are a major component of the normal human microbiota residing on mucous membranes and predominate in many infectious processes, particularly those arising from mucosal sites. These organisms generally cause disease subsequent to the breakdown of mucosal barriers and the leakage of indigenous flora into normally sterile sites. The predominance of anaerobes in certain clinical syndromes can be attributed to the large numbers of these organisms residing on mucous membranes, the elaboration of a variety of virulence factors, the ability of some anaerobic species to resist oxygenated microenvironments, synergy with other bacteria, and resistance to certain antibiotics. Anaerobic bacteria play an important role in the aetiology of mixed aerobic-anaerobic infections. Such mixed infections may afford an optimum situation for the exchange of genetic elements between species of aerobes and anaerobes, resulting in increased virulence and antimicrobial resistance. Clinicians have become more aware in the past few decades of the types of infections caused by anaerobic bacteria. The importance of anaerobes in certain infections is further enhanced by the failure to provide appropriate antibiotic coverage for anaerobes in mixed aerobic-anaerobic infections and an increase in the number of anaerobes that have become resistant to antimicrobial agents [10].

In our study, a total of 50 samples were collected from different clinical conditions like brain abscess, pyopneumothorax, perineal abscess, dental abscess, liver abscess, breast abscess, abdominal abscess, etc. Different samples which were collected and considered for anaerobic culture were pus aspirates and tissue samples. A total of 50 clinical samples, of which 46 were pus aspirates and 4 were tissue samples, 40 samples have given the positive results. It is stated that pus aspirate has the advantage of being the best transport medium [6].

4.1. Rate of Isolation of Obligate Anaerobes

Suitable commercially available anaerobic-transport media along with bedside inoculation of specimens and rapid sealing of inoculated anaerobic media plates in anaerobic environment increases the isolation of obligate anaerobes. The total rate of isolation of obligate anaerobes in our study was 66 %. According to Ion Rosenblatt, there has been a tremendous increase in the role of anaerobic bacteria in clinical infections. Older studies show an incidence of 2-10% whereas recent studies indicate recovery of anaerobes from 85% of clinical specimens [2]. The rate of isolation of obligate anaerobes from the total clinical samples was statistically significant. Similar studies by Anuradha De, Alka *et al* [7]. Joseph W Holland *et al* [1]. Yooswon Park *et al*. [8] showed an isolation rate of 7.9 %, 48.8 % and 65.3% respectively. A similar study was done by Ajitha Mehta wherein 154 post-operative sepsis cases were studied from abdominal & Gynaec-obstetric surgery. Samples collected were subjected to aerobic & anaerobic culture. Out of 130 cases, aerobes were isolated from 81.33%, anaerobes from 41.33%. Anaerobic gram negative bacilli (58.07%) were predominant than Gram positive cocci (37.4%) [9].

4.2. Synergism

Polymicrobial infection can be more virulent than those involving single organisms. Synergism between aerobic and anaerobic bacteria has been recognized in a variety of clinical infections. The ability of anaerobic bacteria to act synergistically during polymicrobial infection contributes to the pathogenesis of anaerobic infections. Studies in experimental models demonstrate that facultative and obligate anaerobes synergistically potentiate abscess formation [10]. Both facultative and obligate anaerobes were inoculated into the mice together and found that there was an increased virulence and mortality rate when inoculated together than separate [7,11].

In our study, synergism was seen among most of the culture positive samples.

Total positive cultures – 40

Synergism seen in – 21 (51.2%)

No synergism (single organism) seen in – 10 (48.8%)

Total synergism was observed in 21 samples out of which 11 samples showed synergism between facultative anaerobes and ten samples with obligate anaerobes. Common aerobes which were found in synergy with obligate anaerobes were *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

4.3. Association of Predisposing Conditions

Out of the total 39 samples, 34 samples were associated with predisposing conditions. 34 patients had predisposing conditions like tooth decay (15), trauma (5), chronic suppurative otitis media (3), congenital heart diseases (2) and others (4) like fissure in ano, injection, appendicitis, cholecystectomy, lactation, etc. The most common predisposing condition found in our study was tooth decay. A similar correlation has been found in the study done by J. Craig Baumgartner *et al* where in 8 of 22 (36%) cases showed association with tooth decay. Association of the predisposing factors has been found to be associated with the isolation of anaerobes from clinical sample. [12]

