Iranian Journal of Microbiology
Volume 8 Number 3 (June 2016) 181-186

ORIGINAL ARTICLE

Anaerobic infections in surgical wards: a two year study

Padmaja Ananth-Shenoy[1], Shashidhar Vishwanath[1*], Ryumzook Targain[1], Seema Shetty[1], Gabriel Sunil-Rodrigues[2], Chiranjay Mukhopadhyay[1], Kiran Chawla[1]

[1]*Department of Microbiology, Kasturba Medical College, Manipal University, Manipal, Karnataka, India*
[2]*Department of Surgery, Kasturba Medical College, Manipal University, Manipal, Karnataka, India*

Received: December 2015**, Accepted:** May 2016

ABSTRACT

Background and Objectives: Anaerobic bacteria are recognized as important pathogens in surgical infections. However, they are the most overlooked microorganisms by the clinic and the laboratory because of the tedious culture techniques with longer turn-around times. The study was aimed to analyze the frequency of anaerobic bacterial surgical infections and their predisposing factors.
Materials and Methods: A retrospective study was conducted over a period of two years including patients with surgical infections. The specimens were processed by Gram staining, aerobic and anaerobic culture. The anaerobic bacteria were isolated using standard procedures. The predisposing factors and clinical presentation were studied in these patients.
Results: A total of 261 specimens were received from patients with diverse infections from surgical wards. Ninety-one anaerobes were isolated from 64 (24.5%) surgical patients with a predominance of Gram-negative bacilli (37.4%). Anaerobic bacteria as monomicrobial isolates were seen in 21.9% isolates. Anaerobic bacterial isolation along with aerobic bacteria was seen in 71.9% of patients and polymicrobial anaerobic growth was detected in 6.3% of patients. Diabetes mellitus (28, 43.8%) was found to be the most frequent predisposing factor. *Bacteroides fragilis* group (20.9%) were the most frequent anaerobic Gram-negative bacilli followed by *Prevotella* spp. (12.1%). *Peptostreptococcus anaerobius* was the predominant anaerobic cocci isolated (14.3%). Necrotizing fascitis (34.4%) was the most common clinical presentation with anaerobic etiology followed by deep seated abscesses (23.4%).
Conclusion: Anaerobic bacteria were isolated from a significant proportion of surgical infections. To avoid therapeutic failures, anaerobic bacteria in surgical infections need to be recognized by surgeons and laboratorians.

Keywords: Anaerobic bacteria, Gram negative bacilli, Polymicrobial, Surgical infections

INTRODUCTION

Anaerobic bacteria constitute a significant part of our indigenous flora (1). Any event compromising the oxidation-reduction potential within the tissues facilitates the anaerobic growth. Though large numbers of anaerobic organisms are spread throughout the gastrointestinal tract, a relatively limited number of organisms are responsible for clinical disease in patients with surgical infections (2).

Anaerobic bacterial infections are common and may be serious and life threatening, but still are usually overlooked (3). They are common cause of various polymicrobial infections involving different sites which mainly include post-operative wound

*Corresponding author: Dr. Shashidhar Vishwanath, Department of Microbiology, Kasturba Medical College, Manipal University, Manipal – 576104. Karnataka. INDIA.
Tel: +91 820 2922717
Fax: +91 820 2571927
E-mail: drshashidharv@gmail.com

infections, intra-abdominal, oro-dental, pulmonary, gynecological/obstetric and various skin and soft tissue infections (4, 5).

Anaerobic bacteria have been isolated from a variety of infections in surgical patients ranging from abscesses, diabetic foot infections and peritonitis to life threatening infections such as necrotizing fasciitis and gas gangrene (6-8). The important anaerobic bacteria encountered in surgery include *Bacteroides fragilis* group, *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Peptostreptococcus* spp., *Clostridium* spp. and *Actinomyces* spp. (2). Different isolation rates of anaerobic bacteria from surgical infections have been reported earlier (2, 9, 10).

The delay in isolation of anaerobic bacteria from clinical specimens because of their polymicrobial nature and technical difficulty in susceptibility testing very often leads to early initiation of empirical antibiotic treatment for patients. Many clinical microbiology laboratories do not perform culture and susceptibility testing routinely for anaerobic bacteria.

A study was conducted to study the occurrence frequency of anaerobic infections among surgical patients.

