Original Article

Automation with Basic Microbiology Techniques: The Synergy Vibrates at a Higher Octave for Clinical Anaerobic Bacteriology and Antimicrobial Stewardship

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Abstract

Background: Anaerobic bacteria are the most common normal flora that colonizes the skin and lines the mucosal surfaces of our body. Anaerobic infections are most often overlooked, and failure to provide specific antimicrobial coverage to these organisms may result in therapeutic failure. The aims of our study were to look for the Microbiology profile, antimicrobial susceptibility, and clinical correlates of various anaerobic bacteria isolated from various clinical specimens of patients using automated techniques, i.e., Anoxomat and MALDI-TOF MS/Vitek-2.

Methods: All the specimens received for anaerobic culture were processed as per standard guidelines. Direct microscopic examination of each of the specimens was also done. Identification of anaerobic bacteria was done by MALDI-TOF Vitek MS and/or Vitek-2. Antimicrobial susceptibility testing was performed using the E-test method, and interpretation was done as per CLSI guidelines.

Results: A total of 910 specimens were received for anaerobic culture during the study period. Out of 910 specimens, a total of 71 bacteria were isolated anaerobically. Out of these 71 isolates, 54 were obligate anaerobes and 17 were facultative anaerobes. Out of 54 obligate anaerobes, *Bacteroides* sp. was isolated maximally (27.7%) followed by *Prevotella* sp. (24.0%). The various clinical specimens from which these anaerobes were recovered mainly included abdominal pus, perianal abscess, High vaginal swab, Urethral pus, and Breast abscess. Metronidazole resistance in *Bacteroides* sp. was found to be 30.7%, whereas in *Prevotella* sp. it was comparatively low (25%). Facultative anaerobes like various species of *Streptococcus* from deep-seated abscesses like Brain abscess were isolated anaerobically only.

Conclusion: Accurate and rapid detection of anaerobes due to automated methods synergizes with basic microbiological techniques, which leads to early institution of targeted therapy and thus helps in antimicrobial stewardship. Initial isolation of facultative anaerobes under anaerobic conditions only is also an interesting area of research.

Keywords: Anaerobes; Anoxomat; MALDITOF-MS; Antimicrobial stewardship; Metronidazole; Bacteroides

Introduction

Anaerobic bacteria are the most common normal flora that colonizes the skin and lines the mucosal surfaces of our body. They do not cause any infection in their natural state, but upon breach in the mucosal lining due to surgery, injury, or

trauma may cause infections. Anaerobic infections have been observed in almost every organ and anatomic region of the body. They may cause infections with varying severity from mild to life-threatening ones.[1, 2] Isolation and identification of anaerobic bacteria is a great challenge because of the efforts required for their cultivation and diagnosis. Anaerobic infections are most often overlooked, and failure to provide specific antimicrobial coverage to these organisms may result in therapeutic failure.[2] This study was done to illustrate the impact of automated techniques for the isolation and identification of anaerobic bacteria rapidly and reliably.

The aims and objectives of the study were to look for the Microbiology profile, antimicrobial susceptibility pattern, and clinical correlates of various anaerobic bacteria isolated from various clinical specimens of patients using automated techniques, i.e., Anoxomat and Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)/Vitek-2 (Biomerieux, France) and to see the impact of isolation of anaerobic bacteria on patient management and antimicrobial stewardship.

Materials and Methods

This study was done in a tertiary health care center from May 2018 to August 2019. The permission to conduct this retrospective study was obtained from the Institutional Ethics committee. Data collection was done through the Department of Microbiology and the Medical record department. The findings which were captured from Microbiology records were demographics of the patient, type of sample, direct microscopy findings, name of bacterial isolate, Obligate anaerobe/facultative aerobe/facultative anaerobe, identification method, i.e., MALDI-TOF MS or Vitek-2, in-vitro antimicrobial susceptibility. Clinical data of the patients with anaerobic bacterial infection was collected from the medical record section. Data was compiled as per the type of sample and bacterial isolate, correlation between conventional and automated techniques, mixed bacterial infections, antimicrobial susceptibility pattern, and clinical correlation.

