Iranian Journal of Microbiology
Volume 13 Number 3 (June 2021) 274-281

ORIGINAL ARTICLE

Anaerobic infections in patients admitted in various surgical units of a tertiary care hospital of north India: neglected but important

Charu Singh[1], Anshul Sood[2], Kiran Bala[2], Cherring Tandup[3], Pallab Ray[2], Archana Angrup[2*]

[1]Department of Microbiology, IMS-BHU, Varanasi, India
[2]Department of Medical Microbiology, Research Block A, PGIMER, Chandigarh, India
[3]Department of General Surgery, PGIMER, Chandigarh, India

Received: December 2020**, Accepted:** April 2021

ABSTRACT

Background and Objectives: Anaerobic infections are usually caused by the host's endogenous flora due to a breach in the anatomical barriers and *Bacteroides* spp. are the most notorious organisms associated with anaerobic infections. The identification of anaerobes has been a challenge since times. MALDI-TOF-MS is a boon for aiding the rapid detection of anaerobic organisms and has helped us to enlist the distribution of various anaerobic pathogens.

Materials and Methods: This retrospective analysis (January 2018 to December 2019) was carried out in a tertiary care hospital in North India, in which the anaerobic microbiological profile of all patients admitted to surgical wards, ICU, and OPD of various departments (Orthopedics, Surgery, Gynecology, and Obstetrics) was reviewed. Samples received were immediately processed aerobically (5% sheep blood agar and Mac Conkeyagar) as well as anaerobically (RCM and freshly prepared sheep blood agar) as per the laboratory protocols.

Results: *Bacteroides fragilis* (19.12%) was the most common anaerobe whereas among aerobes *Escherichia coli* (30.2%) followed by *Klebsiella pneumoniae* (10.34%) were most commonly isolated. The majority of patients were males (56%) and the most common presentation was with abscesses (21.4%). Polymicrobial infections (69.51%) outnumbered monomicrobial ones (30.48%).

Conclusion: There is a paucity of literature on anaerobe isolation from surgical infections from our country which motivated us to study anaerobic infections and the high sample size in our institute enabled us to study surgical infections from an anaerobic perspective. This will add to the knowledge of microbiologists and clinicians. MALDI-TOF MS helped in rapid and accurate identification and hence we could report a wider spectrum of organisms in our study.

Keywords: Anaerobic bacteria; Metronidazole; Resistance; *Bacteroides fragilis*; Matrix assisted laser desorption/ionization-time of flight mass spectrometry; Oxidation-reduction potential

INTRODUCTION

Anaerobes constitute a significant proportion of the normal microbiota colonizing the skin and the mucosal surfaces (1). A breach in the normal mucosal barriers either due to trauma, surgery or a pathological lesion like tumor leads to invasion of the sterile cavities or tissues by this endogenous flora resulting in a variety of infections (2, 3). These conditions also disrupt the oxidoreductive potential within the tissues and facilitate anaerobic growth. A substantial number of anaerobes are responsible for surgical infections (4). The most commonly encountered anaer-

*Corresponding author: Archana Angrup, MD, Department of Medical Microbiology, Research Block A, PGIMER, Chandigarh, India.
Tel: +91-1722755273
Fax: +91-1722755155
Email: archanaangrup@yahoo.com

obes in clinical samples include *Bacteroides fragilis* group, pigmented *Prevotella* and *Porphyromonas* species, *Fusobacterium* species, *Peptostreptococcus* species, *Clostridium* species, and *Actinomyces* species (3).

Knowledge of the microbial etiology is essential in choosing an appropriate antimicrobial agent for treating any infection. Since the majority of the surgical infections are polymicrobial, failure to take into consideration the anaerobic flora as one of the causes of mixed surgical site infection is associated with a high rate of therapeutic failure (2).

The role of a microbiologist is crucial in providing information to the clinician regarding the etiological agent in the clinical sample. Anaerobic sample processing is a time-consuming process that has been a major deterrent in organism identification. The advent of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been a boon to microbiologists in reducing the turnaround time and has assisted in the quick identification of organisms at the species level without the need to wait for results of conventional biochemical identification.

The studies related to anaerobic infections and the contributing organisms are rare from this part of the country. Therefore, an approach was made to study the aerobic and anaerobic organisms with the major emphasis on anaerobes, associated with surgical infections in patients admitted in various surgical units at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh.

