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Anaerobic Profile of Intra-abdominal Infections – A 23-Year Retrospective Study

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Abstract

Obligate anaerobes, which are part of normal intestinal flora are now gaining pathogenic potential by becoming more virulent and causing moderate to severe abdominal infections. Moreover, there is delay in initiation of appropriate antimicrobial therapy. The study aimed to describe and analyse 23 years data on anaerobic intra – abdominal infections in regards to the distribution and antimicrobial susceptibility patterns of the obligate anaerobes which were isolated from various intra – abdominal infections. The demographic and microbiological data was retrieved from the microbiology departmental registers. Total number of cases/specimen were 1124. *Bacteroides fragilis* group (238) (56%) and Peptostreptococcus sp (109) (25%) amounted to the majority of the isolates. Rare anaerobes like *Clostridium sporogenes, Propionibacterium sp, Clostridium bifermentans* and *Fusobacterium varium* were also isolated. Majority of mixed anaerobic infections were contributed by *Bacteroides fragilis* group and *Peptostreptococcus* sp (99) out of 102 mixed anaerobic infections). Chronic alcoholism was the most common predisposing condition (p value <0.05). Among the antimicrobials which were used by the clinicians for treating the infection, only Metronidazole was tested for its susceptibility pattern. One isolate was resistant to metronidazole (Diameter of inhibition zone was 6 mm). As they are fastidious they usually go unnoticed. Hence, this descriptive study intends to bring light on the large number of various obligate anaerobes and the potential diseases that they can cause and also the need for their antibiotic susceptibility testing to look for antimicrobial resistance among the isolates.

Keywords: Anaerobes, *Bacteroides Fragilis* Group, *Peptostreptococcus* sp, Metronidazole

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INTRODUCTION

Intra-abdominal infections encompasses a plethora of pathological conditions that involve lesions of all the intra-abdominal organs. They range from inflammation of a single organ to peritonitis which can be primary, secondary or tertiary. They also include intraperitoneal, retroperitoneal and parenchymal abscesses.[1] In uncomplicated intra-abdominal infections the infectious process only involves a single organ and does not proceed to peritoneum. In complicated intra-abdominal infections, the infectious process proceeds beyond the organ, and causes either localized peritonitis or diffuse peritonitis and are associated with morbidity and poor prognosis.[1,2] Although the facultative anaerobes like *E.coli, Klebsiella* and other members of Enterobacteriaceae are the most common organisms to be implicated in intra-abdominal infections, obligate anaerobes also have the potential to cause intra abdominal infections. Obligate anaerobes, which are part of normal intestinal flora are now gaining pathogenic potential by becoming more virulent and causing moderate to severe abdominal infections. The obligate anaerobes were largely neglected as their mere incapability of causing serious abdominal infections. Infections due to anaerobic isolates can sometimes be missed because of the special measures required for their transportation (anaerobic transportation). These obligate anaerobes (like *Bacteroides fragilis* group, *Peptostreptococcus* sp) unlike the sturdy gram negatives and gram positives, are very fastidious and do not grow on common bacteriological media; hence, difficult to isolate them and hence, there is delayed diagnosis. Hence, successful isolation of these microorganisms in the microbiology laboratory requires incubation in anaerobic atmosphere, the use of specialized culture media, and prolonged incubation of culture. Moreover, there is delay in initiation of appropriate antimicrobial therapy. Despite the establishment and distribution of treatment guidelines,[3,4] there is still lack of standardization in empiric management, specifically for certain indications where antibiotic therapy is most common that is for obligate anaerobes.[5-7] In recent years, higher resistance rates of these microorganisms to some antimicrobial agents have been observed. Hence it is essential to know when an anaerobic infection is vital in order to use appropriate microbiologic methods to identify the bacteria and to select the correct treatment.[8] In this regard, this study was aimed to describe and analyse the distribution of various obligate anaerobes causing varied anaerobic intra abdominal infections, and also to document any antimicrobial resistance among the obligate anaerobes.