MATERIALS AND METHODS

A retrospective study was conducted in the Microbiology laboratory attached to a tertiary care teaching hospital over a period of two years from November 2013 to October 2015 following clearance from Institutional Ethics Committee. The Demographic information, clinical presentations and co-morbidities were extracted from medical records. For microbiological analysis, specimens including tissue, pus aspirate, drain fluid and wound swabs were aseptically inoculated into a wide mouth sterile container and RCM (Robertson's cooked meat) medium during surgical procedure in the operating theatre or in the ward. When wound swabs were the only collected samples, they were inoculated at bedside into the RCM broth medium. The collected specimens were transported immediately to the microbiology laboratory (within 30 minutes). The specimens were processed for Gram staining and anaerobic cultures were done following standard techniques on 5% sheep blood agar, neomycin blood agar, and phenyl ethyl alcohol agar with metronidazole discs (5U) for preliminary identification. The specimens were inoculated into RCM if bedside inoculation was not performed. The plates were incubated in anaerobic workstation (Don Whitley Scientific, Shipley, UK) and inspected daily for anaerobic growth. Inoculated RCM broth media were incubated for seven days and subcultures were done on 5% sheep blood agar if any additional morphotypes were noted on Gram staining. The specimens were also cultured aerobically on 5% sheep blood agar and MacConkey agar and isolates were identified using standard methods (11).

Anaerobic isolates were identified by Gram staining, aerotolerance test (on chocolate agar incubated at 37 °C in CO_2 incubator), fluorescence under long wave (365 nm) ultraviolet light (UV), antibiotic disc tests (vancomycin 5µg, kanamycin 1000µg and colistin 10µg) and biochemical reactions such as catalase, nitrate reduction, growth in the presence of 20% bile, esculin hydrolysis, lipase, lecithinase, susceptibility to sodium polyanethol sulfonate (SPS), urease and sugar fermentation tests in Viande-Levure broth. Vitek 2 automated system (bioMerieux Inc.) or MALDI-TOF (bioMerieux Inc.) were used for species identification. The inoculated culture plates and RCM broth were discarded if there was no growth observed after seven days of incubation (12).

RESULTS

Over a period of two years, a total of 261 specimens were received in microbiology laboratory for anaerobic culture from patients admitted with diverse infections in surgical wards. Significant growth of pathogenic bacteria including anaerobic and aerobic bacteria was noted in 178 patients (68.2%). Among studied 261 patients, anaerobic bacteria were isolated from 64 patients (24.5%) (Table 1).

Among 64 patients with anaerobic infections, 21.9% had anaerobic bacteria as monomicrobial isolates (n=14). Anaerobes were isolated along with aerobic bacteria in 71.9% (n=46) and polymicrobial anaerobic growth was seen in 6.3% (n=4) patients. Anaerobic bacteria which were isolated as monomicrobial flora included *Clostridium* spp. (n=9), *Bacteroides fragilis* (n=2), *Peptostreptococcus anaerobius* (n=1), *Porphyromonas asaccharolyticus* (n=1) and *Veillonella parvula* (n=1). A total of 91 anaerobes were isolated from 64 surgical patients with an average of 1.42 isolates per specimen. Among these, the most common isolated microorganisms were anaer-

Table 1. Demographic and clinical details of patients infected with anaerobic bacteria (N=64)

Characteristic	No. of patients	Percentage (%)
Age (yrs)		
21 to 40	19	29.9
41 to 60	26	40.6
61 to 80	16	25.0
> 80	3	4.7
Gender		
Male	52	81.3
Female	12	18.8
Specimen		
Tissue	47	73.4
Pus aspirate	15	23.4
Wound swab	1	1.6
Pigtail drain	1	1.6
Type of microbial growth		
Monomicrobial anaerobic growth	14	21.9
Aerobic + Anaerobic growth	46	71.9
Polymicrobial anaerobic growth	4	6.2
Sites of infection		
Necrotising fasciitis	22	34.3
Abscess	15	23.4
Diabetic foot infection	11	17.2
Chronic non healing ulcer	11	17.2
Gas gangrene	3	4.7
Burn wound infection	1	1.6
Cellulitis	1	1.6

obic Gram-negative bacilli (n=34, 37.4%) followed by anaerobic Gram-positive cocci (n=29, 31.9%) and anaerobic Gram-positive bacilli (n=24, 26.4%). *Bacteroides fragilis* group (n=19, 20.9%) were the most frequent anaerobic Gram-negative bacilli followed by the pigmented *Prevotella* spp. (n=11, 12.1%). *Peptostreptococcus anaerobius* (n=13, 14.3%) was the predominant isolated anaerobic cocci (Table 2).