MATERIALS AND METHODS

Study population. This was a retrospective analysis performed in the Department of Medical Microbiology, PGIMER, Chandigarh which is a tertiary care hospital in North India catering to a large number of patients. The isolates were recovered from various specimens such as pus, abscess fluid, tissue, blood, and other body fluids received at the Bacteriology Laboratory over two years (January 2018 to December 2019). Patient samples meeting the inclusion and exclusion criteria were included and the clinical data were retrieved from the Central Registration Department of the institute.

Inclusion criteria consisted of all clinical specimens collected from patients admitted in surgery, ICU, and OPD of various departments in our hospital (Orthopedics, Surgery, Gynecology, and Obstetrics). Both clean and unclean surgeries were included. Patients of all age groups were included in the study.

Exclusion criteria consisted of samples that were likely to be contaminated with anaerobic commensals e.g stool samples. Samples in which the anaerobic environment was likely to be compromised e.g opened and leaky containers were not accepted. Suboptimal specimens like swabs were generally rejected and only accepted in cases where no other sample was obtained.

Sample collection and transfer. Robertson's cooked meat (RCM) broth was provided by the laboratory on placing a requisition for the same to various clinical wards, Intensive Care Units, and Operation Theatres. The collected sample was directly placed in the RCM broth which provided an anaerobic environment from the initial stage after sample collection leading to an increase in the yield of anaerobes. The samples were then transferred to the microbiology laboratory without any delay.

Processing and identification. All the samples received in the laboratory (as per laboratory protocol) were processed aerobically as well as anaerobically. Preliminary bacterial identification was done using conventional methods like Gram staining followed by the colony morphology and presumptive susceptibility to metronidazole (5 µg) disc test (5). For aerobic inoculation, 5% sheep blood agar and MacConkey agar were used whereas for anaerobic culture, RCM broth along with freshly prepared sheep blood agar was used. After application of a heavy inoculum on the primary plate, metronidazole disc (5 µg) was placed at the center for the presumptive testing of anaerobes. The plates were incubated at 37°C for 48 hours in an anaerobic jar using an automated anaerobic gas generation system, Anoxomat Mart II (Mart Microbiology BV, Lichtenvoorde, Netherlands). In each run, one obligate aerobic and anaerobic strain i.e. *Pseudomonas aeruginosa* ATCC 27853 and *Bacteroides fragilis* ATCC 25285 were used for quality control as biological indicators for the establishment of anaerobic conditions. After incubation, the anaerobic plates were examined for the presence of any zone of inhibition around the metronidazole disc. The presence of a zone was suggestive of anaerobic growth and was further subcultured to get isolated colonies. In case

of no zone of inhibition, the anaerobic plates were correlated with the aerobic plates for the presence of metronidazole-resistant anaerobes. RCM broth was examined for turbidity for up to three days. Turbid cultures were further inoculated on blood agar followed by aerobic and anaerobic incubation and identification. Identification of aerobic and anaerobic isolates to species level was done using the MALDI-TOF Biotyper 2.0 database (Bruker Daltonics, Germany). The Biotyper software compares the mass spectra of each sample to the reference mass spectra in the database and generates an identification score. MALDI scores were interpreted as follows: score ≥2.0, accurate identification to species level; scores of ≥1.7 but <2.0, accurate identification to genus level; and scores less than 1.7 were considered unreliable identification. The isolates were stored for future reference in 60% *Brucella* broth with 40% glycerol in a frozen state at -80°C.

RESULTS

A total of 22,177 samples were processed anaerobically during the study period out of which 1,106 (5%) samples had grown either pure anaerobic (polymicrobial and monomicrobial) or mixed aerobic and anaerobic growth. From these 1,106 samples, 1,524 anaerobic bacteria were isolated (1.38 average isolates per sample). Out of these 1,106 samples, only 187 (16.9%) were received from various surgical units of our hospital and the sample distribution was as follows: pus (158), tissue (14), swabs (12), abdominal mesh (1), bone (1), placenta (1). There were 105 (56%) male and 82 (44%) female patients included in the study. The patients with surgical interventions presented primarily with abscess, surgical site infections, infected wound, cyst, gangrene, fracture, soft tissue discharge, pancreatitis, amputation, appendicitis, knee joint infection, diabetic foot infection, perforated appendix, pyometra as mentioned in Table 1.

