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An audit on anaerobic bacteria isolated in the year 2012, in a tertiary care centre in Tamil Nadu

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Abstract : The definitive diagnosis of non-spore forming anaerobic infections is dependent on the laboratory isolation and identification of these pathogens as such infections do not have any characteristic clinical features. Here, we present retrospective data for the year 2012 on anaerobic micro-organisms isolated at our centre. Of the 636 samples sent for anaerobic culture, anaerobes were isolated in 37 samples of which 31 (83.87) were non-spore forming anaerobes. Mixed infections were seen in 2431 (77.41). Bacteroides spp. Peptostreptococcus spp. were the most commonly isolated anaerobes. Infection due to this organism was almost exclusively seen in adults, age 20 years. This retrospective study re-iterates that anaerobic infections still occur and the clinicians should be aware, so that appropriate specimens can be sent. Prospective studies are being planned to determine the prevalence of anaerobic infections using the newer and more efficient automated systems.

Keyword : Anaerobic bacteria, non-spore forming anaerobes, Bacteroides spp., Clostridium spp.

Introduction:

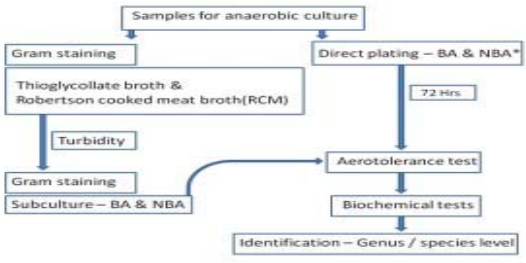
Anaerobes were first discovered by Louis Pasteur in 1862. They made their first appearance in the clinical microbiology laboratory in 1893, when the first isolate, *Bacteroides fragilis* was isolated (1). Subsequently, over the next three decades, anaerobes were documented to be the major causative agents of puerperal sepsis, lung abscesses and intra-abdominal sepsis. Anaerobic microbial bacteriology went from a period of intense neglect to a period of intense activity. The year 1965 marked the start of the renaissance of anaerobic microbiology, largely spearheaded by Sidney Finegold, who is often referred to as the father of anaerobic microbiology (2-4). In India, interest in anaerobic microbiology started a little later but soon caught up, and by 1980s, anaerobes had been cultured from all types of infections, starting with brain abscesses, lung abscesses, otitis media, oro-dental infections, cutaneous abscesses, intra abdominal sepsis, pelvic infections (3,4). The success of anaerobic culture is largely determined by the quality of the samples, speed of processing and effective means for the maintenance of anaerobic environment for the organism to grow. There is difficulty in culturing anaerobes because of the need for highly trained and motivated personnel, and costly infrastructure (5). In recent times, automation has been introduced to overcome these limitations.

Anaerobic infections are most often treated with metronidazole and clindamycin in our hospital. The cost of anaerobic culture is twice that of aerobic culture, in addition the culture report takes a minimum of 6-8 days (based on our data). Therefore empirical treatment with metronidazole and/or clindamycin has become a routine among clinicians at our centre. Unless judicious use ofantimicrobials is planned for the anaerobic bacteria based on their susceptibility patterns, they may soon follow their counterparts, the aerobes, in developing into 'superbugs' which do not respond to commonly used drugs. Therefore it is imperative for microbiology laboratories to isolate and identify these pathogens from an epidemiological viewpoint, so that future clinical disasters from resistant microorganisms can be prevented (5).

Methodology:

This is a retrospective observational study. Totally 636 samples were sent for anaerobic culture in the year 2012. Fig1. describes the algorithm followed in our laboratory for isolation and identification of anaerobes. Based on the individual biochemical reactions like glucose, sucrose, lactose, mannite, trehalose fermentation, production of indole, lecithinase, lipase, gelatinase, esculin hydrolysis, and reactions in litmus milk, the genus/species level identification was made (7). Details of the patients such as age, sex, culture report were collected. The collected data were documented using Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA). For preliminary analysis, we classified the culture report as aerobic organisms, anaerobic organisms, contaminants and no growth.