Among the mixed aerobic and anaerobic infections, 14 patients (21.9%) had anaerobic growth in association with *E. coli* followed by *K. pneumoniae* in 10 patients (15.6%). *P. anaerobius* was the most common anaerobic bacterium found in association with aerobic bacterial pathogens.

Necrotizing fasciitis was the most common clinical presentation with anaerobic etiology followed by deep seated abscesses, infected non healing ulcer and diabetic foot infection (Table 1).

Diabetes mellitus (n=28, 43.8%) was found to be the most frequent underlying risk factor followed by the history of trauma (n=10, 15.6%) and prior surgery (n=3, 4.7%). One patient (1.6%) had a history of snake bite.

DISCUSSION

Anaerobic infections are derived from the host's own endogenous flora, with few exceptions like *Clostridium* spp. In a non-diseased state, these microorganisms form an important part of the normal flora that inhabit the mucosal surfaces and play a key role in preventing the colonization of pathogenic, exogenous microbial populations. Any structural and functional defect including breech in the normal mucosal barriers, any localized vascular insufficiencies or obstruction leads to the infections (2).

Surgical infections are largely polymicrobial and both aerobic and anaerobic bacteria have been involved in the pathogenesis of these infections (13).

Table 2. Distribution of anaerobic bacterial isolates from surgical infections

Isolates	No.	Total Percentage (%)
Anaerobic gram-positive cocci		
Peptostreptococcus anaerobius	13	14.3
Finegoldia magna	8	8.8
Peptoniphilus asaccharolyticus	7	7.7
Anaerococcus prevotii	1	1.1
Anaerobic gram-negative cocci		
Veillonella parvula	4	4.4
Anaerobic gram-negative bacilli		
Bacteroides fragilis subsp. fragilis	12	13.2
Bacteroides fragilis subsp. vulgatus	1	1.1
Bacteroides fragilis subsp. thetaiotaomicron	3	3.3
Bacteroides fragilis subsp. ovatus	3	3.3
Fusobacterium nucleatum	3	3.3
Prevotella bivia	8	8.8
Prevotella melaninogenica	2	2.2
Prevotella buccae	1	1.1
Porphyromonas asaccharolytica	1	1.1
Anaerobic gram-positive bacilli		
Clostridium perfringens	3	3.3
Clostridium bifermentans	4	4.4
Clostridium ramosum	3	3.3
Clostridium clostridioforme	3	3.3
Clostridium sporogenes	4	4.4
Clostridium baratii	3	3.3
Clostridium cadaveris	1	1.1
Clostridium subterminale	3	3.3
Total	**91**	**100%**

However, the anaerobic etiological diagnosis in infections is rarely sought for by the clinicians considering the longer turnaround time for anaerobic cultures and use of metronidazole as empirical therapy. On the other hand, many laboratories do not provide anaerobic workup considering the tedious process involved in anaerobic cultures, lack of standards for disk diffusion tests and absence of automated anaerobic susceptibility systems. A general decrease in susceptibility to metronidazole among anaerobes and clinical failure due to antibiotic resistance 1in anaerobic bacteria has been reported (14). Gram-positive anaerobes including *Actinomyces, Bifidobacterium, Eubacterium, Lactobacillus*, and *Propionibacterium* spp., have shown intrinsic resistance to metronidazole (14). Hence, it is essential for the laboratories to routinely offer anaerobic cultures and generate local data of anaerobic antimicrobial resistance profiles to aid the clinicians in choosing the empirical therapy.

The primary focus of this study was to analyse the role of anaerobic pathogens in surgical infections. The isolation of anaerobes in surgical infections depends on the type and site of infection, predisposing factors and the etiological agents in the target population. In our study, the overall isolation rate of anaerobic bacteria was 24.5% in which 78.1% were polymicrobial types including anaerobes alone or mixed with aerobic flora. This finding is similar to the reported literature on anaerobic infections (14). Earlier studies analyzing different surgical infections have reported varying anaerobic isolation rates reaching more than 87% in diabetic foot infections (2). In our study, we found the majority of the surgical infections in the age range of 41-60 years (40.6%) and male gender predominance was noted (81.2%) which was mirroring the other studies (7, 9, 15, 16). This could be related to their occupation and outdoor jobs which could pose risk factors for injuries and further infections.