A total of 251 anaerobic isolates were isolated from 187 samples, amounting to an average of 1.3 (251/187) isolates per patient. 228/251 (90.8%) anaerobic organisms were identified to species level by MALDI-TOF-MS and 23/251 (9.16%) organisms could not be identified and were labeled as non-reliable identification (NRI) (Table 2). 56/187 (29.94%) samples yielded monomicrobial anaerobic growth whereas 131 (70.05%) yielded polymicrobial (aerobic/anaerobic, both or more) growth (Fig. 1).

Gram-positive cocci were the most commonly isolated anaerobes (83/187, 33.1%) followed by Gram-negative bacilli (GNB) contributing to 27.1% (68/187). 39/187 (15.6%) organisms were identified as Gram-positive bacilli (GPB) and 38/187 (15.1%) as Gram-negative cocci (GNC) whereas there was only one organism (0.4%) identified as Gram-negative coccobacilli (GNCB). *Bacteroides* (19.12%) were the most predominantly recovered genera followed by *Peptoniphilus* (15.94%), *Veillonella* (13.55%), and *Clostridium* (9.16%).

Of the mixed aerobic and anaerobic infections, the highest number of anaerobic infections were seen in association with *E. coli* in 35 (30.17%) followed by *Klebsiella* spp.in 13 (11.21%), *Staphylococcus aureus* in 12 (10.34%), and *Proteus* spp.in 11 (9.43%) (Fig. 2).

Abscess (21.39%) was the most common sample from which anaerobic bacteria were isolated followed by samples from tumor sites (12.29%), surgical site infections (7.48%), infected wound (6.41%), cysts (4.81%), gangrene (3.20%), fracture (3.74%), and, discharge from tissue (4.27%) (Table 1).

DISCUSSION

Anaerobic infections except for gas gangrene which is acquired exogenously, are usually derived from the host's endogenous flora (4). Anaerobes inhabit mucosal surfaces and prevent the colonization of exogenous microbes (also called colonization resistance) (4). Any breach in this mucosal barrier opens the surface for outside invasion and an imbalance of resident flora due to the derangement of oxidation-reduction potential leading to infection. Immunosuppressive states like cancer, recent surgery, hematological disorders, etc. have been recognized as important risk factors in causing anaerobic bacterial infections (6).

A higher percentage of anaerobes have been isolated in gas gangrene, diabetic foot infections, ruptured appendix, colorectal surgery, appendicectomy, abscesses, cellulitis, oral, dental infections, etc (7). The present study noted maximum anaerobic isolation from abscesses (21%) followed by wounds associated with malignant conditions like carcinoma breast, rectum, etc. (Table 1). Antony et al. in their study reported wound infections (23.41%) as the major cause of anaerobic isolation which was only 6%

Table 1. Samples received for anaerobic isolation (n=187)

Attributes	Number	Percentage
Specimen		
Pus	157	84
Tissue	14	7.4
Swab	12	6.4
Others (Abdominal mesh, Central line tip, Bone, Placenta)	4	2.12
Type of infection		
Abscess	40	21.39
Surgical site wound	14	7.48
Infected wound	35	18.7
Cyst	9	4.81
Gangrene	6	3.20
Fracture	7	3.74
Discharge from tissue	8	4.27
Pancreatitis	7	3.74
Amputation	7	3.74
Fistula	5	2.67
Appendicitis	4	2.13
Knee infection	4	2.13
Diabetic foot	4	2.13
Perforated appendix	4	2.13
Pyometra	4	2.13
Others (Periprosthetic infection, Splenic rupture, Osteomyelitis, Ulcer, Bedsore, Gall bladder perforation, Ileal perforation, Lipoma, Renal trauma, Renal trauma, Submandibular space infection, Fibroid, Empyema, Cellulitis,Vascular injury, Mucormycosis, Gunshot Injury, Necrotizing fasciitis, Necrotizing pancreatitis, Sepsis, Secondary infection in breast cancer)	29	15.37