METHODOLOGY

A hospital-based retrospective analytical cross-sectional study was conducted in the Microbiology laboratory, at Jawaharlal Institute of Postgraduate Medical Education and Research, which is a tertiary care centre and a teaching hospital, situated in Puducherry (Union Territory), Tamil Nadu, South India. The study process analysed 1124 intra – abdominal infection cases received over a period of 23 years and 8 months (January 1994 – September 2017). The demographic and microbiological data was retrieved from the microbiology departmental registers maintained as a database in the Microbiology Department of the institute. The demographic data included age, gender and occupation of the patient. The microbiological data included the specimen received, the method of isolation and the isolated obligate anaerobes from various specimens. Attempt was also made to retrieve medical records of the concerned patients to perform a case review. Only past 10 years records (2007 to 2017) were retrieved from the medical record section. The clinical information (like antibiotic usage and risk factors) of the patients were retrieved from the medical records section.

Methods for processing of specimens

Pus and serosanguinous fluid was the specimen of choice. The specimen was collected in the syringe (intraoperative collection), and was immediately transferred into RCM (Robertson Cooked Meat) broth. The broth with the sample was sent to the microbiology laboratory for processing. Once, the broth with the sample is reached, it is incubated for 48 hours at 37°C in the incubator.

Flow chart for processing

RCM (Robertson Cooked Meat) broth with sample (DAY 1)

After 48 hours of incubation

RCM Smear was prepared from the broth and subjected to Gram staining (DAY 3)

In the Gram stain of RCM, the type of organisms (Gram positive or Gram negative) seen were noted down

RCM

ANAEROBIC CULTURE was done on the following media:
PEA agar (Phenyl Ethyl agar)
Neomycin agar
Blood agar
Anaerobic culture was read on DAY 5, i.e. in 48 hours

On Day 5 the anaerobic culture plates were read and colonies with different morphologies were marked and subjected to aerotolerance (done on Blood agar)

DAY 5: ANAEROBIC CULTURE (Aerotolerance)

24 Hours — O2+ Day6

48 Hours — O2 - Day7

If there is growth in anaerobic incubation (O2-) on day 7 and no growth in the corresponding aerobic incubation (O2+), the isolate is confirmed as an obligate anaerobe, and gram staining, 3% KOH, bile esculin and sensitivity are done for the concerned isolate. The biochemical reactions and sensitivity plates are read on day 9 (after 48 hours of incubation).

Presumptive identification (Table 3) was performed with antibiotic discs and spot tests (Wadsworth method)[9]; for example, at the genus level (*Bacteroides fragilis* group) (Figure 1).

Antimicrobial susceptibility testing for obligate anaerobes was started only in 2016. Metronidazole was the only antimicrobial which was tested. It was tested only against *Bacteroides fragilis* group according to standard CLSI guidelines. As the antimicrobial susceptibility testing for obligate anaerobes was started only in 2016, and among the antibiotics like beta-lactamase resistant penicillin, cephalosporin, clindamycin, macrolide, metronidazole and tetracycline, metronidazole is the most active antibiotic against *Bacteroides fragilis* group and it was the most common antibiotic preferred for treating anaerobic infections by the clinicians, hence, in this context, only metronidazole was tested against *Bacteroides fragilis* group. Moreover, there were

Table 1. Age distribution

Range of Age	No. (out of 1124)	Percentage
5 - 10 years	86	7.65
11 - 20 years	144	13
21- 40 years	348	31
40 years and above	546	49

Table 2. No. of cases

Clinical conditions	No. of cases
Liver abscess	180
Appendicular abscess	224
Acute/chronic appendicitis	128
Intestinal perforations/ Perforation peritonitis (secondary peritonitis)	186
Peritonitis (other than perforation peritonitis)	90
Necrotizing pancreatitis	74
Pancreatic abscess	64
Splenic abscess	68
Gut gangrene	56
Perinephric abscess	54
TOTAL	1124

studies documenting metronidazole resistance among the isolates of *Bacteroides fragilis* group.[10-14] As regards the statistical analysis, continuous variables were analysed by mean and standard deviations, and percentages and proportions were used for categorical variables. Chi-square or Fisher's exact tests were used to find significant association between predisposing factors and the clinical condition and also whether the isolated obligate anaerobe was significantly associated with the clinical condition. A univariate logistic regression analysis was also performed to document whether isolated obligate anaerobe was significantly ($P < 0.05$) associated with the clinical condition.