Figure 1: Algorithm for isolation & identification of Anaerobes



* BA – Blood agar plate & NBA- Neomycin Blood agar plate incubated in anaerobic jar

Among the anaerobes, we analyzed the type (spore and non-spore formers) and identified the anaerobe to their genus/ species level. Statistical analysis, chi square test was done using the online software (in-silico.net, Hamburg, Germany) to test our hypotheses regarding the role of age and sex on positive anaerobic culture results in these groups.

Results:

Among the 636 samples received for both aerobic and anaerobic culture in the year 2012, 276 (43.39%) were pus samples, 233 were tissue biopsy samples (36.63%), and 127 were samples from other sites (19.96%). The male: female sex ratio for the samples was 1.6:1 (393:243). The age distribution of the samples are as follows; 105 (16.5%) samples were from individuals 20 years, whereas the rest, 531 (83.49%) were from those whose age was >20 years. Of the 636 samples, 253 (39.77%) showed no growth in both aerobic and anaerobic culture, 293 (46.06%) samples grew aerobic organisms, 53 (8.33%) samples grew contaminants (coagulase negative staphylococci, diphtheroids, alpha- haemolytic streptococci) and 37 (5.8%) grew anaerobic organisms (Fig. 2). Among the 37 anaerobes isolated 6 (16.2%) belonged to the genus *Clostridium*, of which one was *Cl. tetani* and 31 (83.8%) were non-spore formers. Mixed infections were associated with 30 (81.08%) of the 37 samples positive for anaerobes. All clostridia species and 24 (77.4%) of the 31 non spore formers were found to be in mixed infections (Table 1). The non-spore formers isolated included 16 *Bacteroides spp.*, 17 *Peptostreptococcus spp*, 4 *Fusobacterium spp,* and one each of *Prevotella spp, & Porphyromonas spp* (Table2). Of the 37 samples from which anaerobes were isolated, 15 were pus specimens, 13 were surgically excised tissue samples, among the remaining nine, five were from patients with lacrimal canaliculitis.

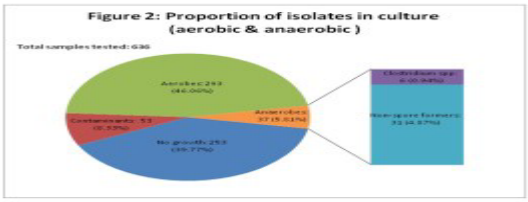


Table 1: Distribution of anaerobes isolated in pure and mixed infection

n = 37	Mixed infection			Pure infection
	Aerobes	Anaerobes	Aerobes + anaerobes	
Non-spore forming anaerobes	17	6[1]	1[1]	7
Clostridium spp.	5	1[2]	-	0
Total	30			7

1 - All the anaerobes were non spore forming anaerobes

2 – Was isolated with another spore former

Table 2: List of various anaerobic organisms isolated in mixed and pure infection

Organism isolated	Mixed infection	Pure infection
Clostridium spp.	6	-
Bacteroides spp.	12	4
Peptostreptococcus spp.	15	2
Fusobacterium spp.	4	1
Porphyromonas spp.	1	-
Prevotella spp.	1	-

Table 3: Chi Square results for significance of role of sex and age on distribution of positive anaerobic culture

	Anaerobe culture positive	Anaerobe culture negative	p value
Male (n=393)	21 (5.35%)	372 (94.65%)	0.516
Female (n=243)	16 (6.58%)	227 (93.42%)	
Age ≤20 yrs (n=105)	1 (0.95%)	104 (99.05%)	0.02
Age >20 yrs (n=531)	36 (6.77%)	495 (93.22%)	

Chi-square test analyses shows that anaerobic culture results are equally distributed over gender whereas those with age >20 years are more likely to be positive than those with age 20 years (Table 3).