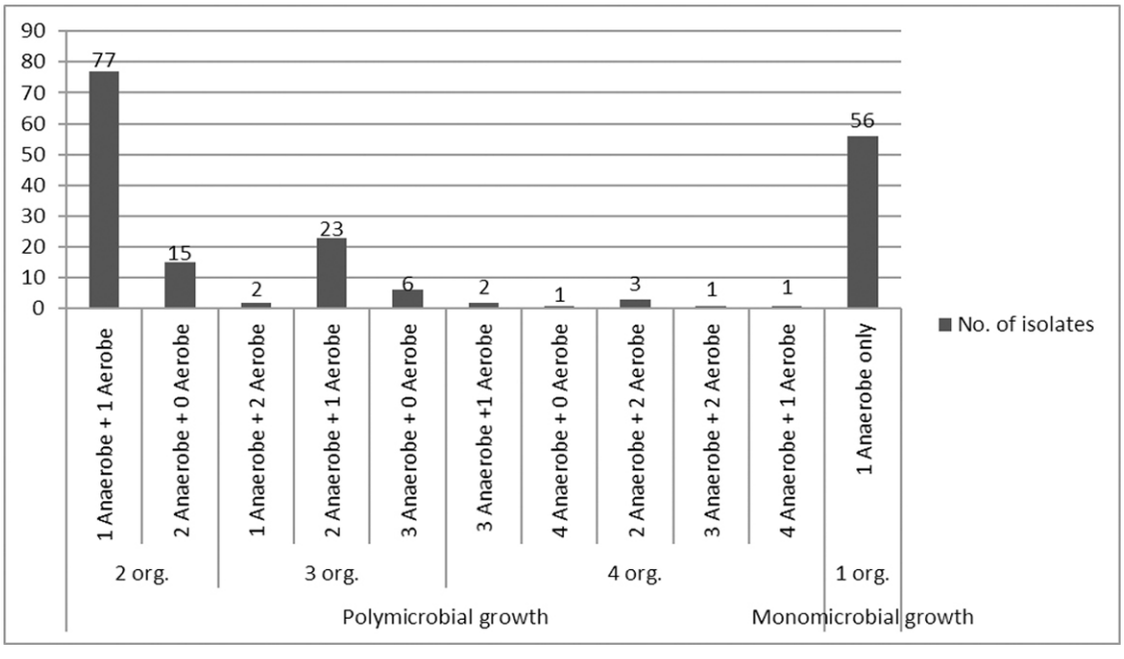

Fig. 1. Type of microbial growth in surgical specimens of study subjects (n=187)
(Abbreviations; org- organism/organisms, no.- number)

in our study (8). Padmaja et al. reported maximum anaerobe isolation from abscesses (23.9%) followed by diabetic foot infection (20%) whereas in our study diabetic foot infections contributed to just 2% of all infections (2). Shenoy et al. in their study isolated anaerobes majorly from necrotizing fasciitis (34%) (as compared to 0.53% in our study) followed by abscesses (23%) (9). The varied etiology of these infections might be due to the geographic variation where the studies were carried out. We have reported more types of anaerobic genera in causing the various infections as compared to the other studies. This could be explained by the number of samples received as our hospital caters to a large population of patients from North India and also to the use of MALDI-TOF MS which has made it possible to speciate the isolates (2, 8-10).

The etiology of the abscesses (n=40) revealed that 18 were monomicrobial and 22 were polymicrobial. In 9 isolates, monomicrobial anaerobes were seen and in 3 samples both aerobe and anaerobes were isolated. In two samples of appendicular abscess *E. coli* and *K. pneumoniae*, and in another specimen of gall bladder emphysema *E. coli* were isolated.

The most common anaerobe isolated was *Peptoniphilus* spp. (15.5%) followed by *Veillonella* spp. (13.5%) of which *V. parvula* contributed to 30% of cases. *P. harei* was the majorly isolated anaerobe amongst genus *Peptoniphilus* (35/39) which was isolated from varied etiologies like pyometra, infected wounds, surgical site infections, sebaceous cyst etc. *Veillonella* species, though considered to be of low virulence, are part of the normal anaerobic flora of the oral cavity, upper respiratory tract, small intestine, and vagina (11). Out of the 12 cases in which *Veillonella* was isolated, 3 cases presented as neck abscesses and one as hip (greater trochanter) abscesses. It emphasizes the role of a commensal microorganism in causing infection under certain conditions.