RESULTS

Total number of cases/specimens (A single specimen was received from a single patient) received over the period of 23 years and 8 months were 1124. Demographically, the majority of the patients were above the age of 40 (49%) (Table 1). The spectra of uncomplicated abdominal infections which were received were, Liver abscess (180), Appendicular abscess (224), Acute/chronic appendicitis (128), Necrotizing pancreatitis (74), Pancreatic abscess (64), Splenic abscess (68), Gut gangrene (56), and Perinephric abscess (54). Similarly, complicated abdominal infections were Intestinal perforations/Perforation peritonitis (secondary peritonitis) (186) and Peritonitis (other than perforation peritonitis) (90). Appendicular abscess (224) and intestinal perforations/Perforation peritonitis (186) were the majority of the intra-abdominal infections (Table 2). Pus or serosanguinous fluid was the major specimen received in the anaerobic section. The specimens were processed anaerobically according to the SOP (Standard Operating Procedures). Out of 1124 cases only 326 (29%) cases were culture positive, whereas 798 cases were culture negative. These 326 cases yielded 428 anaerobic isolates. *Bacteroides fragilis* group (238) (56%) and *Peptostreptococcus* sp (109) (25%) amounted to the majority of the isolates. Rare anaerobes like *Clostridium sporogenes,*

Table 3. Presumptive identification with antibiotic disks of main anaerobes. (Adapted from Wadsworth Anaerobic Bacteriology manual)[18]

Microorganism or group	Vancomycin (5 µg)	Kanamycin (1000 µg)	Colistin (10 µg)	Penicillin (2 U)
Gram-positive	S	V	R	V
Gram-negative	R	R	S	
B. fragilis group	R	R	R	R
Other Bacteroides species	R	R	V	S
Porphyromonas	S	R	R	
Fusobacterium	R	S	S	
Clostridium perfringens	S	S	R	
Prevotella	R	V	V	
Parabacteroides	R	R	R	

S = sensitive; R = resistant; V = variable

Table 4. Spectrum of anaerobic isolates

Isolate	No,	(%)
Bacteroides fragilis group	238	56
Peptostreptococcus sp	109	25
Peptostreptococcus anaerobius	56	13
Clostridium sporogenes	4	0.9
Fusobacterium sp	3	0.7
Obligate non sporing gram positive *bacilli*	5	1.2
Clostridium perfringens	7	1.6
Propionibacterium sp	1	0.2
Clostridium bifermentans	3	0.7
Microaerophilic streptococci	1	0.2
Fusobacterium varium	1	0.2
TOTAL	428	

Table 5. Specimen wise distribution of isolates

Isolate	Clinical conditions	No. of obligate anaerobes isolated from the specimen	Percentage
Bacteroides fragilis group	1) Appendicular abscess	107	45
	2) Intestinal perforation	82	44
	3) Acute/chronic appendicitis	32	25
	4) Necrotizing pancreatitis	8	11
	5) Gut gangrene	7	13
	6) Splenic abscess	2	3
	Total	238	
Peptostreptococcus sp	1) Intestinal perforation	88	47
	2) Appendicular abscess	9	4
	3) Gut gangrene	10	18
	4) Splenic abscess	2	3
	Total	109	
Peptostreptococcus anaerobius	1) Intestinal perforation	48	26
	2) Appendicular abscess	8	3.6
	Total	56	
Clostridium sporogenes	Intestinal perforations/ Perforation peritonitis	4	2
Fusobacterium sp	Intestinal perforation (appendicular perforation)	3	2
Fusobacterium varium	Peritonitis	1	1
Obligate non sporing gram positive bacilli	Intestinal perforations/ Perforation peritonitis	5	2.7
Clostridium perfringens	1) Gut gangrene	4	7
	2) Intestinal perforation	2	8
	3) Necrotising pancreatitis	1	1.4
	Total	7	
Propionibacterium sp	Peritonitis	1	1.1
Clostridium bifermentans	Appendicular abscess	1	0.4
Microerophilic streptococci	Intestinal perforations	1	0.5

Propionibacterium sp, *Clostridium bifermentans* and *Fusobacterium varium* were also Isolated (Table 4 and Table 5). Out of 238, 107 (45%) isolates of *Bacteroides fragilis* group were isolated from appendicular abscess.