4.4. Isolation Rate of Anaerobes from Various Abscesses and Their Statistical Correlation

4.4.1. Empyema Thoracis (Pyothorax)

Empyema is the presence of pus in the pleural cavity and represents an effusion containing a great number of polymorphonuclear leukocytes and fibrin; it is an internal extension of infection from pneumonia, lung, oral, retropharyngeal paravertebral or skin abscess [14]. Among the respiratory conditions, empyema thoracis has been the most common condition in which obligate anaerobes were associated. The total rate of isolation of obligate anaerobes is 80% in our study among empyema cases. A study done by Zhang Y *et al*. showed that the isolation rate of anaerobes was rather high in pyothorax (88.9%) out of 372 specimens collected from surgical patients and the predominant anaerobe was *Bacteroides* (25%) [15].

4.4.2. Brain Abscess

Anaerobic bacteria are common constituents of brain abscesses. Either a single anaerobic species or a mixture of anaerobic or aerobic bacteria, or both, may be found in brain abscesses [10]. Brain abscess most commonly originates from the contiguous site of an existing infection as chronic otitis media, mastoiditis, sinusitis or dental caries but it can also occur directly after penetrating head injury, neurosurgical procedures or hematogenously as in children with congenital heart disease [16]. The rate of isolation of obligate anaerobes from brain abscess in our study was 44.4%. The various anaerobes isolated were *Bacteroides* species which was most commonly seen in 3 brain abscesses. *Peptostreptococcus* was isolated in one sample. No growth was observed in 5 samples and was correlating with direct smear with numerous pus cells and no bacteria. Out of 9 samples collected from patients, male preponderance is observed. A study done by V. Lakshmi *et al*., 2011 showed that in the case of Intracranial abscesses larger number of men being affected and otogenic infections were the most common. Total numbers of *Bacteroides* isolated were four from a total of 39 intracranial abscess samples. [13] Predisposing factors were chronic suppurative otitis media and congenital heart diseases. Out of 9 samples collected, five patients had chronic suppurative otitis media, 3 had congenital heart disease (1 ASD, 2 VSD). According to a study done by Ingham HR *et al*. otogenic cerebral abscesses constitute a major proportion of all cerebral abscesses. [17] A study done by Kashi *et al.* showed predisposing factors were identified in 94 (79.7%) patients, otogenic infection being the most common (31.4%) which is correlating with the present study (50%). A study was done by Sarala Menon *et al*., 2008 [18] showed that chronic suppurative otitis media was the most common predisposing factor for temporal lobe infections. Forty-one (54.70 %) abscesses were found to be due to pyogenic organisms.

4.4.3. Perianal Abscess

Organisms of the gastrointestinal tract were found most often in the intra-abdominal and buttock lesions. Perirectal abscess by direct smear showed the anaerobic organisms and are isolated in the culture. In our study, a total of 7 perianal abscesses were collected out of which five samples showed polymicrobial growth with obligate and facultative organisms. Most common obligate anaerobe isolates were *Bacteroides* species (5) (71.4%), and facultative anaerobe was *Escherichia* coli (6) (85.7%). *Staphylococcus aureus* (1) was isolated in a case of a gluteal abscess. A study done by S. J. Eykyn and R. H. Grace showed that Gut aerobes, predominantly *Escherichia coli*, were isolated from 49 of 53 (92.5%) and 'Gut-specific *Bacteroides*' mostly *Bacteroides fragilis* were isolated from 47 of 53 (88.7%) patients [19].

4.5. Isolation of Aerobic Bacteria

Among 18 samples, facultative anaerobes were found in association with obligate anaerobes, and a total of 23 aerobes were isolated from 50 samples. Most typical aerobic gram negative bacilli being *Escherichia coli* 7 (14%), Second common gram negative bacilli was *Pseudomonas aeruginosa* 5 (10%) followed by *Klebsiella pneumoniae* 3 (6%). Most common gram positive cocci isolated was *Staphylococcus aureus* 7 (14%) followed by *Enterococcus faecalis* 1 (2%). Our study correlates with the study done by Park *et al*. (18) wherein most common organisms were *Escherichia coli* (17.5%), *Staphylococcus aureus* (7.5%) and *Klebsiella pneumoniae* (7.5%). In a study done by S.Saini *et al*., the predominant aerobic isolates were *Escherichia coli*, *Staphylococcus aureus*, Coagulase negative *Staphylococcus* and *Klebsiella pneumo*nia [20].