Of all the anaerobes isolated, 23 could not be identified by MALDI-TOF MS and were reported as non-reliable identification. This may be due to the absence of these isolates in the MALDI -TOF database and require further identification by molecular methods. The isolation rate of 1.34 anaerobes (251/187) per patient in the present study was similar to the findings of a seven-year retrospective study done by Park et al. (12). 69.51% (130/187) of our isolates were polymicrobial which is comparable to the findings of Shenoy et al. (78%) and Antony et al. (57%) (8).

Table 2. Distribution of various anaerobes (n=251) in surgical specimens of study subjects

Gram's stain	Genus	No.	Percentage	Species
GNB	*Bacteroides* spp.	48	19.1%	*B. fragilis* (29); *B. ovatus* (4); *B. thetaiotaomicron* (10); *B. uniformis* (3); *B. vulgatus* (2)
	Prevotella spp.	13	5.2%	*P. bergensis* (2); *P. bivia* (4); *P. buccae* (5); *P. buccalis* (1); *P. corporis* (1)
	Others	7	2.8%	*Dialister pneumosintes* (2); *Fusobacterium canifelinum* (1); *F. varium* (1); *Parabacteroides distasonis* (2); *Campylobacter rectus* (1)
GNC	*Veillonella* spp.	34	13.5%	*V. atypica* (2); *V. parvula* (31); *V. ratti* (1)
	GNCB	1	0.4%	*Gardnerella vaginalis* (1)
	Others	3	1.2%	*Acidaminococcus intestine* (1); *Megasphaera micronuciformis* (2)
GPB	*Clostridium* spp.	23	9.2%	*C. cadaveris* (1); *C. cochlearium* (1); *C. fallax* (1); *C. novyi* (1); *C. parapetrificium* (1); *C. perfringenes* (4); *C. ramosum* (1); *C. sordellii* (3); *C. sporogenes* (9); *C. subterminale* (1)
GPC	Others	16	6.4%	*Cutibacterium acnes* (4); *Cutibacterium avidum* (4); *Acidaminococcus intestine* (1); *Actinomyces odontolyticus* (2); *A. sanguinis* (1); *Bifidobacterium dentium* (1); *Bifidobacterium longum* (2); *Eggerthia catenaformis* (1); *Eubacterium limosum* (1)
	Peptoniphilus spp.	39	15.5%	*P. coxii* (2); *P. harei* (35); *P. lacrimalis* (2)
	Streptococcus spp.	21	8.4%	*S. anginosus* (4); *S. constellatus* (6); *S. haemolyticus* (1); *S. intermedius* (5); *S. lavendulae* (1); *S. oralis* (1); *S. pyogenes* (2); *S. salivarium* (1)
	Finegoldia spp.	14	5.6%	*F. magna* (14);
	Others	9	3.6%	*Peptostreptococcus anaerobius* (6); *Parvimonasmicra* (1); *Actinotignum sanguinis* (1); *L. raffinolactis* (1)
NRI	NRI	23	9.2%	NRI (23)

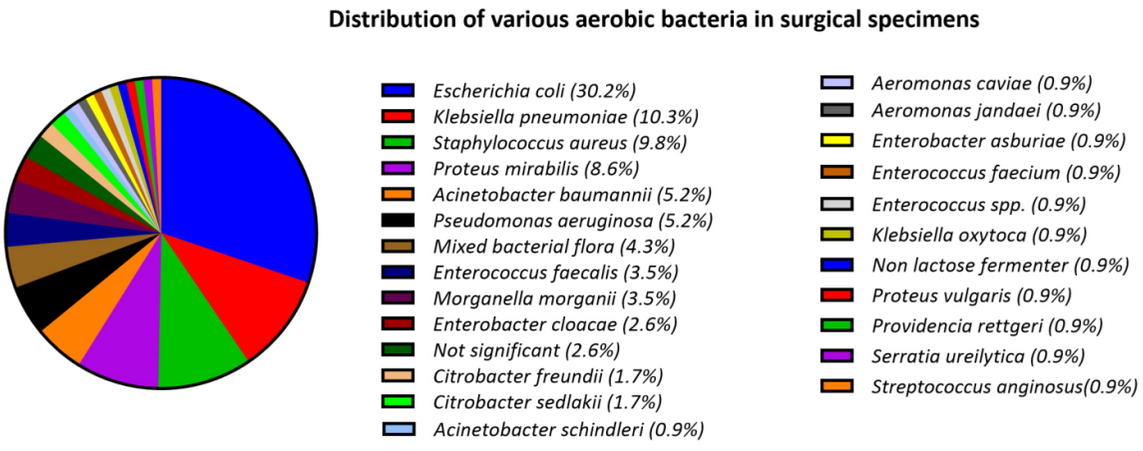

Fig. 2. Distribution of various aerobic bacteria (n=116) in surgical specimens of study subjects (n=187)