We found anaerobic gram-negative bacilli (37.4%) are the predominantly isolated group. Similar results with *Bacteroides* spp. as the most frequently isolated microorganisms from surgical infections have been reported by the others (2, 6, 15-17). Members of the *B. fragilis* group which include 10 species are the frequently recovered isolates from clinical specimens and are also the most resistant bacteria towards antimicrobial agents (18). Infections by *B. fragilis* are associated with mortality of more than 19% and when left untreated, the mortality rate may be up to 60 % (19). We found abscesses as the most frequent site of isolation for *B. fragilis* group. Overall, *Clostridium* spp. (26.4%), *B. fragilis* group (20.9%) and *P. anaerobius* (14.3%) were the most common isolates.

Previous studies have shown that *Peptostreptococcus* spp. could be the most frequent isolated anaerobes in necrotizing fasciitis, infections following trauma in children and in chronic venous ulcers (20-22) as well as leg ulcers (23).

Gram-positive anaerobic cocci account for 25-30% of all anaerobic bacterial isolates from clinical specimens. Among them, *P. anaerobius* is known to be the most frequently associated with abscesses, infections of abdominal cavity and female genitourinary tract and chronic wounds (24). In our study, *P. anaerobius* was also found more commonly in association with necrotizing fasciitis. This anaerobe bacterium was also more frequently found in association with aerobes such as *E. coli* and *Klebsiella* spp. in polymicrobial infections. Microbial synergy provides for enhanced pathogenicity and severity of infections by virtue of enhanced expression of virulence factors, creation of tissue hypoxia and lower redox potential, production of specific nutrients and impairment of host cellular immune function (13).

Clostridium spp. are other frequent pathogens from surgical infections as Kamble et al. found as the predominant isolates in wound infections (3). Gorbach et al. have also shown that the major source of Clostridial infections could be intraabdominal sepsis associated with trauma or prior intestinal surgery (25). *Clostridium* spp. were commonly found in association with diabetic foot infections in our study and *C. perfringens* was isolated from three cases of gas gangrene (4.4%). In our study the majority of the anaerobic microorganisms were isolated from cases suffering from necrotizing fasciitis (34.3%) and deep seated abscess (23.4%) including pelvic, psoas, perinephric and intra-abdominal abscess. De et al. (9) have earlier reported post-operative wounds and diabetic foot ulcers frequently infected with anaerobic bacteria (30.1%). Saini et al. also recovered anaerobes from secondary peritonitis (88%) and necrotizing fasciitis (65%) (6).

In surgical infections, anaerobic etiology may be suspected by the clinician if infections are within the sites that normally harbor anaerobic flora like oropharynx, colon and female genital tract, a foul-smelling discharge, tissue necrosis with abscess formation or gangrene, gas production, showing characteristic forms through Gram staining, failure to recover likely pathogens by aerobic culture, failure to respond to the antibiotics that are inactive against anaerobes, infections associated with malignancy and those following human bites (26). The typical anaerobic bacterial infections include gas gangrene, brain abscess, oral infections, putrid lung abscesses, intra-abdominal abscesses, wound infections following gynecologic and bowel surgery, perirectal abscesses, postabortal infections and septic thrombophlebitis and these infections require sampling for anaerobic cultures (27).

In our study, a wide range of anaerobic bacterial pathogens were isolated which could be attributed to the quick transport of specimens to the laboratory, standardized bacteriological techniques, incubation of cultures for seven days and the automated systems for identification of species following preliminary identification by basic biochemical tests and antibiotic identification discs. However, there is a large gap to be filled in the request for anaerobic cultures when compared to aerobic ones which are presumed to be the important etiological agents in infectious sites.

CONCLUSION

Anaerobic bacteria which are the most overlooked microorganisms in the current aerobic culture based diagnostics were isolated from a significant proportion of surgical patients. It is necessary to perform anaerobic cultures in patients with surgical infections to provide appropriate patient care and to avoid therapeutic failures. The role of anaerobic bacteria in surgical infections needs to be recognized by both surgeons and clinical microbiologists.