4.6. Antimicrobial Susceptibility and Resistance Pattern among Obligate Anaerobes

All Bacteroides isolates were susceptible to Imipenem, and only one isolate showed resistance to Chloramphenicol. *Bacteroides* species showed maximum resistance to penicillin G (76.9%), ciprofloxacin (61.5%) and erythromycin (61.5%). Resistance was also found to metronidazole (46.1%), amoxicillin and clavulanic acid (46.1%) and clindamycin (38.4%). A study conducted by Micaela Gal reported that out of 206 *Bacteroides* isolates 24% showed resistance to metronidazole. Resistance to clindamycin group is commonly seen with *Bacteroides fragilis* group. Mechanism of resistance is by inactivation / altered ribosomal target site. The *erm* F gene involves another mechanism of resistance in some *Bacteroides* species, conjugal transfer of clindamycin resistance has been shown to be plasmid mediated, chromosomally enabled clindamycin resistance is linked to tetracycline resistance. Similar results were found in a study done by Ayyagari A *et al.*, wherein comparatively increased resistance was seen in erythromycin & ampicillin group of the drug among *B. fragilis group*. *Bacteroides* showed resistance to penicillin as they produce beta-lactamase enzymes. There are reports of development of resistance to metronidazole drug among *Bacteroides* spp [20]. A study done by David W. Hecht said that "the national anaerobe survey performed by Tufts–New England Medical Center (Boston) has reported frequencies of Clindamycin resistance among anaerobes in the *B. fragilis* group as low as 3% in 1987, which increases to 16% and 26% in 1996 and 2000, respectively. Individual medical centers in these and other studies have found frequencies of resistance to clindamycin to be as high as 44%." [22]. Rates of resistance to clindamycin among the *Bacteroides fragilis* group have increased in the United States from 3% in 1982 to 16% in 1996 and 26% in 2000, with rates as high as 44% in some series [23]. The medically important *Bacteroides* species are typically resistant to penicillin G (> 97%) [23].

Prevotella spp showed maximum resistance to penicillin G (69.2%) and erythromycin (30.7%). Few isolates showed resistance to metronidazole (15.3%) and clindamycin (7.6%). A study done by David W. Hecht showed that 83% of *Prevotella* isolates were resistant to penicillin G [24]. Ackermann *et al*. have reported clindamycin resistance among *Prevotella* spp. (9% resistant) *Fusobacterium* showed maximum resistance to penicillin G (63.6%) and metronidazole (54.5%) followed by ciprofloxacin (36.3%) and erythromycin (27.2%). F. Baquero *et al*., 1992 reported that two-thirds of all *Fusobacterium* isolates showed resistance to erythromycin [24]. Susan Nyfors *et al*., 2002 reported that 18% of the total *Fusobacterium* isolates were β lactamase producers and are resistant to penicillin group of drugs [26]. *Porphyromonas* spp, *Peptostreptococcus* spp and *Bifidobacterium* spp which showed susceptibility to all the drugs tested. *Peptococcus* spp showed resistance only to ciprofloxacin (33.3%).

5. Conclusion

In conclusion, our study signifies the role of obligate anaerobes in different clinical presentations, their synergism with other facultative, obligate aerobic & anaerobic organisms and also their increasing antibiotic resistance pattern. Diagnostic importance has to be given for the presence of these organisms in various clinical samples, as they are often overlooked. Routine processing of the samples employing anaerobic methods like proper sample collection, appropriate media and conditions for anaerobiosis should be practiced by the laboratories to look for the presence of these organisms. When samples from suspected anaerobic infections are cultured, it is imperative that they be properly collected and transported. Samples should be collected so as to avoid contamination by indigenous flora of mucosal surfaces. It is also important to remember that prior antibiotic therapy reduces cultivability of these bacteria [10]. Antibiotic susceptibility testing should also be done as there are many reports on the emergence of resistance to various antibiotics among these obligate anaerobes [27]. These measures help the clinician to provide an evidence-based therapy for the patient betterment and also avoid usage of empirical therapy which is resulting in over usage of the drugs and causing the emergence of resistance to various antibiotics. As evidenced from our study and various other studies, beyond doubt, there is a significant problem with increasing resistance to antimicrobial agents among anaerobic bacteria it is the need of the hour that antibiotic policies should be formulated and implemented to control and overcome this emerging problem.

Conflict of Interest

The authors have no competing interests.

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