Studies analyzing different surgical infections have reported varying anaerobic isolation rates reaching more than 87% in diabetic foot infections showing polymicrobial growth (4). Park et al. demonstrated 80% of infections being polymicrobial (12).

The surgical infections are largely polymicrobial, both aerobic and anaerobic organisms have been seen to be responsible for the pathogenesis of these infections (13). The isolation of a higher number of isolates has been made possible because of timely sample processing, good culture, and isolation practices which were achievable by good planning and hard work leading to improvement of methodology. The advent of MALDI-TOF has also served as a boon in the early and accurate identification of anaerobic bacteria.

Studies are lacking regarding the prevalence of anaerobes isolated in various infections in various geographical areas of the country. We observed that anaerobic Gram-positive cocci (33%) were the predominant group of anaerobes causing infections in the present study. Though in other studies like that by Shenoy et al. anaerobic Gram-negative bacilli were isolated from the majority of patients i.e *Bacteroides* spp. (20.9%) (9). Antony et al. also isolated *Bacteroides* spp. as the most common anaerobe (8). In our study *Bacteroides* spp. (*B. fragilis* in 11.55%) was isolated in 19.12% of cultures. Brook in his study found out that in 10/30 cases of cultures growing *Bacteroides* spp. 32% presented with abscess (11). Padmaja et al. isolated *Clostridium* species (37.8%) followed by *Bacteroides* (17.6%) in their study unlike our study with 9/40 (22.5%) cases of abscesses caused by *Bacteroides* species and 9.16% by *Clostridium* spp. (2). The next two most common isolates apart from *Bacteroides* spp. in our study were *Peptoniphilus* spp. (15.5%) and *Veillonella* spp. (13.55%). In a study done by Wren et al. *Bacteroides* spp. (40.4%), *Fusobacterium* spp. (10.1%), *Clostridium* spp. (2.2%), Gram-positive non-sporulating bacilli (13.4%) and *Veillonella* spp. (5.6%), were reported from pus samples aspirated from closed abscesses (14). 13/34 (38.23%) patients growing *Veillonella* had an abscess in our study similar to other studies reported in the literature. Brook et al. in their study from abscesses found *Bacteroides* spp. (32%) as the most common bacteria, followed by *E. coli* and *Peptostreptococcus* spp. (11). In our study, the most common aerobic organism isolated from polymicrobial cultures was *E. coli* (30.2%) followed by *S. aureus* (7.56%). A similar finding was seen by Saini et al. where *E. coli* was seen as the most common aerobe involved in polymicrobial infections (15). Park et al. stated that the most common pathogens by rank were *Bacteroides fragilis*, accounting for 41.8% of anaerobic infections followed by, *Clostridium* spp. in 11.8%, *Prevotella* spp. in 9.4%, and *Peptostreptococcus* spp. in 8.4% (12). *E. coli* (17.5%), *S. aureus* (7.5%), and *K. pneumoniae* (7.5%) were common concomitant aerobic organisms observed by Park et al. (12). Sunmonen et al. studied 86 abscesses in intravenous drug users (IVDU); which yielded 173 aerobes and 131 anaerobes, among which *S. aureus* (50%) was the most common aerobe and *Prevotella* spp. most common anaerobe isolated (16). In non IVDU, *S. aureus* (53%), followed by Coagulase- Negative *Staphylococcus* (CONS) (19%) have been reported (14). Coinfection of *E. coli* with *Bacteroides* spp. was seen in 42.8% (15/35) patients

followed by *Veillonella* spp. in 20% (7/35) patients. The infections associated with these organisms were, abscess (11/35), appendicitis (3/35), and cancer (4/35). It has been mentioned in a study that *B. fragilis* is representative of anaerobic Gram-negative rods and commonly isolated from patients suffering from abdominal and soft tissue infections (7). The isolates not identified by MALDI-TOF –MS were 23 in number. Our culture and isolation was accurate and inability to identify the isolates could be attributed to the reason that those isolates pattern might not be there in MALDI-TOF-MS database thus indicating the emergence of new organisms and hence the need for regular updating of the MALDI-TOF-MS database.