All the anaerobes, except for one non-sporing anaerobe, were isolated from adults (20 years of age). The only anaerobe isolated in a person under the age of 20 years was *Peptostreptococcus spp* from an appendicular abscess of a nine year old boy. The lone isolate of *Cl.tetani* was from a 26 year old male who had a history of RTA a week prior to presenting with signs & symptoms of tetanus, as well as no history of vaccination. Tissue from the affected toe was sent for microscopy and anaerobic culture. As the patient had typical clinical features of tetanus, appropriate treatment with tetanus toxoid and tetanus immunoglobulin was initiated (despite negative Gram staining), including intensive care. Subsequently culture grew *Cl. tetani*, patient improved and was discharged in stable condition. *Bacteroides fragilis* was found to be the commonest species among the *Bacteroides spp.* isolated. Most of the *Bacteroides spp.* were found to be in mixed infections – 75% (12 of 16).

Discussion:

B.fragilis and *Peptostreptococcus spp.* were the commonest organisms isolated in our study, and this is confirmed in literature. *B.fragilis* is important medically because it is associated with polymicrobial infections and many strains produce beta lactamases. In addition, they are associated with high mortality as they cause invasive infections and are highly resistant to penicillin, vancomycin, and colistin (6). Most of the *B.fragilis* strains are susceptible to metronidazole, though few strains may be resistant (7).

In this retrospective audit of anaerobic culture for the year 2012, we had an anaerobic culture positivity rate of 5.8% whereas a study conducted by De and Gogate, showed a prevalence of 8%. Our data shows that 77.4% of the anaerobic infections due to non-spore formers were mixed, and this is comparable to the results (78.2%) obtained in the study performed by De and Gogate (8).

There was no significant difference in prevalence of anaerobic infections in males or females. In contrast, anaerobic cultures in individuals above the age of 20 years were more likely to be positive.

Two other studies, have shown similar results vis-à-vis the age of patients. Thirumoorthi et al found that only 2.9% cultures contained anaerobes in children (9). In another report of 70 cases of anaerobic infections only six patients were 19 years or younger (10). A probable explanation for this difference may be related to the fact that underlying conditions that predispose to anaerobic infections in an adult population are less commonly encountered in a pediatric age group (9). Recently a study conducted in Korea by Park and colleagues also has reiterated that anaerobic infections are more common in adults than in children (11).

The isolation rate is low in our study and we believe it to be due to the fact that no special transport media was used. Moreover, most of the samples were first inoculated into thioglycollate (TG) & Robertson cooked meat broth (RCM), though ideally they should have been inoculated and immediately incubated in strict anaerobic conditions (anaerobic jar). During the period of the study, the plates inoculated were incubated in an anaerobic jar using the evacuation replacement technique. As this is a cumbersome, technically demanding and labor intensive procedure, this was performed at fixed times (twice) every day.

This audit of anaerobic culture has provided new insights so that we can undertake appropriate measures to increase the yield. We hope to increase the yield by prompt transport in appropriate containers (to maintain viability of anaerobes) and rapid processing using the newly acquired and commissioned automation for the same. In addition, antimicrobial testing for anaerobes will be implemented to monitor the susceptibility patterns and development of resistance. Furthermore, this study reiterates that anaerobic infections though uncommon still occur. Appropriate and adequate specimens collected, transported and processed rapidly are crucial in maximising anaerobic culture yield. We opine that automated anaerobic culture and commercial transport media will be helpful and further prospective studies using these tools are required to confirm this supposition. In addition, anaerobic infections seem to be commoner in older age groups, this and the role of underlying disease needs to be studied further.

Conclusion:

Anaerobic infections still occur, *Bacteroides spp.* and *Peptostreptococcus spp.* are the commonest anaerobes isolated. Chance of isolating an anaerobe seems to be significantly higher in those above 20 years. This preliminary finding needs to be studied further in a prospective manner to determine its significance. A greater awareness of these bacterial infections amongst the clinicians and use of better and more sophisticated techniques of anaerobic cultures in the laboratory is likely to bring an increase in the isolation of anaerobes.

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