All the specimens received for anaerobic culture were processed in the department of Microbiology as per standard guidelines using the Anoxomat technique.[3, 4] Specimens included in the study were pus and tissues from various sites, abscesses, High Vaginal Swabs, body fluids, and respiratory specimens. Specimens were transported either in transport media or immediately following collection with intimation to the laboratory. Direct microscopic examination of the specimen was also done by Gram's staining (Figure 1). Anaerobic cultures were put up on Brain Heart Infusion Blood Agar/Columbia Blood Agar/Phenyl Ethyl Alcohol Agar along with Aerobic culture on Columbia Blood Agar and MacConkey Agar. Anaerobiosis was achieved by the Anoxomat system, and the plates were incubated for 96 hours. Identification of the isolate was done by VITEK® MS and/or Vitek-2 using ANC cards (M/s Biomerieux India Limited). Antimicrobial susceptibility testing was performed using the E-test method (Biomerieux, France), and interpretation was done as per CLSI guidelines.[5, 6]

Anoxomat: Anoxomat is an automated, evacuation and replacement method of McIntosh & Fildes to create customized anaerobic, microaerophilic, or custom environments, such as capnophilic. It is a substitute for conventionally used methods like anaerobic gas chambers and gas-generating sachet systems which may be resource-intensive, error-prone, and cumbersome to handle. Hydrogen replaces Oxygen after the anaerobic evacuation cycle. The remaining mere 0.6% residual oxygen content is removed by the activated Palladium catalyst. The Anoxomat system allows laboratories to improve workflow, increase productivity—and save on operational costs also.

MALDI TOF-MS: It is an automated mass spectrometry microbial identification system that uses Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology. It contains a comprehensive database for the identification of microbes. In this technique, the microbe from the colony is mixed with a matrix supplied by the manufacturer on a position on the test slide, which is then placed into the mass spectrometer wherein this spot is activated by a laser. The matrix absorbs much of the energy and converts it into heat which vaporizes the outer portion of the specimen. The molecules then move through a vacuum space at a different rate based on the mass to charge ratio (m/z), and this time of flight is determined by the arrival of the different molecules at the detector. A summation of the time of flight for all molecules present produces a spectrum which is then electronically compared with all the spectra in the library of the system to determine the best match and subsequently the identification of the microbe. The accuracy of identification of anaerobes has been described previously as being 84% for species and 92% for genus.[7]

Results

A total of 910 specimens were received during the study period from which 71 anaerobic bacteria were isolated. Out of the 71 isolates, 54 were obligate anaerobes (Table 1) and 17 were facultative anaerobes (Table 2) which were isolated anaerobically only, i.e., they were not isolated aerobically. Out of 54 obligate anaerobic bacteria, *Bacteroides* sp. (n=15) and *Prevotella* sp. (n=15), were the most predominant ones. Table 1 also depicts the species distribution of various obligate anaerobic bacteria.

Table 3 describes the antimicrobial susceptibility pattern of commonly isolated anaerobic bacteria in our study. Table 4 and 5 describes the distribution of isolation of various anaerobic isolates with respect to different types of clinical samples.

Isolation of mixed aerobic and anaerobic bacteria was seen in 26 cases. The distribution of mixed isolates was as per Table 6.

Out of 54 patients with infection due to obligate anaerobes, antibiotics were changed in 26 patients after the culture report based on the type of anaerobic bacteria and antimicrobial susceptibility. In 28 patients, the empirical antibiotics being administered were continued as they were appropriate as per the culture and susceptibility report. In all the seventeen patients with infection due to facultative anaerobes isolated anaerobically only, optimum antibiotics could be administered only after the culture and susceptibility report. In a few patients, the decision of surgical management was also taken only after the culture report.