An emerging issue with anaerobes is the increase in antimicrobial resistance especially in the *B. fragilis* group to metronidazole reported from various parts of the world (17-19). Metronidazole resistance has been linked to *nim* genes in *Bacteroides, Parabacteroides* spp. and *Prevotella* spp. and this may lead to a failure of using it routinely as an empirical therapy (20).

The long turnaround time deters a clinician from requesting anaerobic culture. Moreover, not all laboratories are fully equipped to provide the complete anaerobic workup up to genus and species level and the tedious procedure involved also adds to the problem. The clinicians hold a general belief that the addition of metronidazole will provide adequate anaerobic coverage. This can be misleading since there are many reports of an increase in metronidazole resistance from different parts of the world (17-19).

Apart from metronidazole resistance, mutations in *gyrA* and *parC* have been associated with quinolone resistance (21). Anaerobic microbiota is believed to act as a reservoir of antimicrobial resistance genes and is capable of transferring them to other anaerobic and aerobic organisms with pathogenic potential (22). In *Bacteroides* spp. conjugative transposons have also been stated to be responsible for the transfer of resistance-associated genes (22-24). Due to the practice of empirical antibiotic administration, anaerobic culture is often not requested affecting the patient's outcome. With the increasing reports of resistance in anaerobes, it becomes imperative to know the etiological agent along with the susceptibility profile. With the advent of MALDI-TOF-MS, which is based on microbial identification of characteristic protein fingerprints of bacteria, it takes a few minutes to rapidly identify species of various microorganisms, therefore shortening the time to detection to a great extent and has thus aided in the improvement of diagnostic efficiency of infectious diseases.

CONCLUSION

Varied etiology and lack of knowledge of the prevalence of various anaerobes make it challenging to treat anaerobic infections. Abdominal perforation, ruptured appendix, ruptured bowel, etc are usually associated with polymicrobial infections with aerobic as well as anaerobic organisms in which the gut flora gets access to the earlier non-accessible site. In settings where the provision of anaerobic culture is not available, a sterile culture report can lead to the missing and underreporting of anaerobic bacteria.

Knowledge regarding the types of anaerobes involved in various infections can help the clinician to think in terms of empirical treatment before the laboratory results are available. The use of MALDI-TOF-MS has helped us a lot to detect many anaerobic pathogens which we could have not identified. Besides, MALDI-TOF-MS has helped us to shorten the turnaround time by at least 24-36 hours which is beneficial to the patient. Thus, we attempted to study the bacteriological profile of anaerobes isolated in our center as very few studies have been done from this part of the country and this will be of immense help in terms of patient care.

We could not carry out genotypic analysis of the isolates and determining the source of anaerobe responsible for causing the infection to be extraneous or endogenous in many cases. Moreover, antibiotic susceptibility testing which is not done routinely in our setup was also one of the limitations that could help us to learn the disease outcome.

REFERENCES

1. Murphy EC FI. Gram-positive anaerobic cocci - commensals and opportunistic pathogens. *FEMS Microbiol Rev* 2013; 37:520-553.
2. Shenoy PA, Vishwanath S, Gawda A, Shetty S, Anegundi R, Varma M, et al. Anaerobic bacteria in clinical specimens – frequent, but a neglected lot: A five year experience at a tertiary care hospital. *J Clin Diagn Res* 2017; 11:DC44-DC48.