Mixed anaerobic infections

Majority of mixed anaerobic infections were contributed by *Bacteroides fragilis* group and *Peptostreptococcus* sp (99 out of 102 mixed anaerobic infections). The rest three cases (all the three cases were of intestinal perforation) yielded *Clostridium sporogenes, Bacteroides fragilis* group and *Peptostreptococcus* sp in the first, *Fusobacterium* sp and *Bacteroides fragilis* group in second and *Fusobacterium varium* and *Peptostreptococcus* sp in the third.

Case review

We could retrieve only the last 10 years case records. A total of 656 intra-abdomen infection cases were received in last 10 years (2007 to 2017). The demographic information was compiled from the department registers (not from the case records). Out of 1124 cases, 728 (65%) were males and 396 (35%) were females. Occupation wise, majority of them were labourers (the occupation data was obtained only for 656 cases, as the data had to be retrieved from the case records). Predisposing or underlying conditions were present in 69 (out of 110 culture positive cases) (63%) instances. Chronic alcoholism, leading to gastric/duodenal ulcers and intestinal perforation (43%) was the most common predisposing condition (p value < 0.05). As regards the significant association of obligate anaerobe with the clinical condition, using univariate logistic regression analysis, *Bacteroides fragilis* group (45%) was found to be significantly associated with appendicular abscess (p value < 0.05), and *Peptostreptococcus* sp (47%) was significantly associated with intestinal perforation (p value < 0.05).

Other Predisposing conditions were, recent abdominal surgery in 35% of instances, malignancy in 15% of instances (leukemia), immune deficiency in 7% of instances. The antimicrobials used for treating the infection were beta-lactamase resistant penicillin, cephalosporin, clindamycin, macrolide, metronidazole and tetracycline.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing for the anaerobes was started only in 2016. Among the antimicrobials which were used by the clinicians for treating the infection, only Metronidazole was tested for its susceptibility pattern. It was tested only against *Bacteroides fragilis* group by Kirby – Bauer disk diffusion method according to standard CLSI Guidelines. From January 2016 to September 2017, 17 (out of 238) *Bacteroides fragilis* isolates were obtained from intra – abdominal infections. Among them only one isolate was resistant to metronidazole (Diameter of inhibition zone was 8 mm, Figure 1 and Figure 2).

DISCUSSION

Over a period of 23 years, 1124 intra – abdominal infection cases were received. Majority of the patients were males (65%), and majority were labourers by occupation. Appendicular abscess (224) and intestinal perforations/ Perforation peritonitis (186) were the majority of the intra-abdominal infections, with pus or serosanguinous fluid being the major specimen received. *Bacteroides fragilis* group (238) (56%) and *Peptostreptococcus* sp (109) (25%) amounted to the majority of the isolates. Massimo Sartelli,[10] also documented *Bacteroides fragilis* group to be the major anaerobic isolate.[10] In a similar study done by Brook I and Frazier EH,[11] the most frequently isolated anaerobes were *Bacteroides* spp. (*B. fragilis* group), *Peptostreptococcus*, Clostridium and *Fusobacterium* spp.[11] *Bacteroides fragilis* emerged as the predominant isolate in

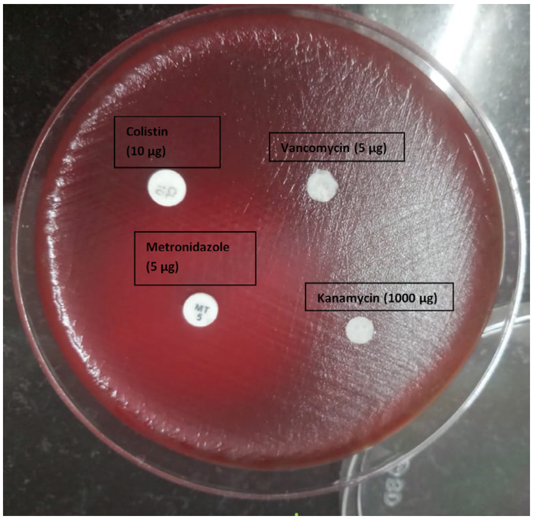

Figure 1. Identification of *Bacteroides fragilis* group using discs Vancomycin (5 µg)-Resistant, Kanamycin (1000 µg) - Resistant and Colistin (10 µg) – Resistant.