Table 1: Frequency of isolation of obligate anaerobic bacteria (with species distribution)

S.No.	Name of anaerobic bacteria	Number of isolates (%) (n=54)
1.	*Bacteroides* sp.	15 (27.8%)
	Bacteroides fragilis	09/15
	Bacteroides thetaiotaomicron	04/15
	Bacteroides vulgatus	02/15
2.	*Prevotella* sp.	15 (27.8%)
	Prevotella bivia	08/15
	Prevotella oralis	03/15
	Prevotella disiens	02/15
	Prevotella timonensis	02/15
3.	*Bifidobacterium* sp.	06 (11.1%)
4.	*Clostridium* sp.	04 (7.4%)
	Clostridium perfringens	02/04
	Clostridium tertium	02/04
5.	*Veillonella* sp.	04 (7.4%)
6.	*Peptostreptococcus anaerobius*	03 (5.6%)
7.	*Actinomyces* sp.	02 (3.7%)
	Actinomyces turicensis	01/02
	Actinomyces odontolyticus	01/02
8.	*Parabacteroides distasonis*	02 (3.7%)
9.	*Eggerthia catenaformis*	02 (3.7%)
10.	*Gardnerella vaginalis*	02 (3.7%)
11.	*Actinotignum schaalii*	01 (1.9%)
12.	*Fusobacterium gonidiaformans*	01 (1.9%)
13.	*Peptoniphilus asaccharolyticus*	01 (1.9%)

Table 2: Facultative anaerobes isolated anaerobically only

S.No	Name of Facultative Anaerobes	Number of isolates (%)
1.	*Staphylococcus aureus*	6 (35.2%)
2.	*Streptococcus constellatus*	3 (17.6%)
3.	*Streptococcus intermedius*	3 (17.6%)
4.	*Staphylococcus hominis*	1 (5.8%)
5.	*Streptococcus anginosus*	1 (5.8%)
6.	*Streptococcus canis*	1 (5.8%)
7.	*Streptococcus pneumoniae*	1 (5.8%)
8.	*Haemophilus parainfluenzae*	1 (5.8%)

Table 3: Antimicrobial susceptibility pattern

Antimicrobial	*Bacteroides* sp. (% Sensitivity)	*Prevotella* sp. (% Sensitivity)	*Peptostreptococcus* sp. (% Sensitivity)
Metronidazole	73.3% (11/15)	86.6% (13/15)	66.6% (02/03)
Amoxycillin+clavulanate	73.3% (11/15)	86.6% (13/15)	100% (03/03)
Piperacillin+tazobactam	86.6% (13/15)	100% (15/15)	100% (03/03)
Ceftriaxone	86.6% (13/15)	100% (15/15)	100% (03/03)
Imipenem	93.3% (14/15)	100% (15/15)	100% (03/03)
Meropenem	93.3% (14/15)	100% (15/15)	100% (03/03)
Tetracycline	26.6% (04/15)	13.3% (02/15)	66.6% (02/03)
Clindamycin	66.6% (10/15)	33.3% (05/15)	100% (03/03)
Chloramphenicol	46.6% (07/15)	66.6% (10/15)	100% (03/03)

Table 4: Distribution of the anaerobic isolates among various clinical specimens (Part 1)

Type of sample	*Bacteroides* sp.	*Prevotella* sp.	*Parabacteroides* sp.	*Fusobacterium* sp.	*Veillonella* sp.	*Peptostreptococcus* sp.
Abdominal pus	09	03	02	-	01	01
HVS	01	03	-	-	-	01
Ischiorectal abscess	02	-	-	-	-	-
Bartholin's abscess	-	01	-	01	-	-
Perianal abscess	02	-	-	-	-	-
Scrotal wall abscess	-	01	-	-	-	-
Respiratory specimen	-	-	-	-	03	-
Breast abscess	-	01	-	-	-	01
Peripenile/urethral abscess	01	01	-	-	-	-
Pyometra	-	-	-	-	-	-
Other's	-	05	-	-	-	-

Table 5: Distribution of the anaerobic isolates among various clinical specimens (Part 2)