3. Cohen-Poradosu R KD (2015). Anaerobic Infections: General concepts. In: Bennet JE, Dolin R, Blaser MJ Eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Elsevier health(-Saunders). Philadelphia: US, pp. 2736-2743.
4. Edmiston CE, Krepel CJ, Seabrook GR, Jochimsen WG. Anaerobic infections in the surgical patient: microbial etiology and therapy. *Clin Infect Dis* 2002; 35(Suppl 1):S112-118.
5. Senne JE, McCarthy LR. Evaluation of a metronidazole disk test for the presumptive identification of anaerobes. *Am J Med Technol* 1982; 48:613-616.
6. Mandell GL, Bennett JE, Dolin R E (2005). Mandell, Douglas, and Bennett's Principles and practice of infectious diseases. 6th ed. Elsevier health (Saunders). Philadelphia: US. pp. 3083-3089.
7. Japanese Society of Chemotherapy Committee on guidelines for treatment of anaerobic infections; Japanese Association for Anaerobic Infections Research. Chapter 1-1. Anaerobic infections (General): epidemiology of anaerobic infections. *J Infect Chemother* 2011; 17 Suppl 1:4-12.
8. Antony B, Justin S, Raveendran R, Shetty AK, Kuruvilla TS, Boloor R. Spectrum of anaerobes encountered in surgical infections in a tertiary care hospital in Mangalore, coastal Karnataka: A prospective study. *Muller J Med Sci Res* 2016; 7:40-43.
9. Ananth-shenoy P, Vishwanath S, Targain R, Shetty S, Rodrigues GS, Chiranjay Mukhopadhyay C, et al. Anaerobic infections in surgical wards: a two year study. *Iran J Microbiol* 2016; 8:181-186.
10. Kaur S, Rajagopalan S, Kaur N, Shafiq N, Bhalla A, Pandhi P, et al. Drug utilization study in medical emergency unit of a tertiary care hospital in north India. *Emerg Med Int* 2014; 2014:973578.
11. Brook I. Bacteroides infections in children. *J Med Microbiol* 1995; 43:92-98.
12. Park Y, Choi JY, Yong D, Lee K, Kim JM. Clinical features and prognostic factors of anaerobic infections: a 7-year retrospective study. *Korean J Intern Med* 2009; 24:13-18.
13. Boyanova L, Kolarov R, Mitov I. Recent evolution of antibiotic resistance in the anaerobes as compared to previous decades. *Anaerobe* 2015; 31:4-10.
14. Wren MWD. The culture of clinical specimens for anaerobic bacteria: A comparison of three regimens. *J Med Microbiol* 1977; 10:195-201.
15. Saini S, Gupta N, Aparna, Lokveer, Griwan MS. Surgical infections: a microbiological study. *Braz J Infect Dis* 2004; 8:118-125.
16. Summanen PH, Talan DA, Strong C, McTeague M, Bennion R, Thompson JE, et al. Bacteriology of skin and soft-tissue infections: comparison of infections in intravenous drug users and individuals with no history of intravenous drug use. *Clin Infect Dis* 1995; 20 Suppl 2:S279-282.
17. Sethi S, Shukla R, Bala K, Gautam V, Angrup A, Ray P. Emerging metronidazole resistance in *Bacteroides* spp. and its association with the nim gene: a study from North India. *J Glob Antimicrob Resist* 2019; 16:210-214.
18. Brazier JS, Stubbs SL DB. Metronidazole resistance among clinical isolates belonging to the *Bacteroides fragilis* group: Time to be concerned? *J Antimicrob Chemother* 1999; 44:580-581.
19. Choudhry R, Mathur P, Dhawan B KL. Emergence of metronidazole resistant *Bacteroides fragilis*, India. *Emerg Infect Dis* 2001; 7:485-486.
20. Kirchner M, Mafura M, Hunt T, Card R, Anjum MF. Antibiotic resistance gene profiling of faecal and oral anaerobes collected during an antibiotic challenge trial. *Anaerobe* 2013; 23:20-22.
21. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 2007; 20:593-561.
22. Salyers AA, Gupta AWY. Human intestinal bacteria as reservoirs for antibiotic resistance genes. *Trends Microbiol* 2004; 12:412-416.
23. Shoemaker NB, Wang GRSA. Evidence for natural transfer of a tetracycline resistance gene between bacteria from the human colon and bacteria from the bovine rumen. *Appl Environ Microbiol* 1992; 58:1313-1320.
24. Shoemaker NB, Vlamakis H, Hayes K SA. Evidence for extensive resistance gene transfer among *Bacteroides* spp. and among *Bacteroides* and other genera in the human colon. *Appl Environ Microbiol* 2001; 67:561-568.