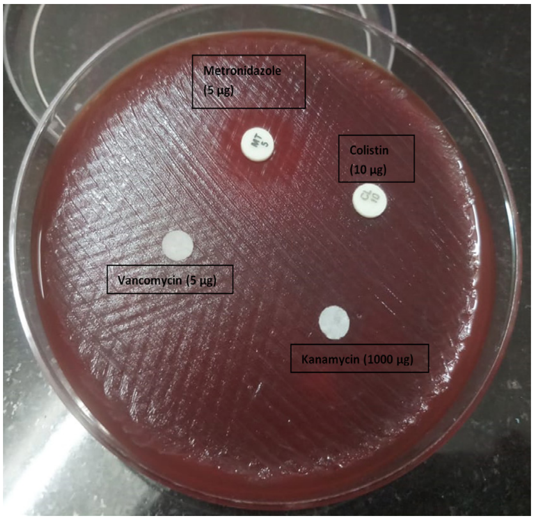

Figure 2. Metronidazole resistant isolate of *Bacteroides fragilis* group

a study by Shree N et al.[12] Simillarly, Chia T C et al. in their study also documented *Bacteroides fragilis* as the major anaerobic isolate.[13] Nicole Lopez et al.[14] also documented Bacteroides spp. (B. fragilis group), and Clostridium as major anaerobic isolates. In our study, we had also isolated rare anaerobes like *Fusobacterium varium, Clostridium bifermentans* and *Propionibacterium* sp. Rare anaerobes like *Fusobacterium* spp was also isolated by Brook I and Frazier EH.[11] The various studies have shown variety of anaerobes causing significant intra–abdominal infections.[15-18] Majority of *Bacteroides fragilis* group (107) were isolated from appendicular abscess, followed by intestinal perforation (82) in our study. The above studies and the present study shows the importance of anaerobes in various intra-abdominal infections. Antibiotic resistance among anaerobic microorganisms has also increased in recent years.[19-22]

In the present study, only metronidazole was tested for its susceptibility pattern and was tested only against *Bacteroides fragilis* group. One isolate of *Bacteroides fragilis* group was resistant to metronidazole in the present study. In a study by Karlowsky JA et al.,[23] regarding the *Bacteroides fragilis* group, resistance to penicillin was observed in 80–90% of isolates, while a higher proportion of the strains (20%) were also resistant to amoxicillin clavulanate.[23] The overall resistance rate to carbapenems was very low.[22,23] In another study, resistance to penicillin was observed to be around 80–90% of *Bacteroides fragilis* group. while a higher proportion of the strains (20%) were resistant to amoxicillin–clavulanate.[24] The overall resistance rate to carbapenems was very low (<1%).[24] But in another study, the authors have reported a high rate (64%) of carbapenem resistance among the *Bacteroides fragilis* group.[25] As in the present study, antibiotic susceptibility for other antibiotics was not performed, the resistant patterns for other antibiotics could not be commented on. In another study, regarding *Prevotella*, resistance rate to penicillin was increasingly documented, and their resistance rate to clindamycin ranged from 11 to 40%.[24,25] Their resistance rate to clindamycin ranged from 11 to 40%. Overall, no resistance to metronidazole was found in *Prevotella* isolates, although in some studies, this resistance has been detected.[26,27] On the other hand, resistance to antibiotics has been found in very few isolates of *Fusobacterium*, except for penicillin due to beta lactamase production.[27] Among Clostridium, *C. difficile* showed a high resistance rate to imipenem (90%) and clindamycin (40%) but no resistance to metronidazole.[28] In a similar study, 4% metronidazole resistance was documented among the anaerobic bacteria (*Bacteroides fragilis* group).[29] But they did not report any carbapenem (Imipenem and Meropenem) resistance among the anaerobic bacteria. In a recent study by Valdezate S et al., they documented *Bacteroides fragilis* strains which were resistant to meropenem.[30] Among *Clostridia, C. difficile* showed a high resistance rate to imipenem (90%).[31]

CONCLUSION

In conclusion, as there is growing antimicrobial resistance among theobligate anaerobes, routine antimicrobial susceptibility testing for anaerobes has become essential and provides information regarding the antimicrobial resistance patterns and permits empirical therapies to be selected in accordance with local data on resistant strains.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by Jipmer Ethics Committee, with reference number JIP/IEC/2018/4653.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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