Type of sample	*Peptoniphilus* sp.	*Bifidobacterium* sp.	*Actinomyces* sp.	*Actinotignum* sp.	*Clostridium* sp.
Abdominal pus	-	02	01	-	02
HVS	-	02	-	-	-
Ischiorectal abscess	-	-	-	-	-
Bartholin's abscess	-	-	-	-	-
Perianal abscess	-	-	-	01	-
Scrotal wall abscess	-	-	-	-	-
Respiratory specimen	-	-	01	-	-
Breast abscess	-	-	-	-	-
Peripenile/urethral abscess	-	-	-	-	-
Pyometra	01	-	-	-	-
Other's	-	-	-	-	-

Table 6: Frequency of occurrence of mixed aerobic and anaerobic bacterial isolates

Number of Mixed aerobic and anaerobic bacteria	Number of clinical samples
One Aerobic bacteria + One Anaerobic bacteria	15
Two Aerobic bacteria + One Anaerobic bacteria	9
Three Aerobic bacteria + One Anaerobic bacteria	2

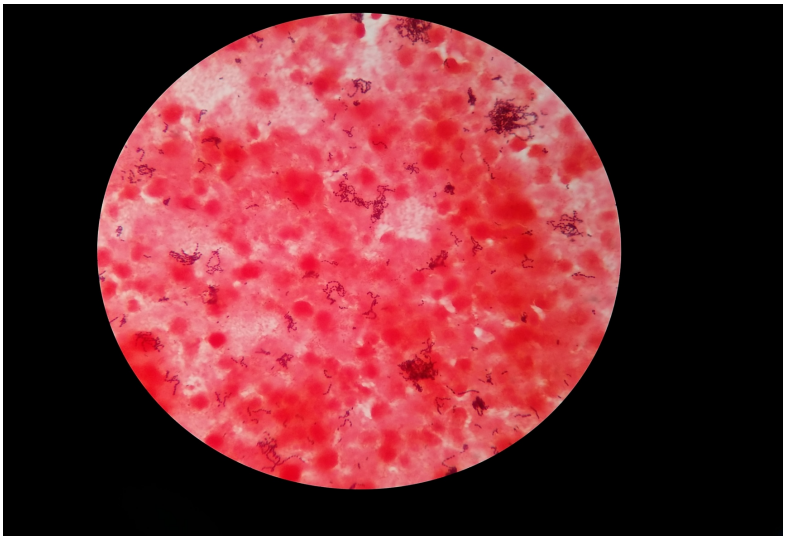

Figure 1: Gram stain examination (1000X)-Showing Gram-positive cocci in chains (Later grown as *Peptostreptococcus* in anaerobic culture)

Discussion

A good recovery of anaerobic bacteria (7.8%) was obtained in our study. Several studies showed variable recovery of anaerobes from various anatomic sites.[2, 8, 9, 10, 11, 12, 13] The majority of the isolates in our study were from intra-abdominal abscesses. Patients with intra-abdominal abscesses where the organisms were recovered from perioperative or

postoperative samples. Intra-abdominal surgeries were performed due to perforation peritonitis, malignancy, post-transplant, etc.

For successful isolation of anaerobic bacteria–specimen collection, transportation, quality and selection of primary isolation media, anaerobic incubation system, and efficient identification systems are crucial.[2] In our study, automated systems in synergy with the conventional method were helpful for a good yield of anaerobic bacteria. The clue towards anaerobic infection was obtained by conventional Gram stain examination which also helped in deciding appropriate culture media. The good anaerobiosis was created by an automated evacuation and replacement technique-based Anoxomat system, and rapid and reliable identification was done by the MALDI TOF-MS System. The better recovery of anaerobes through automated evacuation and replacement systems like anoxomat have been seen in a few comparative studies.[14, 15] Also, there are several studies citing the advantage of MALDI-TOF MS over other phenotypic methods for earlier and optimal detection.[16, 17, 18, 19, 20]