REFERENCES

1. Finegold SM. Overview of clinically important anaerobes. *Clin Infect Dis* 1995; 20:S205-207.
2. Edmiston CE Jr, Krepel CJ, Seabrook GR, Jochimsen WG. Anaerobic infections in the surgical patient: Microbial etiology and Therapy. *Clin Infect Dis* 2002; 35:S112-118.
3. Kamble S, Pol S, Jose T, Gore V, Kagal A, Bharadwaj R. The prevalence of anaerobes from cutaneous and subcutaneous wound infections. *Indian J Basic Appl Med Res* 2014; 3:371-378.
4. Finegold SM (2000). Anaerobic bacteria: General concepts. In: Mandell, Douglas and Bennett's principles and practice of infectious diseases. Ed, GL Mandell, JE Bennet, R Dolin. Churchill Livingstone, 5th ed. Philadelphia, pp. 2519-2537.
5. Jamal W, Al Hashem G, Rotimi VO. Antimicrobial resistance among anaerobes isolated from clinical specimens in Kuwait hospitals: Comparative analysis of 11-year data. *Anaerobe* 2015; 31:25-30.
6. Saini S, Gupta N, Aparna, Lokveer, Griwan MS. Surgical infections: A Microbiological study. *Braz J Infect Dis* 2004; 8:118-125.
7. Mathew A, Mridula M, Vishwanath S, Mukhopadhyay C, Rodrigues G. Clinico-microbiological profile of necrotizing fasciitis secondary to diabetes mellitus in a tertiary care hospital. *Webmed Central General Surgery* 2010; 1(12):WMC001399.
8. Bessman AN, Wagner W. Non clostridial gas gangrene: A report of 48 cases and review of literature. *JAMA* 1975; 233:958-963.
9. De A, Gogate A. Prevalence of Gram negative anaerobic bacilli in routine clinical specimens. *Indian J Pathol Microbiol* 2001; 44:435-438.
10. Garg R, Kaistha N, Gupta V, Chander J. Isolation, identification and antimicrobial susceptibility of anaerobic bacteria: A study re-emphasizing its role. *J Clin Diagn Res* 2014; 8:DL01-DL02.
11. Collee JG, Miles RS, Watt B (1996). Tests for identification of bacteria. In: Mackie and McCartney Practical Medical Microbiology. Ed, JG Collee, AG Fraser, BP Marmion, A Simmons. Churchill Livingstone, 14th ed. New York, pp. 131-150.
12. Jousimies-Somer H, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM (2002). Wadsworth-KTL anaerobic bacteriology manual. 6th ed. Star Publishing Company. California.
13. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001; 14:244-269.
14. Lofmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin Infect Dis* 2010; 50:S16-S23.
15. Akhi MT, Ghotaslou R, Beheshtirouy S, Asgharzadeh M, Pirzadeh T, Asghari B, et al. Antibiotic susceptibility pattern of aerobic and anaerobic bacteria isolated from surgical site infection of hospitalized patients. *Jundishapur J Microbiol* 2015; 8:e20309.
16. Elliott D, Kufera JA, Myers RA. The microbiology of necrotizing soft tissue infections. *Am J Surg* 2000; 179:361-366.
17. Mousa HA. Aerobic, anaerobic and fungal burn wound infections. *J Hosp Infect* 1997; 37:317-323.
18. Liu C, Song Y, McTeague M, Vu AW, Wexler H, Finegold SM. Rapid identification of the species of the *Bacteroides fragilis* group by multiplex PCR assays using group- and species-specific primers. *FEMS Microbiol Lett* 2003; 222:9-16.
19. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. *Clin Microbiol Rev* 2007; 20:593-621.
20. Brook I, Frazier EH. Clinical and microbiological features of necrotizing fasciitis. *J Clin Microbiol* 1995; 33:2382-2387.
21. Brook I. Aerobic and anaerobic microbiology of infections after trauma in children. *J Accid Emerg Med* 1998; 15:162-167.
22. Brook I, Frazier EH. Aerobic and Anaerobic microbiology of chronic venous ulcers. *Int J Dermatol* 1998; 37:426-428.
23. Bowler PG, Davies BJ. The Microbiology of infected and noninfected leg ulcers. *Int J Dermatol* 1999; 38:573-578.
24. Murphy EC, Frick IM. Gram-positive anaerobic cocci-commensals and opportunistic pathogens. *FEMS Microbiol Rev* 2013; 37:520-553.
25. Gorbach SL, Thadepalli H. Isolation of *Clostridium* in human infections: evaluation of 114 cases. *J Infect Dis* 1975; 131:S81-85.
26. Nichols RL, Smith JW. Anaerobes from a surgical perspective. *Clin Infect Dis* 1994; 18:S280-286.
27. Anderson CB, Marr JJ, Ballinger WF. Anaerobic infections in surgery: clinical review. *Surgery* 1976; 79:313-324.