Bacteroides sp. (n=15) and *Prevotella* sp. (n=15) were the most commonly isolated anaerobic bacteria in our study. *Bacteroides* sp. have also been observed as a predominant isolate in several other studies.[2, 8, 21, 22] We found the predominant isolation of *Bacteroides* sp. from intraabdominal abscesses which was also observed by Gorbach et al. in their study. In our study, *Prevotella* sp. were mainly isolated from genitourinary abscesses.[23] In a study by Kaori et al., *Prevotella* sp. was found to be the predominantly isolated anaerobe in Bartholin's gland abscess.[24] A study by George et al. highlighted the prevalence of *Prevotella* sp. in female genital tract infections.[25]

Polymicrobial infections, i.e., isolation of both anaerobic and aerobic pathogens, were also observed in 26 clinical specimens, and the most common aerobic isolates were *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Enterococcus* sp., and *Streptococcus* sp. Microbial synergy with polymicrobial infections involving both aerobic and anaerobic pathogens may lead to enhanced pathogenicity and severity of infection.[2] The optimum isolation and identification of mixed aerobic and anaerobic bacterial flora was possible only due to automation in combination with the conventional Gram stain technique which gave the basic impression about mixed aerobic and anaerobic bacterial flora in a clinical specimen.

One of the best findings of our study was the primary isolation of facultative anaerobes under anaerobic conditions only. There were 17 such isolates, and *Staphylococcus aureus* (n=6) was the predominantly isolated facultative anaerobe. *Staphylococcus* is a facultative anaerobe that shifts more towards reductive conditions by enhancing nitrogen metabolism.[26] There are studies and case reports of facultative anaerobes being primarily isolated anaerobically. Peake et al. reported a case of Septicemia due to an obligate anaerobic strain of *Staphylococcus aureus*.[27] Rowlinson et al. reported a case of Prosthetic hip joint infection due to a strictly anaerobic strain of *Staphylococcus epidermidis*.[28] There are many studies which emphasize that in certain strains of *Staphylococcal* sp. (especially *Staphylococcus aureus* and *Staphylococcus epidermidis*) that grow anaerobically there is an increased production of ica operon. This ica operon encodes for polysaccharide intracellular adhesion, which is associated with biofilm production and device-associated infections.[29, 30] Therefore, there is an implication that anaerobic growth of these organisms can result in biofilm production, which may result in tolerance to antibiotics and thus increased pathogenicity.[28] The other facultative anaerobes in our study were isolated mainly from deep-seated abscesses like abdominal abscess, Brain abscess, and discharging sinuses from post-prosthetic joint infections. The facultative anaerobes that can behave as anaerobes upon initial isolation have been reported as *Streptococcus intermedius*, *Streptococcus constellatus*, *Gemella morbillorum*, and *Staphylococcus saccharolyticus*.[28]

The clinical implications of fast and accurate identification of anaerobes were seen in our study as it resulted in rapid isolation and accurate identification of anaerobic bacteria which led to the timely management of infections (Surgical debridement and/or Medical treatment). Also, there were certain infections which were clinically diagnosed as sexually transmitted diseases due to *Neisseria gonorrhoeae*, etc., turned out to be anaerobic infections, thus resulting in the institution of appropriate antimicrobial therapy. Anaerobic infections like bacterial vaginosis could also be diagnosed and treated optimally.

It has been observed that antimicrobial resistance amongst anaerobes is increasing consistently over the past few decades and the susceptibility of anaerobes to antimicrobials has become much less predictable; because of this, the choice of appropriate empirical therapy has become even more difficult.[31] So, it is imperative to optimally speciate the anaerobic bacteria because there are certain anaerobic bacteria like *Bacteroides* sp. that are inherently resistant to a few antibiotics.[32] Increased instances of resistance of anaerobic bacteria to some commonly used antibiotics call for the antimicrobial susceptibility testing of the isolates.

Conclusion

Accurate and rapid detection of anaerobes due to automated methods synergizes with basic microbiological techniques which lead to early institution of targeted therapy and thus pave the way to antimicrobial stewardship. Initial isolation of facultative anaerobes under anaerobic conditions only is also an interesting area of research. Further studies may be performed to elucidate it in terms of pathogenesis, virulence, and other characteristics of such bacteria.

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